BİTKİ DOKU KÜLTÜRÜ
Temel Prensipleri ve Yöntemleri

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Önsöz

Bitki doku kültürü, modern biyoteknolojinin en önemli uygulama alanlarından biri olarak hem temel bitkisel araştırmalar hem de bitkisel üretim süreçleri açısından vazgeçilmez bir konuma sahiptir. Bu yöntem, bitkilerin hücre, doku ve organ düzeyinde bitki büyüme ve gelişmesi için gerekli besin maddelerini içeren aseptik ortamlarda çoğaltılmasını, genetik özelliklerinin korunmasını ve farklı biyoteknolojik uygulamalarda kullanılmasını mümkün kılmaktadır. Günümüzde mikroçoğaltma, gen transferi, sekonder metabolit üretimi ve bitki gen kaynaklarının muhafazası gibi geniş bir uygulama yelpazesiyle hem akademik dünyada hem de tarımsal üretimde önemli katkılar sunmaktadır.

Elinizdeki kitap, bitki doku kültürünün hem teorik temellerini hem de pratik laboratuvar uygulamalarını sistematik bir biçimde ele almak üzere hazırlanmıştır. Kitapta yer alan bölümler, doku kültürünün tarihsel gelişiminden başlayarak kullanılan temel araç ve gereçlere, besin ortamı bileşenlerinden aseptik tekniklere, kallus kültürü, embriyo kurtarma, somatik embriyogenez, protoplast kültürü, haploid bitkilerin elde edilmesi gibi ileri düzey yöntemlere kadar geniş bir yelpazeyi kapsamaktadır. Ayrıca, bitki biyoteknolojisinde güncel araştırma eğilimlerine ve tarımsal üretimdeki uygulamalar ile ticarileşme konularına da değinilmektedir.

Benim için bu kitabın özel bir anlamı daha bulunmaktadır. Yaklaşık otuz beş yıl önce Türkiye'de ilk modern bitki biyoteknolojisi laboratuvarını kurma imkânını buldum. Bu laboratuvar, yalnızca araştırma faaliyetlerine değil, aynı zamanda birçok öğrencinin yetişmesine de zemin hazırladı. Bugün gururla söyleyebilirim ki, o öğrencilerden birçoğu kendi alanlarında başarılı birer öğretim üyesi olarak yeni kuşaklara rehberlik etmektedir. Bilimsel bilginin nesilden nesile aktarımı, yalnızca bireysel başarıların değil, aynı zamanda bir akademik topluluğun ve ülkemizin bilimsel kapasitesinin güçlenmesi açısından da büyük önem taşımaktadır. Bu kitap, bir anlamda bu uzun yolculuğun ve ortak çabanın bir ürünü olarak da değerlendirilebilir.

Ayrıca, kitabın farklı bölümlerinin yazımını üstlenerek bu eserin oluşmasına değerli katkılar sunan meslektaşlarıma da en içten teşekkürlerimi sunmak isterim. Her birinin kendi uzmanlık alanındaki bilgi birikimi, bu kitabın kapsamını genişletmiş ve okuyucular için çok daha zengin bir içerik ortaya çıkmasını sağlamıştır. Bu iş birliği ruhu, bilimsel üretimin en önemli unsurlarından biri olan paylaşma ve birlikte çalışma kültürünün de en güzel örneklerinden biri olmuştur.

Kitabın hazırlanmasında hem klasik literatürden hem de güncel bilimsel çalışmalardan yararlanılmış, ayrıca uygulama konuları ile laboratuvar pratiğine yönelik ipuçlarına özel önem verilmiştir. Böylece okurun yalnızca kavramsal bilgi edinmekle kalmayıp, aynı zamanda bu bilgileri laboratuvar koşullarında uygulayabilmesi hedeflenmiştir.

Bitki doku kültürü, doğrudan tarımsal üretime ve gıda arz güvencesine hizmet eden somut çıktılar üretmektedir. Özellikle küresel iklim değişikliği, biyotik ve abiyotik stresler, artan nüfus ve tarımsal sürdürülebilirlik gibi hayati güncel sorunlar karşısında bu yöntemlerin önemi daha da belirginleşmektedir. Bu bağlamda, bu kitabın yalnızca bir ders kaynağı olmanın ötesinde, aynı zamanda araştırma ve inovasyon süreçlerine de katkı sağlamasını diliyorum.

Bu eserin hazırlanmasında emeği geçen tüm yazar, katkı sunan meslektaşlarım ve öğrencilerime teşekkür eder, kitabın bitki biyoteknolojisi alanına ilgi duyan araştırmacılar, öğrenciler ve uygulayıcılar için yararlı bir başvuru kaynağı olmasını temenni ederim.

Prof. Dr. Selim ÇETİNER
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Aralık 2025

Editör Önsözü

Değerli okuyucular,

Elinizdeki bu eser, bitki doku kültürü alanında uzun yıllar süren titiz bir çalışmanın ve çok değerli bilim insanlarının ortak emeklerinin ürünüdür. Bir kitabın doğuşu, tıpkı bir bitkinin doku kültüründen yeniden yaratılması gibi, sabır, özen ve bilimsel titizlik gerektiren bir süreçtir. Bu süreçte bizlere eşlik eden, kendi uzmanlık alanlarında en güncel bilgileri ve deneyimlerini cömertçe paylaşan tüm bölüm yazarlarımıza en derin şükranlarımızı sunuyoruz.

Bitki doku kültürü, modern bitki biliminin ve tarımsal biyoteknolojinin en önemli araçlarından biridir. Temel bilimden uygulamalı araştırmalara, hastalıksız bitki üretiminden genetik kaynakların korunmasına, sekonder metabolit üretiminden ticari uygulamalara kadar uzanan geniş bir yelpazede bu teknoloji, günümüz tarımının ve bitki ıslahının vazgeçilmez unsuru haline gelmiştir. Özellikle iklim değişikliğinin tarımsal üretimi tehdit ettiği, gıda güvenliğinin küresel bir öncelik olduğu günümüzde, bitki doku kültürü teknikleri daha da kritik bir anlam kazanmaktadır.

Bu kitap, alanın temel prensiplerinden en güncel uygulamalarına, geleneksel yöntemlerden yapay zeka destekli modern yaklaşımlara kadar geniş bir içeriği kapsamaktadır. On sekiz bölümden oluşan eserimiz, hem teori hem de pratik uygulamaları bir arada sunarak, okuyucularına bitki doku kültürü konusunda kapsamlı bir perspektif kazandırmayı amaçlamaktadır. Laboratuvar organizasyonundan biyoreaktör sistemlerine, mikroçoğaltımdan protoplast kültürüne kadar her bölüm, alanında uzman akademisyenler tarafından özenle hazırlanmıştır.

Umuyoruz ki bu eser, üniversitelerimizdeki lisans ve lisansüstü öğrencilerimiz için temel bir kaynak, genç araştırmacılarımız için yol gösterici bir rehber ve alanda çalışan bilim insanlarımız için güncel bir referans olacaktır. Yıllar boyunca okunacak, not alınacak ve laboratuvarlarda yol gösterecek bir eser olması en büyük arzumuzdur.

Bitki doku kültürü camiamıza, öğrencilerimize ve Türk bilim dünyasına faydalı olması dileğiyle...

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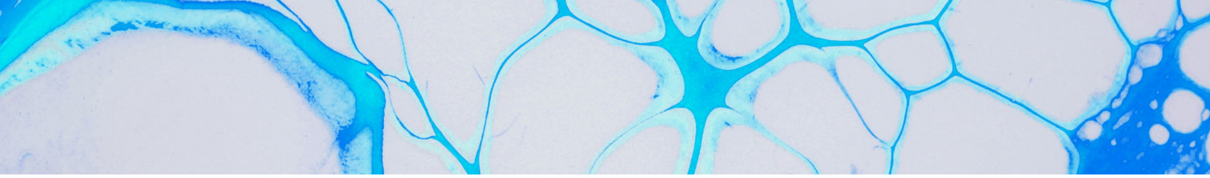

Bölüm 1

BİTKİ DOKU KÜLTÜRLERİNİN TARİHSEL GELİŞİMİ

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Emine AÇAR [2]

1.1 BİTKİ DOKU KÜLTÜRÜNÜN TEMEL KAVRAMSAL DAYANAĞI VE TOTİPOTENSİ İLKESİ

Bitki doku kültürü, *in vitro* olarak tanımlanan fiziksel ve kimyasal koşullar altında bitki hücre, doku, organ ve bunların bileşenlerinin aseptik kültürü olarak tanımlanmaktadır. Bitki doku kültürünün teorik temeli ilk kez Gottlieb Haberlandt tarafından 1902 yılında Alman Bilim Akademisi'ne yaptığı konuşmada ortaya konmuştur. Haberlandt, izole edilmiş fotosentetik yaprak hücreleri ve işlevsel olarak farklılaşmış bitki hücreleri ile deneyler gerçekleştirmiş, bu çalışmalarda doğrudan bitki rejenerasyonu sağlayamamış olsa da tek bir bitki hücresinden yapay embriyoların geliştirilebileceğini öngörmüştür. Böylece, bir hücrenin tüm bir bitkiye dönüşebilme kapasitesini ifade eden "totipotensi" kavramı ilk kez bilimsel bir çerçevede tanımlanmıştır. Bu öncü yaklaşımı nedeniyle Haberlandt, haklı olarak bitki doku kültürünün babası olarak kabul edilmektedir (Thorpe, 2007). Haberlandt'ın ortaya koyduğu bu kuramsal yaklaşım, izleyen yıllarda deneysel çalışmalarla desteklenmeye başlanmıştır. Farklı bir yaklaşımla, Haberlandt'ın öğrencisi olan Kotte (1922) ve Robbins (1922), izole edilmiş kök uçlarını kültüre almayı başarmış ve meristematik hücrelerin *in vitro* koşullarda daha yüksek rejenerasyon kapasitesine sahip olduğunu göstermiştir. Bu yaklaşım daha sonra White (1934) tarafından domates kök uçlarının kullanımıyla ileriye taşınmış; böylece tamamen tanımlanmış ortamlarda kök kültürü sistemleri geliştirilebilmiştir. Kök kültürleri başlangıçta viral etmenlerin incelenmesinde, daha sonraki yıllarda ise fizyolojik ve metabolik çalışmalar için önemli bir araç hâline gelmiştir (Street, 1969). Bu erken dönem çalışmalarda özellikle apikal meristemlerin yüksek totipotent

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Abdalla, N., El-Ramady, H., Seliem, M., El-Mahrouk, M., Taha, N., Bayoumi, Y., Shalaby, T. A., & Dobránszki, J., 2022. An academic and technical overview on plant micropropagation challenges. Horticulturae, 8(8), 677. https://doi.org/10.3390/horticulturae8080677

Ahuja, M., 2021. Fate of forest tree biotechnology facing climate change. Silvae Genetica, 70(1), 117-136. https://doi.org/10.2478/sg-2021-0010

Alam, N., Anis, M., 2019. Untitled. Research Journal of Life Sciences Bioinformatics Pharmaceutical and Chemical Sciences, 05(02). https://doi.org/10.26479/2019.0502.34

Ali, M., Okubo, H., Fujieda, K., 1991. *In vitro* multiplication of intra- and interspecific solanum hybrids through somatic embryogenesis and adventitious organogenesis. Journal of the Japanese Society for Horticultural Science, 60(3), 601-612. https://doi.org/10.2503/jjshs.60.601

Alok, A., Sandhya, D., Jogam, P., Rodrigues, V., Bhati, K., Sharma, H., & Kumar, J., 2020. The rise of the crispr/cpf1 system for efficient genome editing in plants. Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.00264

Amankwaah, V., Prempeh, R., Quain, M., 2023. Plant cryopreservation importance, approaches and future trends. https://doi.org/10.5772/intechopen.108806

Arab, M., Yadollahi, A., Eftekhari, M., Ahmadi, H., Akbari, M., Sarikhani, S., 2018. Modeling and optimizing a new culture medium for *in vitro* rooting of g×n15 prunus rootstock using artificial neural network-genetic algorithm. Scientific Reports, 8(1). https://doi.org/10.1038/s41598-018-27858-4

Arab, M., Yadollahi, A., Shojaeiyan, A., Ahmadi, H., 2016. Artificial neural network genetic algorithm as powerful tool to predict and optimize *in vitro* proliferation mineral medium for g × n15 rootstock. Frontiers in Plant Science, 7. https://doi.org/10.3389/fpls.2016.01526

Aremu, A., Moyo, M., Amoo, S., Grúz, J., Šubrtová, M., Plíhalová, L., Doležal, K., & Staden, J., 2014. Effect of a novel aromatic cytokinin derivative on phytochemical levels and antioxidant potential in greenhouse grown *Merwilla plumbea*. Plant Cell Tissue and Organ Culture (Pctoc), 119(3), 501-509. https://doi.org/10.1007/s11240-014-0550-0

Ashmore, S., Hamilton, K., Offord, C., 2010. Conservation technologies for safeguarding and restoring threatened flora: case studies from Eastern Australia. In Vitro Cellular & Developmental Biology - Plant, 47(1), 99-109. https://doi.org/10.1007/s11627-010-9320-9

Badr-Elden, A., Nower, A., Nasr, M., Ibrahim, I., 2010. Isolation and fusion of protoplasts in sugar beet (*Beta vulgaris* L.). Sugar Tech, 12(1), 53-58. https://doi.org/10.1007/s12355-010-0010-z

Bettoni, J., Costa, M., Gardin, J., Kretzschmar, A., Pathirana, R. 2016. Cryotherapy: A new technique to obtain grapevine plants free of viruses. Revista Brasileira De Fruticultura, 38(2). https://doi.org/10.1590/0100-29452016833

Bhowmik, P., Konkin, D., Polowick, P., Hodgins, C., Subedi, M., Xiang, D., Yu, B., Patterson, N., Rajagopalan, N., Babic, V., Ro, K., Bandara, M., Smyth, S. J., Cui, Y., & Kagale, S., 2021. Crispr/cas9 gene editing in legume crops: Opportunities and challenges. Legume Science, 3(3). https://doi.org/10.1002/leg3.96

Bortesi, L., Fischer, R., 2015. The crispr/cas9 system for plant genome editing and beyond. Biotechnology Advances, 33(1), 41-52. https://doi.org/10.1016/j.biotechadv.2014.12.006

Bouzroud, S., Gasparini, K., Hu, G., Barbosa, M., Rosa, B., Fahr, M., Bendaou, N., Bouzayen, M., Zsögön, A., Smouni, A., & Zouine, M., 2020. Down regulation and loss of auxin response factor 4 function using crispr/cas9 alters plant growth, stomatal function and improves tomato tolerance to salinity and osmotic stress. Genes, 11(3), 272. https://doi.org/10.3390/genes11030272

Bunn, E., Turner, S., Dixon, K., 2011. Biotechnology for saving rare and threatened flora in a biodiversity hotspot. In Vitro Cellular & Developmental Biology - Plant, 47(1), 188-200. https://doi.org/10.1007/s11627-011-9340-0

Cadavid, J., Lamouri, S., Grabot, B., Pellerin, R., Fortin, A., 2020. Machine learning applied in production planning and control: a state-of-the-art in the era of industry 4.0. Journal of Intelligent Manufacturing, 31(6), 1531-1558. https://doi.org/10.1007/s10845-019-01531-7

Campos, N., Panis, B., Carpentier, S., 2017. Somatic embryogenesis in coffee: The evolution of biotechnology and the integration of omics technologies offer great opportunities. Frontiers in Plant Science, 8. https://doi.org/10.3389/fpls.2017.01460

Cantone, I. Fisher, A., 2013. Epigenetic programming and reprogramming during development. Nature Structural & Molecular Biology, 20(3), 282-289. https://doi.org/10.1038/nsmb.2489

Čapla, J., Zajác, P., Čurlej, J., Hanušovský, O., 2025. The current state of carbon footprint quantification and tracking in the agri-food industry. Scifood, 19, 110-127. https://doi.org/10.5219/scifood.28

Ceasar, S., Ignacimuthu, S., 2010. Effects of cytokinins, carbohydrates and amino acids on induction and maturation of somatic embryos in kodo millet (*Paspalum scorbiculatum* linn.). Plant Cell Tissue and Organ Culture (Pctoc), 102(2), 153-162. https://doi.org/10.1007/s11240-010-9716-6

Chavan, J., Patil, P., Patil, A., Deshmukh, A., Panari, P., Mohite, A., Lawand, P., Yadav, P., Bodhe, M., Kadam, A., Namdas, D., Pawar, B., Jadhav, A., Shekhawat, M., & Santa-Catarina, C., 2024. *Salacia* spp.: Recent insights on biotechnological interventions and future perspectives. Applied Microbiology and Biotechnology, 108(1). https://doi.org/10.1007/s00253-023-12998-z

Coelho, N., Gonçalves, S., Romano, A., 2020. Endemic plant species conservation: biotechnological approaches. Plants, 9(3), 345. https://doi.org/10.3390/plants9030345

Coenen, C., Christian, M., Lüthen, H., Lomax, T., 2003. Cytokinin inhibits a subset of diageotropica-dependent primary auxin responses in tomato. Plant Physiology, 131(4), 1692-1704. https://doi.org/10.1104/pp.102.016196

Condic, M., (2014). Totipotency: What it is and what it is not. Stem Cells and Development, 23(8), 796-812. https://doi.org/10.1089/scd.2013.0364

Das, B., Bhattarai, A,. 2025. The versatility of algae in addressing the global sustainability challenges. Frontiers in Bioengineering and Biotechnology, 13. https://doi.org/10.3389/fbioe.2025.1621817

El-Showk, S., Ruonala, R., Helariutta, Y., 2013. Crossing paths: Cytokinin signalling and crosstalk. Development, 140(7), 1373-1383. https://doi.org/10.1242/dev.086371

Esyanti, R., Fadholi, M., Rizki, R., Faizal, A., 2019. Shoot multiplication and growth rates of *Aquilaria malaccensis* lamk. shoot cultures in temporary immersion system (tis)-rita® and bubble column bioreactors. Pakistan Journal of Botany, 51(4). https://doi.org/10.30848/pjb2019-4(36)

Fakhrzad, F., Jowkar, A., Hosseinzadeh, J., 2022. Mathematical modeling and optimizing the *in vitro* shoot proliferation of wallflower using multilayer perceptron non-dominated sorting genetic algorithm-ii (mlp-nsgaii). Plos One, 17(9), e0273009. https://doi.org/10.1371/journal.pone.0273009

Feng, D., Xia, G., Zhao, S., Chen, F., 2004. Two quality-associated hmw glutenin subunits in a somatic hybrid line between *Triticum aestivum* and *Agropyron elongatum*. Theoretical and Applied Genetics, 110(1), 136-144. https://doi.org/10.1007/s00122-004-1810-x

Fki, L., Bouaziz, N., Kriaâ, W., Benjemaa-Masmoudi, R., Gargouri-Bouzid, R., Rival, A., & Drira, N., 2011. Multiple bud cultures of 'barhee' date palm (*Phoenix dactylifera*) and physiological status of regenerated plants. Journal of Plant Physiology, 168(14), 1694-1700. https://doi.org/10.1016/j.jplph.2011.03.013

Fonsah, E., Adamu, C., Okole, B., Mullinix, B., 2007. Field evaluation of cavendish banana cultivars propagated either by suckers or by tissue culture, over six crop cycles in the tropics. Fruits, 62(4), 205-212. https://doi.org/10.1051/fruits:2007016

Gamborg, O. L., 2002. Plant tissue culture. Biotechnology milestones. In Vitro Cellular & Developmental Biology – Plant, 38(2), 84–92.

Gao, C., 2021. Genome engineering for crop improvement and future agriculture. Cell, 184(6), 1621-1635. https://doi.org/10.1016/j.cell.2021.01.005

Gautheret, R. J., 1985. History of plant tissue and cell culture: A personal account. Cell Culture and

Somatic Cell Genetics of Plants, 2, 1-59.

Georgiev, V., Schumann, A., Pavlov, A., Bley, T., 2014. Temporary immersion systems in plant biotechnology. Engineering in Life Sciences, 14(6), 607-621. https://doi.org/10.1002/elsc.201300166

Greplová, M., Polzerová, H., Vlastníková, H., 2008. Electrofusion of protoplasts from *Solanum tuberosum*, *S. bulbocastanum* and *S. pinnatisectum*. Acta Physiologiae Plantarum, 30(6), 787-796. https://doi.org/10.1007/s11738-008-0183-1

Grosser, J. Gmitter, F., 2010. Protoplast fusion for production of tetraploids and triploids: Applications for scion and rootstock breeding in citrus. Plant Cell Tissue and Organ Culture (Pctoc), 104(3), 343-357. https://doi.org/10.1007/s11240-010-9823-4

Grzebelus, E., Szklarczyk, M., Barański, R., 2011. An improved protocol for plant regeneration from leaf- and hypocotyl-derived protoplasts of carrot. Plant Cell Tissue and Organ Culture (Pctoc), 109(1), 101-109. https://doi.org/10.1007/s11240-011-0078-5

Haberlandt, G., 1902. Cellular totipotency. Elsevier Science Publishing Co., New York, 71–90.

Hang, J., Zhang, D., Chen, P., Zhang, J., Wang, B., 2019. Classification of plant leaf diseases based on improved convolutional neural network. Sensors, 19(19), 4161. https://doi.org/10.3390/s19194161

Hesami, M., Condori-Apfata, J., Valencia, M., Mohammadi, M., 2020. Application of artificial neural network for modeling and studying *in vitro* genotype-independent shoot regeneration in wheat. Applied Sciences, 10(15), 5370. https://doi.org/10.3390/app10155370

Hesami, M., Naderi, R., Tohidfar, M., 2019. Modeling and optimizing medium composition for shoot regeneration of chrysanthemum via radial basis function-non-dominated sorting genetic algorithm-ii (rbf-nsgaii). Scientific Reports, 9(1). https://doi.org/10.1038/s41598-019-54257-0

Hoque, F., Robbani, M., Hasan, M., Parvin, J., 2018. Standardization of protocol for *in vitro* propagation of banana (*Musa sapientum*). Journal of the Bangladesh Agricultural University, 16(1), 27-30. https://doi.org/10.3329/jbau.v16i1.36477

Ikeuchi, M., Sugimoto, K., Iwase, A., 2013. Plant callus: mechanisms of induction and repression. The Plant Cell, 25(9), 3159-3173. https://doi.org/10.1105/tpc.113.116053

Iovene, M., Aversano, R., Savarese, S., Caruso, I., Matteo, A., Cardi, T., Frusciante, L., & Carputo, D., 2012. Interspecific somatic hybrids between *Solanum bulbocastanum* and *S. tuberosum* and their haploidization for potato breeding. Biologia Plantarum, 56(1), 1-8. https://doi.org/10.1007/s10535-012-0008-3

Irish, E., Nelson, T., 1988. Development of maize plants from cultured shoot apices. Planta, 175(1), 9-12. https://doi.org/10.1007/bf00402876

Jadhav, N., Rajnivas, B., Subaprıya, V., Sivaramakrishnan, S., Premalatha, S., 2023. Enhancing crop growth efficiency through iot-enabled smart farming system. Eai Endorsed Transactions on Internet of Things, 10. https://doi.org/10.4108/eetiot.4604

Jaganathan, D., Ramasamy, K., Sellamuthu, G., Shilpha, J., Venkataraman, G., 2018. Crispr for crop improvement: An update review. Frontiers in Plant Science, 9. https://doi.org/10.3389/fpls.2018.00985

Jaiswal, S., Hammatt, N., Bhojwani, S., Cocking, E., Davey, M., 1990. Plant regeneration from cotyledon protoplasts of *Brassica carinata*. Plant Cell Tissue and Organ Culture (Pctoc), 22(3), 159-165. https://doi.org/10.1007/bf00033630

Jamil, S., Shahzad, R., Talha, G., Sakhawat, G., Sultana, R., Iqbal, M., 2017. Optimization of protocols for *in vitro* regeneration of sugarcane (*Saccharum officinarum*). International Journal of Agronomy, 2017, 1-8. https://doi.org/10.1155/2017/2089381

Jeong, Y., Lee, H., Kim, S., Noh, Y., Seo, P., 2021. Optimization of protoplast regeneration in the model plant *Arabidopsis thaliana*. Plant Methods, 17(1). https://doi.org/10.1186/s13007-021-00720-x

Jing, Y., Beleski, D., Vendrame, W., 2023. Micropropagation and acclimatization of *Monstera deliciosa* liebm. 'thai constellation'. Horticulturae, 10(1), 1. https://doi.org/10.3390/horticultu-

rae10010001
Jiroutová, P., Sedlák, J., 2020. Cryobiotechnology of plants: A hot topic not only for gene banks. Applied Sciences, 10(13), 4677. https://doi.org/10.3390/app10134677
Jova, M., Gómez-Kosky, R., Pérez, M., Pino, A., Vega, V., Torres, J., Cabrera, A., García, M., & de Ventura, J., 2005. Production of yam microtubers using a temporary immersion system. Plant Cell Tissue and Organ Culture (Pctoc), 83(1), 103-107. https://doi.org/10.1007/s11240-005-4853-z
Kang, H., Naing, A., Kim, C., 2020. Protoplast isolation and shoot regeneration from protoplast-derived callus of *Petunia hybrida* cv. mirage rose. Biology, 9(8), 228. https://doi.org/10.3390/biology9080228
Katel, S., Yadav, S., Turyasingura, B., Mehta, A., 2023. Salicornia as a salt-tolerant crop: Potential for addressing climate change challenges and sustainable agriculture development. Turkish Journal of Food and Agriculture Sciences, 5(2), 55-67. https://doi.org/10.53663/turjfas.1280239
Kaur, K., Dolker, D., Behera, S., Pati, P., 2022. Critical factors influencing *in vitro* propagation and modulation of important secondary metabolites in *Withania somnifera* (L.) dunal. Plant Cell Tissue and Organ Culture (Pctoc), 149(1-2), 41-60. https://doi.org/10.1007/s11240-021-02225-w
Khatun, M., Hossain, M., Khalekuzzan, M., Rownaq, A., 2016. Comparative study on meristem culture of three potato cultivars diamant, cardinal and granula and their shoot formation. Asian Journal of Medical and Biological Research, 1(3), 537-544. https://doi.org/10.3329/ajmbr.v1i3.26477
Kim, J., Sasaki, T., Ueda, M., Sako, K., Seki, M., 2015. Chromatin changes in response to drought, salinity, heat, and cold stresses in plants. Frontiers in Plant Science, 6. https://doi.org/10.3389/fpls.2015.00114
Kotte, W. 1922. Kulturversuche mit isolierten Wurzelspitzen. Beiträge zur Allgemeinen Botanik, 2, 413–434.
Kunakhonnuruk, B., Inthima, P., Kongbangkerd, A., 2019. *In vitro* propagation of rheophytic orchid, epipactis flava seidenf. A comparison of semi-solid, continuous immersion and temporary immersion systems. Biology, 8(4), 72. https://doi.org/10.3390/biology8040072
Lee, K., Zhang, Y., Kleinstiver, B., Guo, J., Aryee, M., Miller, J., Malzahn, A., Zarecor, S., Lawrence-Dill, C. J., Joung, J. K., Qi, Y., & Wang, K., 2018. Activities and specificities of crispr/cas9 and cas12a nucleases for targeted mutagenesis in maize. Plant Biotechnology Journal, 17(2), 362-372. https://doi.org/10.1111/pbi.12982
Lee, H. T., Oh, S., Yoo, H., Kwon, Y. W., 2020. The key role of DNA methylation and histone acetylation in epigenetics of atherosclerosis. Journal of Lipid and Atherosclerosis, 9(3), 419.
Li, Q., Deng, C., Ma, Y., Kong, L., Zhang, H., Zhang, S., & Wang, J., 2017. Identification of novel mirnas and mirna expression profiling in embryogenic tissues of *Picea balfouriana* treated by 6-benzylaminopurine. Plos One, 12(5), e0176112. https://doi.org/10.1371/journal.pone.0176112
Li, Z., Jarret, R., Pittman, R., Dunbar, K., Demski, J., 1993. Efficient plant regeneration from protoplasts of *Arachis paraguariensis* chod. et hassl. using a nurse culture method. Plant Cell Tissue and Organ Culture (Pctoc), 34(1), 83-90. https://doi.org/10.1007/bf00048467
Liu, N., Han, Q., Wang, L., Liu, C., Yu, H., Zhou, H., Wei, M., & Li, Z., 2025. Zfp560 facilitates kap1-dependent chromatin repression to regulate exit from totipotency. https://doi.org/10.21203/rs.3.rs-7290036/v1
Loan, N., Giáp, Đ., Tun, T., Hien, N., Huy, N., Du, T. X., & Nht, D., 2018. Primary and secondary somatic embryogenesis in *Jatropha curcas* L. from leaf transverse thin cell layers. Vietnam Journal of Biotechnology, 14(4), 661-671. https://doi.org/10.15625/1811-4989/14/4/12299.
Loudon, P., Nelson, R., Ingram, D., 1989. Studies of protoplast culture and plant regeneration from commercial and rapid-cyclingbrassica species. Plant Cell Tissue and Organ Culture (Pctoc),

19(3), 213-224. https://doi.org/10.1007/bf00043348

Maćkowska, K., Stelmach, K., Grzebelus, E., 2023. Early selection of carrot somatic hybrids: A promising tool for species with high regenerative ability. Plant Methods, 19(1). https://doi.org/10.1186/s13007-023-01080-4

Malnoy, M., Viola, R., Jung, M., Koo, O., Kim, S., Kim, J., Velasco, R., & Nagamangala Kanchiswamy, C., 2016. Dna-free genetically edited grapevine and apple protoplast using crispr/cas9 ribonucleoproteins. Frontiers in Plant Science, 7. https://doi.org/10.3389/fpls.2016.01904

Martinelli, L., Gribaudo, I. 2001. Somatic embryogenesis in grapevine. In Molecular Biology & Biotechnology of The Grapevine. Dordrecht: Springer Netherlands. 327-351. https://doi.org/10.1007/978-94-017-2308-4_13

Martos, V., Ali, A., Cartujo, P., Ordóñez, J., 2021. Ensuring agricultural sustainability through remote sensing in the era of agriculture 5.0. Applied Sciences, 11(13), 5911. https://doi.org/10.3390/app11135911

Melviana, A., Esyanti, R., Setyobudi, R., Mel, M., Adinurani, P., Burlakovs, J., 2021. Gene expression related to steviol glycoside synthesis produced in *Stevia rebaudiana* (bert.) shoot culture induced with high far-red led light in tis rita® bioreactor system. Sarhad Journal of Agriculture, 37(1). https://doi.org/10.17582/journal.sja/2021/37.1.1.8

Meyer, L., Serek, M., Winkelmann, T., 2009. Protoplast isolation and plant regeneration of different genotypes of petunia and calibrachoa. Plant Cell Tissue and Organ Culture (Pctoc), 99(1), 27-34. https://doi.org/10.1007/s11240-009-9572-4

Mliki, A., Jardak, R., Reustle, G., Ghorbel, A., 2003. Isolation and culture of leaf protoplasts from tunisian grapes. Oeno One, 37(3), 145. https://doi.org/10.20870/oeno-one.2003.37.3.952

Mohr, S., Kühl, R., 2021. Acceptance of artificial intelligence in german agriculture: An application of the technology acceptance model and the theory of planned behavior. Precision Agriculture, 22(6), 1816-1844. https://doi.org/10.1007/s11119-021-09814-x

Mok, M., Martin, R., Dobrev, P., Vaňková, R., Ho, P., Yonekura-Sakakibara, K., Sakakibara, H., & Mok, D., 2005. Topolins and hydroxylated thidiazuron derivatives are substrates of cytokinin o-glucosyltransferase with position specificity related to receptor recognition. Plant Physiology, 137(3), 1057-1066. https://doi.org/10.1104/pp.104.057174

Mordocco, A., Brumbley, J., Lakshmanan, P., 2008. Development of a temporary immersion system (rita®) for mass production of sugarcane (*Saccharum* spp. interspecific hybrids). In Vitro Cellular & Developmental Biology - Plant, 45(4), 450-457. https://doi.org/10.1007/s11627-008-9173-7

Mosoh, D., Khandel, A., Verma, S., Vendrame, W., 2024. Optimizing callus induction and indirect organogenesis in non-dormant corm explants of *Gloriosa superba* (L.) via media priming. Frontiers in Horticulture, 3. https://doi.org/10.3389/fhort.2024.1378098

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15, 473–497

Musa, S., Basir, K., Luah, E., 2021. The role of smart farming in sustainable development. International Journal of Asian Business and Information Management, 13(2), 1-12. https://doi.org/10.4018/ijabim.20220701.oa5

Muthuramalingam, P., Jeyasri, R., Rakkammal, K., Satish, L., Shamili, S., Karthikeyan, A., Valliammai, A., Priya, A., Selvaraj, A., Gowri, P., Wu, Q.-S., Karutha Pandian, S., Shin, H., Chen, J.-T., Baskar, V., Thiruvengadam, M., Akilan, M., & Ramesh, M., 2022. Multi-omics and integrative approach towards understanding salinity tolerance in rice: A review. Biology, 11(7), 1022. https://doi.org/10.3390/biology11071022

Naranjo-Torres, J., Mora, M., Fredes, C., Valenzuela, A., 2021. Disease and defect detection system for raspberries based on convolutional neural networks. Applied Sciences, 11(24), 11868. https://doi.org/10.3390/app112411868

Nasirahmadi, A., Hensel, O., 2022. Toward the next generation of digitalization in agriculture based on digital twin paradigm. Sensors, 22(2), 498. https://doi.org/10.3390/s22020498

Özsan, T., Onus, A., 2020. Callogenesis optimization of some globe artichoke [*Cynara cardunculus* var. scolymus (L.) fiori] cultivars based on *in vivo* and *in vitro* leaf explants. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 48(4), 1873-1884. https://doi.org/10.15835/nbha48412089

Paek, K., Chakrabarty, D., Hahn, E., 2005. Application of bioreactor systems for large scale production of horticultural and medicinal plants. Plant Cell Tissue and Organ Culture (Pctoc), 81(3), 287-300. https://doi.org/10.1007/s11240-004-6648-z

Patil, R., Kumar, S., Chiwhane, S., Rani, R., Pippal, S., 2022. An artificial-intelligence-based novel rice grade model for severity estimation of rice diseases. Agriculture, 13(1), 47. https://doi.org/10.3390/agriculture13010047

Pepe, M., Hesami, M., Small, F. Jones, A., 2021. Comparative analysis of machine learning and evolutionary optimization algorithms for precision micropropagation of *Cannabis sativa*: Prediction and validation of *in vitro* shoot growth and development based on the optimization of light and carbohydrate sources. Frontiers in Plant Science, 12. https://doi.org/10.3389/fpls.2021.757869

Phillips, G., Collins, G., 1979. Virus symptom-free plants of red clover using meristem culture1. Crop Science, 19(2), 213-216. https://doi.org/10.2135/cropsci1979.0011183x001900020010x

Phillips, G. C., Garda, M., 2019. Plant tissue culture media and practices: An overview. In Vitro Cellular & Developmental Biology – Plant, 55(3), 242–257.

Philpott, M., Pence, V., Bassüner, B., Clayton, A., Coffey, E., Downing, J. L., Edwards, C. E., Folgado, R., Ligon, J. J., Powell, C., Ree, J. F., Seglias, A. E., Sugii, N., Zale, P. J., & Zeldin, J., 2022. Harnessing the power of botanical gardens: Evaluating the costs and resources needed for exceptional plant conservation. Applications in Plant Sciences, 10(5). https://doi.org/10.1002/aps3.11495

Pischke, M., Huttlin, E., Hegeman, A., Sussman, M., 2006. A transcriptome-based characterization of habituation in plant tissue culture. Plant Physiology, 140(4), 1255-1278. https://doi.org/10.1104/pp.105.076059

Qiang, B., Miao, J., Phillips, N., Wei, K., Gao, Y., 2020. Recent advances in the tissue culture of american ginseng (*Panax quinquefolius*). Chemistry & Biodiversity, 17(10). https://doi.org/10.1002/cbdv.202000366

Raghavan, V. 1980. Embryo culture. International Review of Cytology. Supplement, 11B, 209–240.

Raghu, A., Unnikrishnan, K., Geetha, S., Martin, G., Balachandran, I., 2011. Plant regeneration and production of embelin from organogenic and embryogenic callus cultures of *Embelia ribes* burm. f. A vulnerable medicinal plant. In Vitro Cellular & Developmental Biology - Plant, 47(4), 506-515. https://doi.org/10.1007/s11627-011-9365-4

Ramasubramanian, A., Mathew, R., Kelly, M., Hargaden, V., Papakostas, N., 2022. Digital twin for human–robot collaboration in manufacturing: review and outlook. Applied Sciences, 12(10), 4811. https://doi.org/10.3390/app12104811

Rasmussen, J., Rasmussen, O., 1995. Characterization of somatic hybrids of potato by use of rapd markers and isozyme analysis. Physiologia Plantarum, 93(2), 357-364. https://doi.org/10.1034/j.1399-3054.1995.930222.x

Reinert, J. 1958. Morphogenese und ihre Kontrolle an Gewebekulturen aus Karotten. Naturwissenschaft, 45, 344–345.

Richardson, K., Wetten, A., Caligari, P., 2001. Cell and nuclear degradation in root meristems following exposure of potatoes (*Solanum tuberosum* L.) to salinity. Potato Research, 44(4), 389-399. https://doi.org/10.1007/bf02358598

Robbins, W. J., 1922. Cultivation of excised root tips and stem tips under sterile conditions. Botanical Gazette, 73, 376–390.

Sarkar, D., Tiwari, J., Sharma, S., Poonam, P., Sharma, S., Gopal, J., Singh, B. P., Luthra, S. K., Pandey, S. K., & Pattanayak, D., 2011. Production and characterization of somatic hybrids between *Solanum tuberosum* L. and *S. pinnatisectum* dun. Plant Cell Tissue and Organ Culture (Pctoc), 107(3), 427-440. https://doi.org/10.1007/s11240-011-9993-8

Sedlák, P., Sedláková, V., Vašek, J., Zeka, D., Čílová, D., Melounová, M., Orsák, M., Domkářová, J., Doležal, P., & Vejl, P., 2022. Phenotypic, molecular and biochemical evaluation of somatic hybrids between *Solanum tuberosum* and *S. bulbocastanum*. Scientific Reports, 12(1). https://doi.org/10.1038/s41598-022-08424-5

Shi, J., Gao, H., Wang, H., Lafitte, H., Archibald, R., Yang, M., Hakimi, S. M., Mo, H., & Habben, J., 2016. Argos8 variants generated by crispr-cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnology Journal, 15(2), 207-216. https://doi.org/10.1111/pbi.12603

Skoog, F., Miller, C. O. 1957. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symposium of the Society for Experimental Biology, 11, 118–131.

Smyda-Dajmund, P., Śliwka, J., Wasilewicz-Flis, I., Jakuczun, H., Zimnoch-Guzowska, E., 2016. Genetic composition of interspecific potato somatic hybrids and autofused 4x plants evaluated by dart and cytoplasmic DNA markers. Plant Cell Reports, 35(6), 1345-1358. https://doi.org/10.1007/s00299-016-1966-2

Smýkalová, I., Doležal, K., Plačková, L., Plíhalová, L., Zatloukal, M., Griga, M., 2025. The effect of supplementing in vitro flax (*Linum usitatissimum* L.) culture media with a synthetic cytokinin derivative (bap9 thp) and a cytokinin metabolism modulator (incyde). Plant Growth Regulation, 105(4), 1125-1138. https://doi.org/10.1007/s10725-025-01326-2

Steward, F. C., Mapes, M. O., Mears, K., 1958. Growth and development of cultured cells. II. Organization in cultures grown from freely suspended cells. American Journal of Botany, 45, 705–708.

Streczynski, R., Clark, H., Whelehan, L., Ang, S., Hardstaff, L., Funnekotter, B., Bunn, E., Offord, C. A., Sommerville, K. D., & Mancera, R., 2019. Current issues in plant cryopreservation and importance for *ex situ* conservation of threatened australian native species. Australian Journal of Botany, 67(1), 1-15. https://doi.org/10.1071/bt18147

Street, H. E., 1969. Growth in organized and unorganized systems. In: Steward, F. C. (Ed.), Plant physiology, Vol. 5B. Academic Press, New York, 3–224.

Su, Y., Tang, L., Zhao, X. Zhang, X., 2020. Plant cell totipotency: insights into cellular reprogramming. Journal of Integrative Plant Biology, 63(1), 228-243. https://doi.org/10.1111/jipb.12972

Sun, C., Ali, K., Yan, K., Fiaz, S., Dormatey, R., Bi, Z., & Bai, J., 2021. Exploration of epigenetics for improvement of drought and other stress resistance in crops: A review. Plants, 10(6), 1226. https://doi.org/10.3390/plants10061226

Suprasanna, P., Jain, S., 2023. Editorial: Innovative technologies and advancements in designing custom-made ornamental plants. Frontiers in Plant Science, 14. https://doi.org/10.3389/fpls.2023.1348949

Surani, M. 2001. Reprogramming of genome function through epigenetic inheritance. Nature, 414(6859), 122-128. https://doi.org/10.1038/35102186

Szewczyk, A., Grabowski, M., Zych, D., 2023. *Ruta chalepensis* L. *in vitro* cultures as a source of bioactive furanocoumarins and furoquinoline alkaloids. Life, 13(2), 457. https://doi.org/10.3390/life13020457

Thorpe, T. A. 2007. History of plant tissue culture. Molecular Biotechnology, 37(2), 169–180.

Tusa, N., Grosser, J., Gmitter, F., 1990. Plant regeneration of `valencia' sweet orange, `femminello' lemon, and the interspecific somatic hybrid following protoplasm fusion. Journal of the American Society for Horticultural Science, 115(6), 1043-1046. https://doi.org/10.21273/jashs.115.6.1043

Varadharajan, V., Radhika, R., Muthuramalingam, P., Runthala, A., Madhesh, V., Swaminathan, G., Murugan, P., Srinivasan, H., Park, Y., Shin, H., & Ramesh, M., 2025. Multi-omics approaches against abiotic and biotic stress—a review. Plants, 14(6), 865. https://doi.org/10.3390/plants14060865

Verma, V., Kumar, A., Chaudhary, P., Chauhan, S., Thakur, M., Bhargava, B., 2022. Meta-topolin mediated *in vitro* propagation in an ornamentally important crop iris × hollandica tub. cv.

professor blaauw and genetic fidelity studies using scot markers.. https://doi.org/10.21203/rs.3.rs-1583057/v1

Villegas-Sánchez, E., Macías-Alonso, M., Osegueda-Robles, S., Herrera-Isidrón, L., Núñez-Palenius, H., Marrero, J., 2021. *In vitro* culture of rosmarinus officinalis l. in a temporary immersion system: Influence of two phytohormones on plant growth and carnosol production. Pharmaceuticals, 14(8), 747. https://doi.org/10.3390/ph14080747

Vollmer, R., Espirilla, J., Espinoza, A., Villagaray, R., Castro, M., Pineda, S., Sánchez, J. C., Mello, A. F. S., & & Azevedo, V., 2024. Effect of gas exchange rate, vessel type, planting density, and genotype on growth, photosynthetic activity, and ion uptake of *in vitro* potato plants. Plants, 13(19), 2830. https://doi.org/10.3390/plants13192830

Wang, J., Sun, Y., Yan, S., Daud, M., Zhu, S., 2008. High frequency plant regeneration from protoplasts in cotton via somatic embryogenesis. Biologia Plantarum, 52(4), 616-620. https://doi.org/10.1007/s10535-008-0121-5

Wawrosch, C., Zotchev, S., 2021. Production of bioactive plant secondary metabolites through *in vitro* technologies—status and outlook. Applied Microbiology and Biotechnology, 105(18), 6649-6668. https://doi.org/10.1007/s00253-021-11539-w

White, P. R., 1934. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiology, 9, 585–600.

Wu, J., Ferguson, A., Mooney, P., 2005. Allotetraploid hybrids produced by protoplast fusion for seedless triploid citrus breeding. Euphytica, 141(3), 229-235. https://doi.org/10.1007/s10681-005-7009-7

Xu, L., Najeeb, U., Raziuddin, R., Shen, W. Q., Shou, J. Y., Tang, G. X., & Zhou, W. J., 2009. Development of an efficient tissue culture protocol for callus formation and plant regeneration of wetland species Juncus effusus L. In Vitro Cellular & Developmental Biology-Plant, *45*(5), 610-618. https://doi.org/10.1007/s11627-009-9228-4

Yadav, B., Pandit, D., Banjade, D., Mehata, D., Bhattarai, S., Bhandari, S., Ghimire, N. P., Yadav, P., & Paudel, P., 2024. Insights into the germplasm conservation and utilization: implications for sustainable agriculture and future crop improvement. Archives of Agriculture and Environmental Science, 9(1), 180-193. https://doi.org/10.26832/24566632.2024.0901026

Yong, J., Ge, L., Ng, Y., Tan, S. 2009. The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water. Molecules, 14(12), 5144-5164. https://doi.org/10.3390/molecules14125144

Zhang, T., Xiao, J., Zhao, Y., Zhang, Y., Jie, Y., Shen, D., Yue, C., Huang, J., Hua, Y., & Zhou, T., 2021. Comparative physiological and transcriptomic analyses reveal ascorbate and glutathione coregulation of cadmium toxicity resistance in wheat genotypes. BMC Plant Biology, 21(1). https://doi.org/10.1186/s12870-021-03225-w

Zhou, L., Dean, J., 2015. Reprogramming the genome to totipotency in mouse embryos. Trends in Cell Biology, 25(2), 82-91. https://doi.org/10.1016/j.tcb.2014.09.006

Ziarani, M., Tohidfar, M., Navvabi, M., 2022. Modeling and optimizing *in vitro* percentage and speed callus induction of carrot via multilayer perceptron-single point discrete ga and radial basis function. BMC Biotechnology, 22(1). https://doi.org/10.1186/s12896-022-00764-4

Zimmerman, J., 1993. Somatic embryogenesis: a model for early development in higher plants. The Plant Cell, 5(10), 1411. https://doi.org/10.2307/3869792

Bölüm 2

TEMEL TEKNİKLERİ VE LABORATUVAR ORGANİZASYONU

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2.1 BİTKİ DOKU KÜLTÜRLERİNİN UYGULAMA ALANLARI

Bitki doku kültürü teknikleri, bitki ıslahı çalışmalarına çok yönlü katkılar sağlamakta ve klasik yöntemlerle uzun yıllar sürebilen ıslah süreçlerinin daha kısa sürede tamamlanmasına olanak tanıyan önemli biyoteknolojik araçlar arasında yer almaktadır. Bu teknikler sayesinde, sağlıklı ve uniform bitki materyali kısa sürede elde edilebilmekte, genetik çalışmalar hızlandırılabilmekte ve yeni çeşit geliştirme süreçleri daha etkin bir şekilde yürütülebilmektedir. Bitki doku kültürü tekniklerinin başlıca uygulama alanları aşağıda özetlenmiştir.

2.1.1 Mikroçoğaltım ve Hastalıksız Bitki Üretimi

Bitkilerde meristem dokularından elde edilen bitkiler çoğunlukla hastalıklardan ari olmakta ve bu dokuların uygun *in vitro* kültür koşullarında bitkiye dönüştürülmesiyle özellikle virüsler ve sistemik patojenlerden arındırılmış sağlıklı bitkiler elde edilebilmektedir (Thanuja ve ark., 2025). Ayrıca, kriyoterapi ve sıcaklık uygulamaları (termoterapi) gibi destekleyici yöntemlerin meristem kültürü ile kullanılması, hastalık etmenlerinin eliminasyonunda başarı oranını önemli ölçüde artırmaktadır (Elansary ve ark., 2024). Meris-

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etkileri ile düşük ve yüksek sıcaklık streslerinin embriyogenez ve hiperhidrisite üzerindeki etkileri, doku kültürü çalışmalarında kontrol edilmesi gereken kritik faktörler olarak öne çıkmaktadır. Geçici daldırma sistemleri gibi yenilikçi biyoreaktör teknolojileri ise, klasik yarı katı ortam sistemlerine kıyasla daha yüksek verim, daha iyi gaz alışverişi, düşük hiperhidrisite riski ve büyük ölçekli üretime uygunluk gibi önemli avantajlar sunmaktadır. Bu yönüyle biyoreaktör temelli üretim sistemleri, geleceğin ticari mikroçoğaltım uygulamalarında merkezi bir rol üstlenmeye adaydır. Sonuç olarak bu bölüm, bitki doku kültürü uygulamalarının yalnızca teorik bir biyoteknoloji alanı olmadığını; aynı zamanda kontrollü laboratuvar organizasyonu, titiz sterilizasyon uygulamaları ve optimize edilmiş çevresel koşullar altında yüksek verimlilikle uygulanabilen güçlü bir üretim ve ıslah teknolojisi olduğunu ortaya koymaktadır. Giderek artan iklim stresi, hastalık baskısı ve sürdürülebilir üretim gereksinimleri göz önüne alındığında, bitki doku kültürü tekniklerinin tarımın geleceğinde stratejik önemi daha da artacaktır.

KAYNAKLAR

Algopishi, U.B., Abdelghffar, A.M., Al Aboud, N.M., Safhi, F.A., Fayad, E., Alharbi, K., Hassanin, A.A., 2025. The genetic applications of plant cell and tissue culture techniques: essential tools for genetic manipulation and crop improvement. *Not Bot Horti Agrobo*, 53(3), 14718–14718.

Altpeter, F., Springer, N.M., Bartley, L.E., Blechl, A.E., Brutnell, T.P., Citovsky, V., Conrad, L.J., Gelvin, S.B., Jackson, D.P., Kausch, A.P., Lemaux, P.G., Medford, J.I., Orozco-Cárdenas, M.L., Tricoli, D.M., Van Eck, J., 2016. Advancing crop transformation in the era of genome editing. *Plant Cell*, 28(7), 1510–1520. https://doi.org/10.1105/tpc.16.00196

Askari, N., Aliniaeifard, S., Visser, R.G., 2022. Low CO_2 levels are detrimental for in vitro plantlets through disturbance of photosynthetic functionality and accumulation of reactive oxygen species. *Horticulturae*, 8(1), 44. https://doi.org/10.3390/horticulturae8010044

Bairu, M.W., Aremu, A.O., Van Staden, J., 2011. Somaclonal variation in plants: causes and detection methods. *Plant Growth Regul*, 63(2), 147–173. https://doi.org/10.1007/s10725-010-9554-x

Behera, B., Behera, S., Mohapatra, D., Barik, D., Naik, S., 2021. Regeneration of plants from alginate-encapsulated axenic nodal segments of *Paederia foetida* L., a medicinally important and vulnerable plant species. *J Plant Biotechnol*, 48(4), 255–263. https://doi.org/10.5010/jpb.2021.48.4.255

Bortesi, L., Fischer, R., 2015. The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnol Adv*, 33(1), 41–52. https://doi.org/10.1016/j.biotechadv.2014.12.006

Castillo, B., Smith, M., Yadava, U., 1998. Plant regeneration from encapsulated somatic embryos of *Carica papaya* L. *Plant Cell Rep*, 17(3), 172–176. https://doi.org/10.1007/s002990050373

Dewir, Y.H., Chakrabarty, D., Ali, M.B., Hahn, E.J., Paek, K.Y., 2024. Hyperhydricity in plant tissue culture: causes and mitigation strategies. *Plant Cell Tiss Organ Cult*, 156, 1–15. https://doi.org/10.1007/s11240-023-02693-5

Dewir, Y.H., et al., 2023. Light and dark modulation of somatic embryogenesis in horticultural crops. *Plants*, 12, 2871.

Dwivedi, S.L., et al., 2015. Haploids: constraints and opportunities in plant breeding. *Biotechnol Adv*, 33(6), 812–829. https://doi.org/10.1016/j.biotechadv.2015.07.001

Elansary, D.W.A., et al., 2024. Efficacy of plant tissue culture techniques for eliminating viruses in horticultural crops: shoot-tip culture, thermotherapy, cryotherapy and their combinations.

Plants, 13(21), 2959.

Esyanti, R., Fadholi, M., Rizki, R., Faizal, A., 2019. Shoot multiplication and growth rates of *Aquilaria malaccensis* Lamk. shoot cultures in temporary immersion system (TIS)-RITA® and bubble column bioreactors. *Pak J Bot*, 51(4). https://doi.org/10.30848/pjb2019-4(36)

Fehér, A., 2015. Somatic embryogenesis—stress-induced remodeling of plant cell fate. *BBA Gene Regul Mech*, 1849(4), 385–402.

George, E.F., Hall, M.A., De Klerk, G.J., 2007. *Plant propagation by tissue culture. Vol. 1: The background*. Springer, Dordrecht.

Georgiev, V., Schumann, A., Pavlov, A., Bley, T., 2014. Temporary immersion systems in plant biotechnology. *Eng Life Sci*, 14(6), 607–621. https://doi.org/10.1002/elsc.201300166

Germanà, M.A., 2011. Gametic embryogenesis and haploid technology as valuable support to plant breeding. *Plant Cell Rep*, 30(5), 839–857. https://doi.org/10.1007/s00299-011-1061-7

Hartmann, H.T., Kester, D.E., 1959. *Plant propagation: principles and practices*. Prentice-Hall, New Jersey.

Hung, C., Trueman, S., 2011. Alginate encapsulation of shoot tips and nodal segments for short-term storage and distribution of the eucalypt *Corymbia torelliana* × *C. citriodora*. *Acta Physiol Plant*, 34(1), 117–128. https://doi.org/10.1007/s11738-011-0810-0

Iqbal, M., Ali, A., Rashid, H., Raja, N., Naveed, N., Mashwani, Z., Chaudhry, Z., 2019. Evaluation of sodium alginate and calcium chloride on development of synthetic seeds. *Pak J Bot*, 51(5). https://doi.org/10.30848/pjb2019-5(36)

Jiroutová, P., Sedlák, J., 2020. Cryobiotechnology of plants: a hot topic not only for gene banks. *Appl Sci*, 10(13), 4677.

Leifert, C., Cassells, A.C., 2001. Microbial hazards in plant tissue and cell cultures. *In Vitro Cell Dev Biol-Plant*, 37(2), 133–138.

Lyam, P., Musa, M., Jamaleddine, Z., Okere, U., Odofin, W., 2012. The potential of temporary immersion bioreactors (TIBs) in meeting crop production demand in Nigeria. *J Biol Life Sci*, 3(1). https://doi.org/10.5296/jbls.v3i1.1156

Mangena, P., 2021. Synthetic seeds and their role in agriculture: status and progress in Sub-Saharan Africa. *Plant Sci Today*, 8(3). https://doi.org/10.14719/pst.2021.8.3.1116

Martins, J.P.R., Rodrigues, L.C.A., Santos, E.R., Gontijo, A.B.P.L., Falqueto, A.R., 2020. Impacts of photoautotrophic, photomixotrophic and heterotrophic conditions on anatomy and photosystem II of in vitro-propagated *Aechmea blanchetiana*. *In Vitro Cell Dev Biol-Plant*, 56(3), 350–361.

Mendonça, E.G., Stein, V.C., Carvalho, H.H., Santos, B.R., Beijo, L.A., Paiva, L.V., 2016. Continuous temporary immersion bioreactor system and semisolid medium for production of *Eucalyptus camaldulensis* clones. *Cienc Florest*, 26(4), 1211–1224. https://doi.org/10.5902/1980509825112

Meucci, A., Ghelardi, C., Chietera, G., Mensuali, A., 2024. Synthetic seed production and slow growth storage of in vitro cultured plants of *Iris pallida* Lam. *Horticulturae*, 10(3), 272.

Mishra, M., Rajan, S., Damodaran, T., 2024. New paradigm shifts in micropropagation of fruit crops through bioreactors – a review. *Indian J Hortic*, 81(1), 1–10. https://doi.org/10.58993/ijh/2024.81.1.1

Murthy, H.N., Joseph, K.S., Hahn, J.E., Lee, H.S., 2023. Suspension culture of somatic embryos for production of high-value secondary metabolites. *Physiol Mol Biol Plants*, 29(8), 1–18. https://doi.org/10.1007/s12298-023-01365-x

Pepe, M., Marie, T.R., Leonardos, E.D., Hesami, M., Rana, N., Jones, A.M.P., Grodzinski, B., 2022. Tissue culture coupled with gas exchange system for phenotyping developmental biology of *Solanum lycopersicum* 'MicroTom'. *Front Plant Sci*, 13, 1025477.

Polivanova, O.B., Bedarev, V.A., 2022. Hyperhydricity in plant tissue culture. *Plants*, 11(23), 3313.

Rahayu, A., Umami, L., Cen, S., Abdusshamad, B., Sean, L., Budiman, K., Semiarti, E., 2023. Automatic dual sequence control temporary immersion bioreactor systems for *Coelogyne pandura-*

ta. *Bio Web Conf*, 80, 06002. https://doi.org/10.1051/bioconf/20238006002
Rai, M.K., Kalia, R.K., Singh, R., Gangola, M.P., Dhawan, A.K., 2011. Developing stress tolerant plants through in vitro selection. *Environ Exp Bot*, 71(1), 89–98.
Ranaware, A.S., Kumar, N., Singh, A.K., Bhat, S.R., Prakash, S., 2023. Protoplast technology and somatic hybridisation in *Brassica*. *Plants*, 12(5), 1060. https://doi.org/10.3390/plants12051060
Ribeiro, I.D.S., Ribeiro, L.M., Soares, J.S., Ramos, J.C.M., Sorgato, J.C., 2022. Light condition and flask sealing on germination of *Dendrobium nobile*. *Ornam Hortic*, 28(4), 407–413.
Shuro, A.R., 2020. Review on somatic hybridization and its role in crop improvement. *J Biol Agric Healthc*, 10(6), 1–9.
Silva, D.R., Soares, M.N.B., Silva, M.C.R., Lima, M.C., Silva-Moraes, V.K.O., Alves, G.L., Felipe, S.H.S., 2024. In vitro photoautotrophy for *Eryngium foetidum*. *Horticulturae*, 10(1), 107.
Singh, S., Manoj, K., Asthana, P., Sahoo, L., 2010. Alginate-encapsulation of nodal segments of *Eclipta alba*. *Acta Physiol Plant*, 32(3), 607–610. https://doi.org/10.1007/s11738-009-0444-7
Thanuja, K., Arulmozhiyan, R., Saraswathi, M.S., Selvarajan, R., Jegadeeswari, V., Rajanbabu, V., 2025. In vitro therapies for virus elimination in horticultural crops. *Planta*, 262(1), 15.
Vásquez-Hernández, M., et al., 2023. In vitro mutagenesis for improvement of *Agave* genus. *Plant Physiol Biochem*, 197, 107647.
Wang, H., Hou, H., Jan, C.C., Chao, W.S., 2023. Irradiated pollen-induced parthenogenesis for doubled haploid production in sunflower. *Plants*, 12(13), 2430. https://doi.org/10.3390/plants12132430

Bölüm 3

BİTKİ DOKU KÜLTÜRÜNDE KULLANILAN SOLÜSYON VE BESİN ORTAMLARININ HAZIRLANMASI

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3.1 KULLANILAN TEMEL EKİPMANLAR VE GÖREVLERİ

Bitki doku kültüründe kullanılan temel ekipmanlar, doku kültürü ortamlarının doğru ve hassas bir şekilde hazırlanmasında ve bitki gelişiminde önemli bir role sahiptir. Bitki doku kültüründe kullanılan bu ekipmanların görevleri aşağıda özetlenmiştir.

3.1.1 Manyetik Karıştırıcı

Bitki doku kültüründe çözelti ve besi ortamlarının hazırlanmasında manyetik karıştırıcılar kritik bir role sahiptir. Manyetik karıştırıcıların temel görevi, çözelti içindeki tüm bileşenlerin homojen karışmasını sağlamaktır. Bu sayede besi ortamlarının hazırlanmasında makro ve mikro elementlerin, vitaminlerin ve karbon kaynaklarının eşit dağılması mümkün olur. Homojenliğin sağlanmadığı ortamlarda, iyon çökelmeleri, pH dalgalanmaları ve mikro elementlerin dengesiz dağılımı bitkinin büyümesini doğrudan olumsuz etkilemektedir (George ve ark., 2008).

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KAYNAKLAR

Aremu, A. O., Bairu, M. W., Szüčová, L., Doležal, K., Finnie, J. F., & Van Staden, J., 2012. Assessment of the role of meta-topolins on *in vitro* produced phenolics and acclimatization competence of micropropagated 'Williams' banana. Acta Physiologiae Plantarum, 34(6), 2265-2273.
https://doi.org/10.1007/s11738-012-1027-6

Baqir, H. A., Zeboon, N. H., & Al-behadili, A. A. J., 2019. The role and importance of amino acids within plants: A review. Plant Archives, 19(2), 1402-1410.

Bhojwani, S. S., & Dantu, P. K., 2013. General requirements and techniques. In S. S. Bhojwani & P. K. Dantu, Plant tissue culture: An introductory text, 11-25. Springer. https://doi.org/10.1007/978-81-322-1026-9_2

Cardoso, J. C., Gerald, L. T. S., & Teixeira da Silva, J. A., 2018. Micropropagation in the twenty-first century. In V. M. Loyola-Vargas & N. Ochoa-Alejo, Plant Cell Culture Protocols, 17-46. Methods in Molecular Biology, vol. 1815. Springer. https://doi.org/10.1007/978-1-4939-8594-4_2

Cathey, H. M., 1964. Physiology of growth retarding chemicals. Annual Review of Plant Physiology, 15(1), 271-302. https://doi.org/10.1146/annurev.pp.15.060164.001415

Cheng, K. L., & Zhu, D.-M., 2005. On calibration of pH meters. Sensors, 5(4), 209-232. https://doi.org/10.3390/s5040209

Chu, C. C., Wang, C. C., Sun, C. S., Hsu, C., Yin, K. C., Chu, C. Y., & Bi, F. Y., 1975. Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Scientia Sinica, 18(5), 659-668.

Driver, J. A., & Kuniyuki, A. H., 1984. *In vitro* propagation of paradox walnut rootstock. HortScience, 19(4), 507-509.

Eeuwens, C. J., 1976. Mineral requirements for growth and callus initiation of tissue explants excised from mature coconut palms (*Cocos nucifera*) and cultured *in vitro*. Physiologia Plantarum, 36(1), 23-28. https://doi.org/10.1111/j.1399-3054.1976.tb05022.x

Eryiğit, T., 2011. Bitkilerde büyüme ve gelişmeyi düzenleyici maddeler: Bitki hormonları.

Gamborg, O. L., Miller, R. A., & Ojima, K., 1968. Nutrient requirements of suspension cultures of soybean root cells. Experimental Cell Research, 50(1), 151-158. https://doi.org/10.1016/0014-4827(68)90403-5

Geetha, G., Harathi, K., & Naidu, C. V., 2016. Role of silver nitrate on *in vitro* flowering and shoot regeneration of Solanum nigrum. American Journal of Plant Sciences, 7(7), 1021-1032. https://doi.org/10.4236/ajps.2016.77097

George, E. F., Hall, M. A., & De Klerk, G. J., 2008. Plant propagation by tissue culture. 3rd ed., Vol. 1. Springer. https://doi.org/10.1007/978-1-4020-5005-3

Hedden, P., & Thomas, S. G., 2012. Gibberellin biosynthesis and its regulation. Biochemical Journal, 444(1), 11-25. https://doi.org/10.1042/BJ20120245

Heidarzadeh, A., 2025. Role of amino acids in plant growth, development, and stress responses: A comprehensive review. Discover Plants, 2, 237. https://doi.org/10.1007/s44372-025-00322-0

Huetteman, C. A., & Preece, J. E., 1993. Thidiazuron: A potent cytokinin for woody plant tissue culture. Plant Cell, Tissue and Organ Culture, 33(2), 105-119. https://doi.org/10.1007/BF01983223

Khan, W., Rayirath, U. P., Subramanian, S., Jithesh, M. N., Rayorath, P., Hodges, D. M., Critchley, A. T., Craigie, J. S., Norrie, J., & Prithiviraj, B., 2009. Seaweed extracts as biostimulants of plant growth and development. Journal of Plant Growth Regulation, 28(4), 386-399. https://doi.org/10.1007/s00344-009-9103-x

Kırdar, E., & Allahverdiev, S., 2020. Büyüme düzenleyiciler ve etkileri. In Z. Yahyaoğlu & M. Genç, Fidan standardizasyonu: Standart fidan yetiştirmenin biyolojik ve teknik esasları, 243-250. Süleyman Demirel Üniversitesi Yayınları.

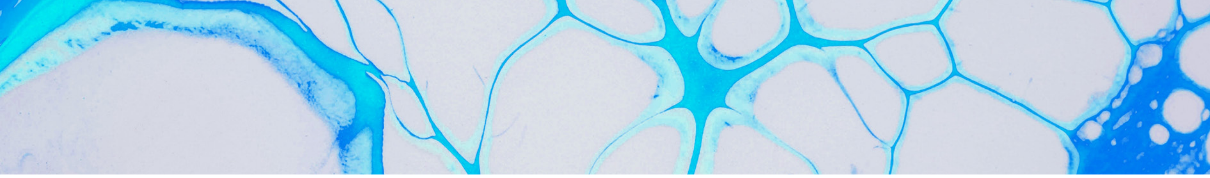
Lloyd, G., & McCown, B., 1980. Commercially feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot tip culture. Proceedings of the International Plant Propagators'

Society, 30, 421-427.
Mahendran, D., Geetha, N., & Venkatachalam, P., 2019. Role of silver nitrate and silver nanoparticles in plant tissue culture and enhanced plant growth and development. In P. Venkatachalam, *In vitro* plant breeding towards novel agronomic traits, 59-74. Springer. https://doi.org/10.1007/978-981-32-9824-8_4
Mao, Z., 2025. A study on the effects of airflow and static electricity on analytical balance weighing. International Journal of Chemistry and Materials Science, 2(1), 45-59. https://doi.org/10.70088/azs1cr49
Miller, B. M., & Bassuk, N. L., 2022. Gibberellic acid 4+7 influences shoot growth of seedling pecan and bitternut hickory. Journal of Environmental Horticulture, 40(2), 39-45. https://doi.org/10.24266/2573-5586-40.2.45
Miller, C. O., Skoog, F., Okumura, F. S., Von Saltza, M. H., & Strong, F. M., 1955. Kinetin, a cell division factor from deoxyribonucleic acid. Journal of the American Chemical Society, 77(5), 1392-1393. https://doi.org/10.1021/ja01610a105
Murashige, T., & Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum, 15(3), 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x
Murthy, H. N., Paek, K. Y., Hahn, E. J., Zhong, J. J., & Jeong, C. S., 2023. Bioreactor systems for micropropagation of plants: Present scenario and future prospects. Frontiers in Plant Science, 14, 1159588. https://doi.org/10.3389/fpls.2023.1159588
Pazuki, A., Aflaki, F., Yücesan, B., & Gürel, S., 2019. Effects of cytokinins, gibberellic acid 3, and gibberellic acid 4/7 on *in vitro* growth, morphological traits, and content of steviol glycosides in Stevia rebaudiana. Plant Physiology and Biochemistry, 137, 154-161. https://doi.org/10.1016/j.plaphy.2019.02.009
Rademacher, W., 2000. Growth retardants: Effects on gibberellin biosynthesis and other metabolic pathways. Annual Review of Plant Physiology and Plant Molecular Biology, 51(1), 501-531. https://doi.org/10.1146/annurev.arplant.51.1.501
Rugini, E., 1984. *In vitro* propagation of some olive (*Olea europaea sativa* L.) cultivars with different root-ability, and medium development using analytical data from developing shoots and embryos. Scientia Horticulturae, 24, 123-134. http://dx.doi.org/10.1016/0304-4238(84)90143-2
Sidik, M. J., Agha, M. S., Alkamil, E. H., Alsayadi, M. M., & Mohammed, S. A., 2024. A mini review of plant tissue culture: The role of media optimization. Journal of Pharmaceutical Negative Results, 15(2), 177-186. https://doi.org/10.70176/3007-973X.1019
Skirvin, R. M., Chu, M. C., Mann, M. L., Young, H., Sullivan, J., & Fermanian, T., 1986. Stability of tissue culture medium pH as a function of autoclaving, time, and cultured plant material. Plant Cell Reports, 5(4), 292-294. https://doi.org/10.1007/BF00269825
Thomas, T. D., 2008. The role of activated charcoal in plant tissue culture. Biotechnology Advances, 26(6), 618-631. https://doi.org/10.1016/j.biotechadv.2008.08.003
Tiburcio, A. F., Altabella, T., Bitrián, M., & Alcázar, R., 2014. The roles of polyamines during the lifespan of plants: from development to stress. Planta, 240(1), 1-18. https://doi.org/10.1007/s00425-014-2055-9
Tomar, R. S., Khamba, S., Kaushik, S., & Mishra, R. K., 2018. Role of vitamins in plant growth and their impact on regeneration of plants under *in vitro* condition. International Journal for Research in Applied Science & Engineering Technology, 6(3), 423-426
Verdú-Navarro, F., Moreno-Cid, J. A., Weiss, J., & Egea-Cortines, M., 2023. The advent of plant cells in bioreactors. Frontiers in Plant Science, 14, 1310405. https://doi.org/10.3389/fpls.2023.1310405
White, P. R., 1943. A handbook of plant tissue culture. Lancaster, PA: The Jacques Cattell Press.
Yan, F., Ma, J., Peng, M., Xi, C., Chang, S., Yang, Y., Tian, S., Zhou, B., & Liu, T., 2024. Lactic acid induced defense responses in tobacco against Phytophthora nicotianae. Scientific Reports, 14(1), 9338. https://doi.org/10.1038/s41598-024-60037-2

Yin, J., 2010. Dicamba and sugar effects on callus induction and plant regeneration from mature embryo culture of wheat. Agricultural Sciences in China, 9(1), 31-37. https://doi.org/10.1016/S1671-2927(09)60064-X

Zhang, C., & Whiting, M. D., 2011. Improving 'Bing' sweet cherry fruit quality with plant growth regulators. Scientia Horticulturae, 127, 341-346. https://doi.org/10.1016/j.scienta.2010.11.006

Zhou, R. J., & Liu, M. J., 2009. Effect of plant growth regulators on tissue culture in Chinese jujube. Acta Horticulturae, 840, 309-314. https://doi.org/10.17660/ActaHortic.2009.840.42



Bölüm 4

MİKROÇOĞALTIM VE HASTALIKSIZ BİTKİ ÜRETİMİ

Ecem KARA [1]
Hatıra TAŞKIN [2]
Gökhan BAKTEMUR [3]

4.1 GİRİŞ

Bitki biyoteknolojisi uygulamaları son yirmi yılda dünya genelinde yaygın biçimde geliştirilmiş ve birçok ülkenin tarım sistemlerine entegre edilmiştir. Bu süreçte doku kültürü araçları önemli bir rol oynamıştır. Günümüzde, bitki biyoteknolojisi transgenik bitkiler de dâhil olmak üzere çeşitli özelliklere sahip çıktılarıyla çiftçiler ve şirketler tarafından en çok benimsenen teknoloji haline gelmiş ve pek çok fayda sunmuştur. Bu teknolojinin hızlı ve geniş ölçekte benimsenmesi, araştırma programlarının doğru uygulanmasına bağlı olarak hem sanayileşmiş hem de gelişmekte olan ülkelerde tarım sistemlerinin yetkinliklerini geliştirmiştir (Sharma ve ark., 2024).

Bitki doku kültürü, son 100 yıl içerisinde bilime çok önemli katkılar sağlamış, özellikle de 20. yüzyılın ikinci yarısında somatik embriyogenesis ve ilişkili diğer tekniklerin keşfiyle birlikte büyük bir ivme kazanmıştır. Bitki doku kültürü, tıbbi bitkiler ve tarımsal ürünlerin ekonomik potansiyellerinden yararlanmak açısından oldukça güvenilir yöntemler sunmaktadır. Bitki doku kültürü; endüstri, tarım ve bitki ıslahında merkezi bir role sahiptir. Mikroçoğaltım, sentetik tohum üretimi, somaklonal varyasyon, melezleme, genetik transformasyon, haploid bitki elde etme, patojen eliminasyonu ve genetik kaynakların (germplazm) korunması gibi uygulamalarla ürün geliştirme ve verim artırma süreçlerini destekler. Ayrıca besi ortamına biyotik/abiyotik elisitörlerin eklenmesi, öncü

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DOI: 10.37609/akya.3992. c3127

KAYNAKLAR

Abdalla, N., El-Ramady, H., Seliem, M. K., El-Mahrouk, M. E., Taha, N., Bayoumi, Y., Shalaby, T. A., Dobránszki, J., 2022. An academic and technical overview on plant micropropagation challenges. Horticulturae, 8, 677. https://doi.org/10.3390/horticulturae8080677

Abido, A. I. A., Aly, M. A. M., Hassanen, S. A., Rayan, G. A., 2013. *In vitro* propagation of grapevine (*Vitis vinifera* L.) Muscat of Alexandria cv. for conservation of endangerment. Middle-East Journal of Scientific Research, 13(3), 328-337. https://doi.org/10.5829/idosi.mejsr.2013.13.3.1926

Adil, S., Singh, V., Anjum, A., Quraishi, A., 2022. A mini-review on electrotherapeutic strategy for the plant viral elimination. Plant Cell, Tissue and Organ Culture (PCTOC), 150, 41-55. https://doi.org/10.1007/s11240-022-02265-w

Anonim, (2025). Mikro çoğaltım [Ders notu]. Ankara Üniversitesi Açık Ders Malzemeleri. Erişim adresi: https://acikders.ankara.edu.tr/mod/resource/view.php?id=89500

Ahmad, N., Fatima, N., Faisal, M., Alatar, A. A., Pathirana, R., 2022. Photosynthetic parameters and oxidative stress during acclimation of crepe-myrtle (*Lagerstroemia speciosa* (L.) Pers.) in a meta-topolin-based micropropagation system and genetic fidelity of regenerated plants. Plants, 11(9), 1163. https://doi.org/10.3390/plants11091163

Ajithkumar, D., Seeni, S., 1998. Rapid clonal multiplication through *in vitro* axillary shoot proliferation of *Aegle marmelos* (L.) Corr., a medicinal tree. *Plant Cell Rep., 17(5),* 422-426. https://doi.org/10.1007/s002990050418

Al-Taleb, M. M., Hassawi, D. S., Abu-Romman, S. M., 2011. Production of virus free potato plants using meristem culture from cultivars grown under Jordanian environment. American-Eurasian J. Agric. & Environ. Sci., 11(4), 467-472.

Bahri, N. B., Bettaieb, T., 2013. *In vitro* propagation of a forest tree *Paulownia tomentosa* (Thunb.) Steud.-A valuable medicinal tree species. Albanian Journal of Agricultural Sciences, 12(1), 37-42.

Barton, M. K., 2010. Twenty years on: the inner workings of the shoot apical meristem, a developmental dynamo. Developmental Biology, 341(1), 95-113. https://doi.org/10.1016/j.ydbio.2009.11.029

Basile, G. N., Tedone, L., Pulvento, C., De Mastro, G., Ruta, C., 2023. Establishment of an efficient *in vitro* propagation protocol for *Cannabis sativa* L. subsp. *ruderalis* Janish. Horticulturae, 9(11), 1241. https://doi.org/10.3390/horticulturae9111241

Benke, A. P., Krishna, R., Khandagale, K., Gawande, S., Shelke, P., Dukare, S., Dhumal, S., Singh, M., Mahajan, V., 2023. Efficient elimination of viruses from garlic using a combination of shoot meristem culture, thermotherapy, and chemical treatment. Pathogens, 12(1), 129. https://doi.org/10.3390/pathogens12010129

Bharat, J. S., Nandkishor, Z. V., Natha, T. S., Avinash, S. A., 2020. Plant tissue culture. Journal of Emerging Technologies and Innovative Research, 7(11), 229-239.

Bhat, A. I., Rao, G. P., 2020. Virus Elimination by Meristem-Tip Culture. In: Characterization of Plant Viruses . Springer Protocols Handbooks. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-0334-5_47

Bi, W. L., Hao, X. Y., Cui, Z. H., Pathirana, R., Volk, G. M., Wang, Q. C., 2018. Shoot tip cryotherapy for efficient eradication of grapevine leafroll-associated virus-3 from diseased grapevine *in vitro* plants. Annals of Applied Biology, 173(3), *261*-270. https://doi.org/10.1111/aab.12459

Brison, M., de Boucaud, M. T., Pierronnet, A., Dosba, F., 1997. Effect of cryopreservation on the sanitary state of a cv *Prunus* rootstock experimentally contaminated with Plum Pox Potyvirus. Plant Science, 123(1-2), 189-196. https://doi.org/10.1016/S0168-9452(97)04581-0

Bonnart, R. M., Chen, K. Y., Volk, G. M., 2022. Plant Tissue Culture Media Preparation. In: Volk G. M. (eds.) Training in Plant Genetic Resources: Cryopreservation of Clonal Propagules. Fort Collins, Colorado: Colorado State University. https://colostate.pressbooks.pub/clonalcryopreservation/chapter/media/

Campol, J. R., Naing, A. H., Aung, H. M., Cho, S. B., Kang, H., Chung, M. Y., Kim, C. K., 2024. Production of genetically stable and *Odontoglossum* ringspot virus-free *Cymbidium* orchid 'New True'plants via meristem-derived protocorm-like body (PLB) subcultures. Plant Methods, 20(1), 145. https://doi.org/10.1186/s13007-024-01269-1

Comlekcioglu, S., Kuden, A. B., Kacar, Y. A., Kamberoglu, M. A., 2007. Meristem culture of two fig cultivars in Turkey. Fruits, 62(2), 125-131. https://doi.org/10.1051/fruits:2007006

Conde, F., Carmona-Martin, E., Hormaza, J. I., Petri, C., 2023. *In vitro* establishment and micropropagation of mango (*Mangifera indica* L.) from cotyledonary nodes. In Vitro Cell. Dev. Biol.-Plant, 59(2), 197-208. https://doi.org/10.1007/s11627-023-10334-8

Cox, K., 2024. Temperature and light in tissue-cultured plant success. The Regular Plant Co. https://theregularplantco.com/temperature-and-light-in-tissue-cultured-plant-success/

Diab, A. A., Khalil, S. M., Ismail, R. M., 2011. Regeneration and micropropagation of grapevine (*Vitis vinifera L.*) through shoot tips and axillary buds. International Journal of Advanced Biotechnology and Research, 2(4), 484-491.

Disasa, T., Feyissa, T., Dagne, K., 2011. *In vitro* regeneration of niger (*Guizotia abyssinica* L.F.) Cass.). Int. J. Biosci., 1(6), 110-118.

Doğan, M., 2019. Zeatin ve Farklı Oksin Kombinasyonlarının Önemli Tıbbi Bitki *Limnophila aromatica* (Lamk.) Merr.'nın *In Vitro* Mikroçoğaltımı Üzerine Etkisi. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *22*, 323-329. https://doi.org/10.18016/ksutarimdoga.vi.555790

Dolatabadi, K., Davarynejad, G., Safarnejad, M. R., Ghayoor, Z., 2023. Developing virus-free grapevine explants by using silver-nanoparticles and its comparison with chemo and thermotherapy-based approaches. Journal of Crop Protection, 12(1), 15-27. https://doi.org/10.48311/jcp.2023.1618

Ebrahimi, M., Habashi, A. A., Emadpour, M., Kazemi, N., 2022. Recovery of virus-free Almond (*Prunus dulcis*) cultivars by somatic embryogenesis from meristem undergone thermotherapy. Scientific Reports, 12(1), 14948. https://doi.org/10.1038/s41598-022-19269-3

Espinosa-Leal, C. A., Puente-Garza, C. A., García-Lara, S., 2018. *In vitro* plant tissue culture: means for production of biological active compounds. Planta, 248(1), 1-18. https://doi.org/10.1007/s00425-018-2910-1

Fouracre, J. P., Poethig, R. S., 2020. Lonely at the top? Regulation of shoot apical meristem activity by intrinsic and extrinsic factors. Curr. Opin. Plant Biol., 58, 17-24. https://doi.org/10.1016/j.pbi.2020.08.008

García-Gonzáles, R., Quiroz, K., Carrasco, B., Caligari, P., 2010. Plant tissue culture: Current status, opportunities and challenges. Cien. Inv. Agr., 37(3), 5-30.

Gaj, M. D., 2004. Factors influencing somatic embryogenesis induction and plant regeneration with particular reference to *Arabidopsis thaliana* (L.) Heynh. Plant Growth Regulation, 43(1), 27-47.

George, E. F., Hall, M. A., De Klerk, G. J. (eds.)., 2008. Plant propagation by tissue culture, 3rd ed.. Springer. https://doi.org/10.1007/978-1-4020-5005-3

Gulzar, B., Mujib, A., Malik, M. Q., Mamgain, J., Syeed, R., Zafar, N., 2020. Plant tissue culture: agriculture and industrial applications. In: Transgenic Technology Based Value Addition in Plant Biotechnology (pp. 25-49). Academic Press. https://doi.org/10.1016/B978-0-12-818632-9.00002-2

Gupta, N., Jain, V., Joseph, M. R., Devi, S., 2020. A review on micropropagation culture method. Asian Journal of Pharmaceutical Research and Development, 8(1), 86-93. https://doi.org/10.22270/ajprd.v8i1.653

Gümüş, C., Ellialtıoğlu, Ş. Ş., 2018. *Kalanchoe blossfeldiana* Poelln. Türünde Yapılan Doku Kültürü Araştırmaları Üzerinde Bir İnceleme. Türk Bilimsel Derlemeler Dergisi, 11(1), 18-26.

Haberlandt, G., 1902. Kulturversuche mit isolierten Pflanzenzellen. Sitzungsber. Akad. Wiss. Wien. Math.-Naturwiss. Kl. Abt. J. 111, 69-92.

Hasnain, A., Naqvi, S. A. H., Ayesha, S. I., Khalid, F., Ellahi, M., Iqbal, S., Hassan, M. Z., Abbas, A., Adamski, R., Markowska, D., Baazeem, A., Mustafa, G., Moustafa, M., Hasan, M. E., Abdelhamid, M. M. A., 2022. Plants *in vitro* propagation with its applications in food, pharmaceuticals and cosmetic industries; current scenario and future approaches. Frontiers in Plant Science, 13, 1009395. https://doi.org/10.3389/fpls.2022.1009395

Herman, E .B., 2015. Media and techniques for growth, regeneration and storage: 2011–2015. In: Lake, M. (ed.). Recent advances in plant tissue culture.Agritech Consultants Inc., Shrub Oak, NY, p. 148.

Hormozi-Nejad, M. H., Mozafari, J., Rakhshandehroo, F., 2010. Elimination of Bean common mosaic virus using an electrotherapy technique. J. Plant Dis. Prot., 117(5), 201-205. https://doi.org/10.1007/BF03356361

Hu, G. J., Hong, N., Wang, L. P., Hu, H. J., Wang, G. P., 2012. Efficacy of virus elimination from in vitro-cultured sand pear (*Pyrus pyrifolia*) by chemotherapy combined with thermotherapy. Crop Protection, 37, 20-25. https://doi.org/10.1016/j.cropro.2012.02.017

Hu, G., Dong, Y., Zhang, Z., Fan, X., Ren, F., 2020. Efficiency of chemotherapy combined with thermotherapy for eliminating Grapevine leafroll-associated virus 3 (GLRaV-3). Sci. Hortic. 271, 109462 https://doi.org/10.1016/j.scienta.2020.109462

James, D., Trytten, P. A., Mackenzie, D. J., Towers, G. H. N., French, C. J., 1997. Elimination of Apple stem grooving virus by chemotherapy and development of an immunocapture RT-PCR for rapid sensitive screening. Annals of Applied Biology, 131(3), 459-470. https://doi.org/10.1111/j.1744-7348.1997.tb05173.x

Jakab, Z. I., Vlaşin, L. D., Chiorean, A. M., 2022. Behaviour of several chokeberry cultivars (*Aronia melanocarpa*) at the *in vitro* micropropagation. Romanian Journal of Horticulture, 3, 31-36. https://doi.org/10.51258/RJH.2022.04

Jiménez, V. M., 2005. Involvement of plant hormones and plant growth regulators on *in vitro* somatic embryogenesis. Plant Growth Regulation, 47(2), 91-110. https://doi.org/10.1007/s10725-005-3478-x

Justine, A. K., Kaur, N., Savita, Pati, P. K., 2022. Biotechnological interventions in banana: current knowledge and future prospects. Heliyon, 8(11), e11636. https://doi.org/10.1016/j.heliyon.2022.e11636

Kabir, A. H., Pal, S. P., Sarker, K. K., Sharmin, S. A., Alam, M. F., 2010. Virus elimination and pathogen-free plantlets regeneration in *Cucurbita pepo* L. Archives of Phytopathology and Plant Protection, 43(6), 527-537.

Kassaye, E., Bekele, B. D., 2015. *In vitro* optimization of the protocol for micropropagation of plum (*Prunus salicina* l. var. Methley) from nodal explants. Biotechnology International, 8(4), 137-148.

Kaur, K., Dolker, D., Behera, S., Pati, P. K., 2022. Critical factors influencing *in vitro* propagation and modulation of important secondary metabolites in *Withania somnifera* (L.) dunal. Plant Cell Tiss. Organ Cult., 149(1), 41-60. https://doi.org/10.1007/s11240-021-02225-w

Kaviani, B., 2015. Some Useful Information about Micropropagation. Journal of Ornamental & Horticultural Plants, 5(1), 29-40.

Kaviani, B., Deltalab, B., Kulus, D., Tymoszuk, A., Bagheri, H., Azarinejad, T., 2022. *In Vitro* Propagation of *Pyracantha angustifolia* (Franch.) CK Schneid. *Horticulturae*, *8*(10), 964. https://doi.org/10.3390/horticulturae8100964

Kaya, E., 2021. Comparison of three different techniques for eradication of Apple mosaic virus (ApMV) from hazelnut (*Corylus avellana* L.). Journal of Plant Protection Research. 61(1), 11-19. https://doi.org/10.24425/jppr.2021.136275

Kaynak, L., Ersoy, N., 1997. Bitki büyüme düzenleyicilerinin genel özellikleri ve kullanım alanları. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 10, 223-236.

Kaynak, L., İmamgiller, B., 1997. Bitki büyüme düzenleyicilerinin f izyolojik olaylardaki rolleri. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 10, 289-299.

Kaynak, L., Memiş, M., 1997. Bitki büyüme engelleyici ve geciktiricilerinin etki mekanizmaları. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 10, 237-248.

Kazemi, N., Zaare Nahandi, F., Habashi, A. A., Masoomi-Aladizgeh, F., 2020. Comparing the efficiency of conventional and novel methods of virus elimination using molecular techniques. Eur. J. Plant Pathol., 157(4), 887-897. https://doi.org/10.1007/s10658-020-02048-z

Kim, M. Y., Cho, K. H., Chun, J. A., Park, S. J., Kim, S. H., Lee, H. C., 2017. Elimination of Grapevine fleck virus from infected grapevines 'Kyoho'through meristem-tip culture of dormant buds. Journal of Plant Biotechnology, 44(4), 401-408.

Köhl, J., Kolnaar, R., Ravensberg, W. J., 2019. Mode of action of microbial biological control agents against plant diseases: Relevance beyond efficacy. Front. Plant Sci., 10, 845. https://doi.org/10.3389/fpls.2019.00845

Kulus, D., Tymoszuk, A., 2024. Advancements in *in vitro* technology: A comprehensive exploration of micropropagated plants. Horticulturae, 10(1), 88. https://doi.org/10.3390/horticulturae10010088

Kumar, S., Khan, M. S., Raj, S. K., Sharma, A. K., 2009. Elimination of mixed infection of Cucumber mosaic and Tomato aspermy virus from *Chrysanthemum morifolium* Ramat. cv. Pooja by shoot meristem culture. Sci. Hortic., 119(2), 108-112. https://doi.org/10.1016/j.scienta.2008.07.017

Kumar, A., Bhuj,B. D., Dhar, S., Dixit, K. M. J., Singh, S. P., 2023. Micropropagation of Fruit Crops: A Review. Adv. Crop Sci. Tech., 11, 595.

Kumlay, A. M., Eryiğit, T., 2011. Bitkilerde Büyüme ve Gelişmeyi Düzenleyici Maddeler: Bitki Hormonları. Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 1(2), 47-56.

Lal, M., Jamwal, M., Sood, Y., Bakshi, P., Sharma, N., Sharma, S., Kumar, S., 2023. Micropropagation of fruit crops: A review. Plant Science Today, 10(1), 108-117. https://doi.org/10.14719/pst.1891

Ling, W. T., Tan, L. V., Khor, S. P., Sriskanda, D., Subramaniam, S., Chew, B. L., 2022. Rapid *in vitro* propagation of fig (*Ficus carica* L.)'Violette de Solliès' supported by molecular and microscopy analyses. Horticulturae, 8(11), 1025. https://doi.org/10.3390/horticulturae8111025

Maheshwari, R., 1969. Applications of plant tissue and cell culture in the study of physiology of parasitism. Proc. Indian Acad. Sci. 69, 152-172. https://doi.org/10.1007/BF03052523

Marino, L.A., Ruffa, P., Mozzanini, E., Patono, D. L., Sereno, A., Pavese, V., 2025. LEDs in Plant Tissue Culture: Boosting Micropropagation of *Castanea sativa* Cultivars. J. Plant Growth Regul., 44, 6046–6060. https://doi.org/10.1007/s00344-025-11812-6

Mazeikiene, I., Kviklys, D., Siksnianiene, J. B., Zinkus, D., Stanys, V., 2019. Influence of Ribavirin on *Prunus domestica* L. regeneration, genome stability and virus eradication *in vitro*. Proceedings of the Latvian Academy of Sciences,73(3), 238-243. https://doi.org/10.2478/prolas-2019-0038

Meira, F. S., Shao, M., Niedz, R. P., Thomson, J. G., 2024. Evaluation of 21 different media on shoot regeneration in 11 cultivars of citrus using juvenile tissue. Plant Cell Tiss. Organ Cult., 158, 8. https://doi.org/10.1007/s11240-024-02785-7

Meybodi, D. E., Mozafari, J., Babaeiyan, N., Rahimian, H., 2011. Application of electrotherapy for the elimination of potato potyviruses. J. Agr. Sci. Tech., 13, 921-927.

Muñoz-Concha, D., Quintero, J., Ercişli, S., 2021. Media and hormones influence in micropropagation success of blackberry cv.'Chester'. Res. J. Biotechnol., 16, 5.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant., 15, 473-497.

Muhammad, A., Rashid, H., Hussain, I., Naqvi, S. S., 2007. Proliferation-rate Effects of BAP and Kinetin on Banana (*Musa* spp. AAA Group) 'Basrai'. HortScience, 42(5), 1253-1255. https://doi.org/10.21273/HORTSCI.42.5.1253

Mostafa, F. M. A., Shaaban, M. M., Elazab, D. S., Kamel, M. T., 2015. *In vitro* Propagation of Four Grape Cultivars. Assiut Journal of Agricultural Sciences, 46(4), 65-76.

Nongdam, P., Beleski, D. G., Tikendra, L., Dey, A., Varte, V., El Merzougui, S., Pereira, V. M., Barros, P. R., Vendrame, W. A., 2023. Orchid Micropropagation Using Conventional Semi-Solid and Temporary Immersion Systems: A Review. Plants, 12(5), 1136. https://doi.org/10.3390/plants12051136

Neri, J. C., Meléndez-Mori, J. B., Tejada-Alvarado, J. J., Vilca-Valqui, N. C., Huaman-Huaman, E.,

Oliva, M., Goñas, M., 2022. An optimized protocol for micropropagation and acclimatization of strawberry (*Fragaria× ananassa* Duch.) variety 'Aroma'. Agronomy, 12(4), 968. https://doi.org/10.3390/agronomy12040968
Panattoni, A., Luvisi, A., Triolo, E., 2013. Elimination of viruses in plants: twenty years of progress. Span. J. Agri Res., 11, 173-188. https://doi.org/10.5424/sjar/2013111- 3201
Papafotiou, M., Vlachou, G., Martini, A. N., 2023. Investigation of the effects of the explant type and different plant growth regulators on micropropagation of five mediterranean *Salvia* spp. native to Greece. Horticulturae, 9(1), 96. https://doi.org/10.3390/horticulturae9010096
Pasternak, T. P., Steinmacher, D., 2024. Plant Growth Regulation in Cell and Tissue Culture *In Vitro*. Plants, 13(2), 327. https://doi.org/10.3390/plants13020327
Phillips, G. C., Garda, M., 2019. Plant tissue culture media and practices: an overview. *In Vitro* Cell. Dev. Biol.-Plant, 55, 242-257. https://doi.org/10.1007/s11627-019-09983-5
Pourhassan, A., Kaviani, B., Kulus, D., Miler, N., Negahdar, N., 2023. A complete micropropagation protocol for black-leaved *Zamioculcas zamiifolia* (Lodd.) Engl.'Dowon'. Horticulturae, 9(4), 422. https://doi.org/10.3390/horticulturae9040422
Quiroz, K. A., Berríos, M., Carrasco, B., Retamales, J. B., Caligari, P. D. S., García-Gonzáles, R., 2017. Meristem culture and subsequent micropropagation of Chilean strawberry (*Fragaria chiloensis* (L.) Duch.). Biol. Res., 50, 20. https://doi.org/10.1186/s40659-017-0125-8
Raj, R., Kaur, C., Agrawal, L., Kumar, S., Chauhan, P. S., Raj, S. K., 2022. Development of a protocol for the elimination of *Cyrtanthus elatus* virus-A from *Narcissus tazetta* by *in vitro* chemotherapy in combination with electrotherapy. J. Virol. Methods, 300, 114368. https://doi.org/10.1016/j.jviromet.2021.114368
Ranaweera, K. K., Gunasekara, M. T. K., Eeswara, J. P., 2013. *Ex vitro* rooting: A low cost micropropagation technique for Tea (*Camellia sinensis* (L.) O. *kuntze*) hybrids. Scientia Horticulturae, 155, 8-14. https://doi.org/10.1016/j.scienta.2013.03.001
Sarıdaş, M. A., Baktemur, G., Taşkın, H., Kargı, S. P., 2021. Effect of plant hormones on micropropagation potential of superior strawberry genotypes and their parents via shoot-tip culture. Acta Sci. Pol. Hortorum Cultus, 20(3), 63-75. https://doi.org/10.24326/asphc.2021.3.7
Sarropoulou, V., Grigoriadou, K., Maliogka, V. I., Sassalou, C. L., Ziogas, V., 2024. The Elimination of Viroids through *In vitro* Thermotherapy and a Meristem Tip Culture from a New Limonime Hybrid (*Citrus* x *limon* var. *limon* (L.) Burm. f. x *Citrus latifolia* var. *latifolia*). BioTech, 13(3), 37. https://doi.org/10.3390/biotech13030037
Schwach, F., Vaistij, F. E., Jones, L., Baulcombe, D. C., 2005. An RNA-dependent RNA polymerase prevents meristem invasion by potato virus X and is required for the activity but not the production of a systemic silencing signal. Plant Physiol., 138(4), 1842-1852. https://doi.org/10.1104/pp.105.063537
Shahzad, A., Parveen, S., Sharma, S., Shaheen, A., Saeed, T., Yadav, V., Akhtar, R., Ahmad, Z., Upadhyay, A., 2017. Plant tissue culture: applications in plant improvement and conservation. In: Plant Biotechnology: Principles and Applications (pp. 37-72). Singapore: Springer Singapore.
Sharma, A. T., Jadhav, D. S., Dachawar, R. R., Pawde, D. V., Zunzulde, S. S., 2024. Plant Tissue Culture: A Review. International Journal of Pharmacy and Pharmaceutical Research, 30(5), 595-612.
Singh, A., 2015. Micropropagation of plants. In: Plant Biology And Biotechnology: Volume II: Plant Genomics And Biotechnology (pp. 329-346). New Delhi: Springer India.
Singh, V., Adil, S., Quraishi, A., 2022. Elimination of BBTV via a systemic *in vitro* electrotherapy approach. Journal of Virological Methods, 300, 114367. https://doi.org/10.1016/j.jviromet.2021.114367
Sultana, K. W., Das, S., Chandra, I., Roy, A., 2022. Efficient micropropagation of *Thunbergia coccinea* Wall. and genetic homogeneity assessment through RAPD and ISSR markers. Sci. Rep., 12(1), 1683. https://doi.org/10.1038/s41598-022-05787-7
Szabó, L. K., Desiderio, F., Kirilla, Z., Hegedűs, A., Várallyay, É., Preininger, É., 2024. Elimination of Cherry virus A from *Prunus domestica* 'Besztercei Bt. 2'using *in vitro* techniques. Plant Cell

Tiss. Organ Cult., 157, 45. https://doi.org/10.1007/s11240-024-02770-0
Taşkın, H., Baktemur, G., Kurul, M., Büyükalaca, S., 2013. Use of tissue culture techniques for producing virus-free plant in garlic and their identification through real-time PCR. The Scientific World Journal, 2013(1), 781282. https://doi.org/10.1155/2013/781282
Thanuja, K., Arulmozhiyan, R., Saraswathi, M. S., Selvarajan, R., Jegadeeswari, V., Rajanbabu, V., 2025. A comprehensive review on *in vitro* therapies for virus elimination and novel methods for virus protection in key horticultural crops. Planta, 262(1), 15. https://doi.org/10.1007/s00425-025-04718-w
VanDerZanden, A. M., 2012. How hormones and growth regulators affect your plants. OSU Extension Service. https://extension.oregonstate.edu/gardening/techniques/how-hormones-growth-regulators-affect-your-plants
Vivek, M., Modgil, M., 2018. Elimination of viruses through thermotherapy and meristem culture in apple cultivar 'Oregon Spur-II'. Virus Disease, 29(1), 75-82. https://doi.org/10.1007/s13337-018-0437-5
Yan, H.B., Liang, C. X., Yang, L. T., Li, Y. R., 2010. *In vitro* and *ex vitro* rooting of Siratia grosvenorii, a traditional medicinal plant. Acta Physiol. Plant, 32, 115-120. https://doi.org/10.1007/s11738-009-0386-0
Walsh, C. S., 2003. Plant Hormones. Concise Encyclopedia of Temperate Tree Fruit. In: Singha, S., Baugher T (eds.). and, Haworth Press. ISBN 1560229411.
Wang, Q., Cuellar, W. J., Rajamäki, M. L., Hirata, Y., Valkonen, J. P., 2008. Combined thermotherapy and cryotherapy for efficient virus eradication: relation of virus distribution, subcellular changes, cell survival and viral RNA degradation in shoot tips. Mol. Plant Pathol., 9(2), 237-250. https://doi.org/10.1111/j.1364-3703.2007.00456.x
Wang, Q. C., Valkonen, J. P. T., 2008. Efficient elimination of sweetpotato little leaf phytoplasma from sweetpotato by cryotherapy of shoot tips. Plant Pathology, 57(2), 338-347. https://doi.org/10.1111/j.1365-3059.2007.01710.x
Wang, Q., Valkonen, J. P., 2009. Cryotherapy of shoot tips: novel pathogen eradication method. Trends Plant Sci., 14, 119-122. https://doi.org/10.1016/j. tplants.2008.11.010
Wang, Q. C., Panis, B., Engelmann, F., Lambardi, M., Valkonen, J. P. T., 2009. Cryotherapy of shoot tips: a technique for pathogen eradication to produce healthy planting materials and prepare healthy plant genetic resources for cryopreservation. Annals of Applied Biology, 154(3), 351-363. https://doi.org/10.1111/j.1744-7348.2008.00308.x
Wang, M. B., Masuta, C., Smith, N. A., Shimura, H., 2012. RNA silencing and plant viral diseases. Mol. Plant Microbe Interact., 25(10), 1275-1285. https://doi.org/10.1094/MPMI-04-12-0093-CR
Wang, M. R., Cui, Z. H., Li, J. W., Hao, X. Y., Zhao, L., Wang, Q. C., 2018. *In vitro* thermotherapy-based methods for plant virus eradication. Plant Methods, 14, 87. https://doi.org/10.1186/s13007-018-0355-y
Waseem, K., Jilani, M. S., Khan, M. S., 2009. Rapid plant regeneration of chrysanthemum (*Chrysanthemum morifolium* L.) through shoot tip culture. African Journal of Biotechnology, 8(9), 1871-1877.
Wöhner, T., Höfer, M., 2024. Evaluation of cryotherapy and meristem isolation from stolons to eliminate viruses in *Fragaria* germplasm. J. Plant Pathol., 106, 729-735. https://doi.org/10.1007/s42161-023-01570-3
Zarbakhsh, S., Shahsavar, A. R., Soltani, M., 2024. Optimizing PGRs for *in vitro* shoot proliferation of pomegranate with bayesian-tuned ensemble stacking regression and NSGA-II: a comparative evaluation of machine learning models. Plant Methods, 20(1), 82. https://doi.org/10.1186/s13007-024-01211-5
Zhang, A. L., Bettoni, J. C., Shi, X., Liu, Y., Yang, B., Liu, Z., 2024. *In vitro* chemotherapybased methods for virus elimination from *Actinidia macrosperma*. Sci. Hortic., 337, 113543. https://doi.org/10.1016/j.scienta.2024.

Bölüm 5

ORGANOGENESİS

Hüsamettin Aycan ALP [1]

5.1 TANIM, TARİHÇE VE TEMEL KAVRAMLAR

Bitki biyoteknolojisi araçlarından biri olan doku kültürü teknikleri, donör (ana) bitkiden izole edilen hücre, doku organ gibi bitki parçalarından (eksplantlardan) yeni bir bitki elde edilmesi işlemidir. Temel botanik araştırmalarında, transgenetik bitki üretimlerinde, *in vitro* klonal çoğaltım, gen kaynaklarının muhafazası gibi pek çok farklı alanda kullanılan farklı teknikleri içermektedir (George, 2008a). Teoride, her diploid bitki hücresi, bir bireyin oluşumu için gerekli olan genetik bilgiyi içermektedir ve bu nedenle, her diploid hücre, tam bir bireye dönüşebilme yeteneğine sahiptir. Totipotansi olarak adlandırılan bu dönüşüm yeteneği Steward (1958) tarafından yapılmış olan çalışmalarla ortaya konulmuş olmakla birlikte, ilk çalışmalar 20. yüzyılın ilk yıllarına Alman botanikçi Haberlandt'ın (1903) çalışmalarına dayanmaktadır (Caponetti ve ark., 2005).

Sürgün, kök ve embriyo gibi yeni (*de novo*) organlar, meristematik dokudan yoksun olan bitki parçalarından üretilebilmektedir. Bu gibi yeni üretilmiş olan organlar adventif (adventive veya adventitious) olarak adlandırılır. Daha önceden eksplant üzerinde yer alamayan yeni form veya farklılaşmış hücre organizasyonları adventif organogenesis veya embriyo oluşması durumunda somatik embriyogenesis olarak adlandırılmaktadır. Organ gelişimleri, eksplant üzerinde kallus dokusunun (kallogenesis) oluşmasının ardından meydana geldiği durumlar indirekt organogenesis olarak adlandırılır. Şayet organlar eksplant üzerinde doğrudan (kallus oluşmadan) gelişmeye başlarsa, bu durum direkt organogenesis olarak isimlendirilir. Adventif gelişim, sürgün yapısı olarak ortaya çıkması durumunda kaulogenesis (caulogenesis), kök yapısı oluşması durumunda ise rizogenesis (rhizogenesis) olarak adlandırılmaktadır (George, 2008b). Organogenesis; hücre bölünmesinin,

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çalışmaların, organogenesis sürecinde rol oynayan moleküler mekanizmaların ve sinyal iletim ağlarının daha ayrıntılı biçimde ortaya konmasına katkı sağlaması beklenmektedir. Ayrıca, farklı türlere özgü kültür koşullarının optimize edilmesi ve biyoteknolojik yöntemlerle desteklenmesi, bu tekniklerin hem bilimsel araştırmalarda hem de endüstriyel uygulamalarda daha etkin ve güvenilir bir şekilde kullanılmasını mümkün kılacaktır. Böylelikle organogenesis tabanlı doku kültürü yaklaşımlarının, sürdürülebilir tarım ve bitki biyoteknolojisi alanında daha geniş ölçekte değer yaratması öngörülmektedir.

KAYNAKLAR

Abhinava, J.V., 2018. Organogenesis, Embryogenesis, Synthetic seeds in tissue culture. Published on: Feb 7, 2018 https://www.slideshare.net/featured/category/science (erişim tarihi: Ağustos 2020)

Al-Mayahi, A.M.W., 2016. Effect of red and blue light emitting diodes "CRB-LED" on *in vitro* organogenesis of date palm (*Phoenix dactylifera* L.) cv. Alshakr. World J Microbiol Biotechnol 32, 160 (2016). https://doi.org/10.1007/s11274-016-2120-6

Alp, H. A., 2020. Türkiye'de Yetişen Bazı Sıklamen Türlerinin Yumrularında Somatik Embriyogenesis ve Sentetik Tohum Üretimi Araştırmaları. Çukurova Üniversitesi Fen bilimleri Enst. Doktora tezi.

Anonim, 2020. Steward Experiment and Application of Totipotency. https://www.biologydiscussion.com (Erişim tarihi: Ağustos, 2025)

Bach, A., Kapczyńska, A., Dziurka, B., Dziurka, M., 2018. The importance of applied light quality on the process of shootorganogenesis and production of phenolics and carbohydratesinLachenaliasp. culturesin vitro. South African Journal of Botany 114 (2018) 14–19 https://doi.org/10.1016/j.sajb.2017.10.015

Bhatia, S., 2015. Application of Plant Biotechnology, Editor(s): Saurabh Bhatia, Kiran Sharma, Randhir Dahiya, Tanmoy Bera, Modern Applications of Plant Biotechnology in Pharmaceutical Sciences, Academic Press

Beyl, C. A., 2005. Getting Started with Tissue Culture: Media Preparation, Sterile Technique, and Laboratory Equipment. Pg: 19-38. Plant Development And Biotechnology. CRC Press LLC 2005. Edit: Trigiano R. N., Gray D. J.

Brown, D.C.W., Thorpe, T.A., 1986. Plant regeneration by organogenesis. Cell Culture and Somatic Cell Genetic of Plants Volume 3. Plant Regeneration and Genetic Variability. Ed: Vasil, I. K. Florida, USA. Academic Press Inc.

Burger, D.W., Hackett, W.P., 1986. Gradients of adventitious bud formation on excised epicotyl and root sections of Citrus. Plant Sci. 43, 229-232.

Caponetti, J. D., Gray, D. J., Trigiano, R.N., 2005. History Of Plant Tissue And Cell Culture. Pg: 9-17. Plant Development And Biotechnology. CRC Press LLC 2005. Edit: Trigiano R. N., Gray D. J.

Chen, C., 2004. Humidity in Plant Tissue Culture Vessels. Biosystems Engineering (2004) 88 (2), 231–241 doi:10.1016/j.biosystemseng.2004.02.00

De Klerk, G. J., 2009. Adventitious Organogenesis. Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology, ed: Michael C. Flickinger. pub: John Wiley & Sons, Inc

Decout, E., Dubois, T., Guedira, M., Dubois, J., Audran, J.C., Vasseur, J., 1994. Role of temperature as a triggering signal for organogenesis or somatic embryogenesis in wounded leaves of chicory cultured *in vitro*. Journal of Experimental Botany, Vol. 45, No. 281, pp. 1859-1865, December 1994

Eckstein, A., Zięba, P., Gabryś, H. 2012. Sugar and light effects on the condition of thephotosynthe-

tic apparatus of Arabidopsis thalianacultured *in vitro*. Journal of PlantGrowth Regulation 31, 90–101.
Jackson, M.B., 2005. Aeration stress in plant tissue cultures. In: Hvoslef-Eide, A.K., Preil, W. (eds) Liquid Culture Systems for *in vitro* Plant Propagation. Springer, Dordrecht. https://doi.org/10.1007/1-4020-3200-5_35
Garcia-Ramirez, Y., 2023 Temporary immersion system for *in vitro* propagation via organogenesis of forest plant species. Trees (2023) 37:611–626 https://doi.org/10.1007/s00468-022-02379-w
Ganapathi, A., Anbazhagan, V.R., Amutha, S., Anand, R.P., 2003. *In Vitro* Organogenesis. Jaiwal P.K., Singh R.P. (eds) Improvement Strategies of Leguminosae Biotechnology. Focus on Biotechnology, vol 10A. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-0109-9_3
Gautheret, R.J., 1939. Sur La Possibilite De Realiser La Culture İndefinie Des Tissue De Tubercules De Carotte. C. R. Hebd. Seances Acad. Sci. 208, 118-121.
George, E.F., Hall, M.A., Klerk, G.J.D., 2008a. "Plant Tissue Culture Procedure – Background". In: George E.F., Hall M.A., Klerk GJ.D. (eds) Plant Propagation by Tissue Culture. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-5005-3
George, E.F., Hall, M.A., Klerk, G.J.D., 2008b. Adventitious Regeneration. In: George E.F., Hall M.A., Klerk GJ.D. (eds) Plant Propagation by Tissue Culture. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-5005-3_10
Gürel, E., Uçar-Türker, A., 2001. Organogenesis, Bitki Biyoteknolijisi I–Doku Kültürü ve Uygulamaları s:36-70. Ed: M. Babaoğlu, E. Gürel, S. Özcan. Selçuk Üniversitesi Basımevi, Selçuk Üniversitesi Vakfı Yayınları.
Karam, N.S., Al-Majathoub, M., 2000. *In vitro* shoot regeneration from mature tissue of wild Cyclamen persicum Mill. Scientia Horticulturae, Volume 86, Issue 4, December 2000, Pages 323-333. https://doi.org/10.1016/S0304-4238(00)00160-6
Kaur, A., Kumar, A., 2020. The Effect of Gelling agent, medium pH and silver nitrate on adventitious shoot regeneration in Solanum tuberosum. bioRxiv 2020.01.03.894063; doi: https://doi.org/10.1101/2020.01.03.894063
Kiferle, C., Lucchesini, M., Maggini, R., Pardossi, A., Mensuali-Sodi, A., 2014. *In vitro* culture of sweet basil: gas exchanges, growth, and rosmarinic acid production. Biol Plant 58, 601–610 (2014). https://doi.org/10.1007/s10535-014-0434-5
Kocak, M., Izgu, T., Sevindik, B., Tutuncu, M., Curuk, P., Simsek, O., Aka Kacar, Y., Teixeira da Silva, J.A., Yalcin Mendi, Y., 2014. Somatic embryogenesis of Turkish Cyclamen persicum Mill. Scientia Horticulturae 172 (2014) 26–33 DOI:10.1016/j.scienta.2014.03.044
Kozai, T, Kitaya, Y, Fujiwara, K, Smith, M.A.L, Aitken-Christie, J., 1995. Environmental measurement and control systems. In: Automation and Environmental Control in Plant Tissue Culture (Aitken-Chistie, J, ed.), pp 539–574. Kluwer Academic Publishers, Dordrecht, The Netherlands
Haberlandt, G., 1903. Culturversuche mit isolierten Pflanzenzellen. Sitzungsberichte. Abt. 1, Mineralogie, Krystallographie, Botanik, Physiologie der Pflanzen, Zoologie, Paläontologie, Geologie, physische Geographie und Reisen. V:111 Pg:69-91 https://www.zobodat.at/pdf/SBAWW_111_0069-0091.pdf (erişim tarihi: Mart 2025)
Mandal, S., Gadgil, V. N., 1979. Differentiation of shoot buds and roots in fruit callus of Solanum nigrum L. Ind. J. Exp. Biol., 17, 876-878.
Markovskaya, E.F., Sysoeva, M.I., Vasilevskaya, N.V., 2007. Daily Temperature Gradients and Processes of Organogenesis in Apical Meristem of Cucumis sativus L. Russian Journal of Developmental Biology, 2007, Vol. 38, No. 1, pp. 7–14.
Mathews, H., 1987. Morphogenetic responses from in vitro cultured seedling explants of mung bean (Vigna radiata L. Wilczek). Plant Cell Tissue Organ Cult. 11, 233-240.
Miller, C., Skoog, F., 1953. Chemical Control Of Bud Formation In Tobacco Stem Segments. American Journal Of Botany. 40, 768-773.
Miller, C., Skoog, F., Von Saltza, M. H., Strong, F.M., 1955. Kinetin, A Cell Division Factor From

Deoxyribonucleic Acid. Journal American Chemical Society 77: 1392

Mimura, T.C., Shindo, M., Kato, E., Yokota, E., Sakano, K., Ashihara, H., Shimmen, T., 2000. Regulation of cytoplasmic pH under extreme acid conditions in suspension cultured cells of Catharanthus roseus: a possible role of inorganic phosphate. Plant Cell Physiol. 41:424-431

Murashige, T., Skoog, F., 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiologia Plantarum; 1962, Vol. 15 Issue 3, p473-497, 25p. https://research.ebsco.com/c/ri34vg/search/details/ruv5szir5r?db=edb (eirşim tarihi: Temmuz 2025)

Nobecourt, P. 1939., Sur Les Radicelles Naissant Des Cultures De Tissus Du Tubercule De Carotte. Comptes Rendus des Seances de la Societe de Biologie et de Ses Filiales; 130, 1271-1272.

Paek, K.Y., Chakrabarty, D., Hahn, E.J., 2005. Application of bioreactor systems for large scale production of horticultural and medicinal plants. Liquid Culture Systems for in vitro Plant Propagation. ISBN 1-4020-3200-5 (e-book) Springer

Pestana, M.C., Lacorte, C., De Freitas, V.G., De Oliveira, D.E., Mansuw, E., 1999. *In vitro* Regeneratıon of Peanut (Arachıs hypogaea L.) Through Organogenesıs: Effect of Culture Temperature and Silver Nitrate. *In Vitro* Cell. Dev. Biol.--Plant 35:214-216. May-June 1999

Prasad, A., Sidhic, J., Sarbadhikary, P., Narayanankutty, A., George, S., George, P.B., Abrahamse, H., 2024. Role of metal nanoparticles in organogenesis, secondary metabolite production and genetic transformation of plants under *in vitro* condition: a comprehensive review. Plant Cell Tiss Organ Cult 158, 33 (2024). https://doi.org/10.1007/s11240-024-02833-2

Ramírez-Mosqueda, M.A., Bello-Bello, J.J., 2021. SETIS™ bioreactor increases *in vitro* multiplication and shoot length in vanilla (Vanilla planifolia Jacks. Ex Andrews). Acta Physiol Plant 43, 52 (2021). https://doi.org/10.1007/s11738-021-03227-z

Quack, F., 1961. Heat Treatment And Substances İnhibiting Virus Multiplication In Meristem Culture To Obtain Virus-Free Plants. *Advances* in *Horticultural Science* and Their Applications Proceeding. International Horticultural Congress 15th 1958 1:144–148

Schwarz, J. O., Sharma, R. A., Beaty, R. M., 2005. Propagation From Nonmeristematic Tissues: Organogenesis. Pg: 159-172. Plant Development And Biotechnology. CRC Press LLC 2005. Edit: Trigiano R. N., Gray D. J.

Seyring, M., Ewald, A., Muller, A., Haensch, K. T., 2009. Screening forpropagation suitability *in vitro* of different Cyclamen species. Electron J Biotech. 12:4–7. doi:10.4067/S0717-34582009000400010

Sheridan, W.F., 1974. A long term callus culture of Lilium: relative stability of the karyotype. J. Cell Biol. 63, 313a.

Shukla, M.R., Piunno, K., Saxena, PK., Jones, M.A.P., 2020. Improved *in vitro* rooting in liquid culture using a two piece scaffold system. Engineering in Life Sciences. 2020;20:126–132. DOI: 10.1002/elsc.201900133

Singh, S.S., Pradhan, C., Swain, D., Rout, G.R., 2020. Augmenting in vitro Shoot Multiplication by Growth Regulators and Photoperiod in *Vigna mungo* L. (Hepper.). Current Journal of Applied Science and Technology, 39(34), 36-45. https://doi.org/10.9734/cjast/2020/v39i3431035

Skoog, F., 1944. Growth and Organ Formation in Tobacco Tissue Cultures American Journal of Botany, Vol. 31, No.1 pp. 19-24 https://doi.org/10.2307/2437663

Skoog, F., Miller, C.O., 1957. Chemical Regulation Of Growth And Organ Formation In Plant Tissues Cultured *in vitro*. Symposia of the Society for Experimental Biology; 11, 118-131.

Sugiyama, M. 1999. Organogenesis *in vitro*. Plant Biology, 2:61–64 Elsevier Science Ltd. ISSN 1369-5266 http://biomednet.com/elecref/1369526600200061.

Suttle, G.R.L., 2005 Commercial Laboratory Production. Plant Development and Biotechnology / ed. Robert N. Trigiano and Dennis J. Gray. ISBN 0-8493-1614-6

Steward, F.C., Mapes, M.O., Mears, K., 1958. Growth And Organized Development Of Cultured Cells. II. Organization In Cultures Grown From Freely Suspended Cells. American Journal of Botany 45: 705–708

Tanaka, K., Fujiwara, K., Kozai, T., 1992. Effects of Relative Humidity in The Culture Vessel on

The Transpiration and Net Photosynthetic Rates of Potato Plantlets *in vitro*. ISHS Acta Horticulturae 319: International Symposium on Transplant Production Systems. DOI: 10.17660/ActaHortic.1992.319.3

Thorpe, T., 2012. History Of Plant Tissue Culture. Plant Cell Culture Protocols, Methods In Molecular Biology, Vol. 877, DOI 10.1007/978-1-61779-818-4_2,

White, P.R., 1934. Potentially Unlimited Growth Or Excised Tomato Root Tips In Liquid Medium. Plant Physiology 9:585–600.

White, P.R., 1939. Controlled Differentiation In Plant Tissue Culture. Bulletin Of The *Torrey* Botanical *Club*; 66, 507-513.

White, P.R., 1963. The Cultivation of Animal and Plant Cells, 2nd Edition. Ronald, New York

Bölüm 6

SOMATİK EMBRİYOGENESİS

Mehmet TÜTÜNCÜ [1]

6.1 TANIM, TARİHÇE VE TEMEL KAVRAMLAR

Bitkilerde rejenerasyon tek bir hücre veya dokunun tüm bir bitkiyi tamamlama yeteneği olarak tanımlanır. Bu durum, 1902 yılında Gottlieb Haberlandt'in tek bir hücrenin kendini tamamlayıp yeni ve fonksiyonel bir bitki oluşturabileceği teorisiyle keşfedilmiş ve bitki doku kültürünün temelini oluşturmuştur. Totipotensi olarak adlandırılan bu hipoteze göre, bitkilerdeki farklılaşmış hücreler hücre döngüsüne yeniden girebilir, doku ve organlar oluşturabilir ve hatta tam bir bitki haline gelebilir. Haberlandt'a göre hücre sadece bitkinin bir parçası olmayıp, bitkiye göre daha az karmaşık olan ayrı bir organizmadır. Doku kültürü çalışmalarının artmasıyla bitki hücrelerinin gelişim süreci içerisinde çevre ile olan etkileşiminin sonucunda değişme ve yeniden yapılanma kapasitesine sahip olduğu gözlenmiş ve güçlü bir rejenerasyon yeteneğine sahip olduğu belirlenmiştir. Böylece, belirli bir görevi yerine getirmek üzere programlanmış hücrelerden henüz farklılaşmamış ya da organize olmamış hücreler oluşabileceği gibi, organize olmamış hücrelerden yeniden farklılaşmış hücreler meydana gelebilmektedir (Arnold, 2008). *İn vitro* bitki rejenerasyonu, hücre bölünmesi ve farklılaşmasından sonra eksplantların büyüme dönemleri boyunca organ ve dokular oluşturduğu bir süreçtir. *İn vitro* rejenerasyon somatik embriyogenesis veya organogenesis yoluyla yapılabilir. Organogenesis, genellikle organların diğer kısımlarından gelen yaralara yanıt olarak yeni organların ve hatta bütün bir bitkinin oluştuğu süreçtir. Bitki doku kültürüne alınan bitki parçasından uygun ortam ve şartlarda doğrudan bir organ oluşabilir. Bu süreç direkt organogenesis olarak tanımlanırken, indirekt organogenesiste bitki parçasından öncelikle kallus yapıları meydana gelir. Kalluslar farklılaşarak sürgün, kök gibi organlara dönüşürler (Bidabadi ve Jain, 2020). Organogenesis hakkında daha detaylı bilgiyi Bölüm 5'de bulabilirsiniz.

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KAYNAKLAR

Arnold, S. V., 2008. Somatic Embryogenesis. In Plant Propagation By Tissue Culture. Volume 1: The Background, (Ed. 3) (pp.335-354). Germany, Springer-Verlag GmbH.

Bidabadi, S. S., & Jain, S. M., 2020. Cellular, Molecular, and Physiological Aspects of *In Vitro* Plant Regeneration. Plants, 9(6), 702. https://doi.org/10.3390/plants9060702

Bozhkov, P. V., Filonova, L. H., Von Arnold, S., 2002. A Key Developmental Switch During Norway Spruce Somatic Embryogenesis Is Induced By Withdrawal of Growth Regulators and Is Associated With Cell Death and Extracellular Acidification. Biotechnology And Bioengineering, 77(6), 658-667. https://doi.org/10.1002/bit.10228

De Moura Vale, E., Heringer, A. S., Barroso, T., Da Silva Ferreira, A. T., Da Costa, M. N., Perales, J. E. A., Silveira, V., 2014. Comparative Proteomic Analysis of Somatic Embryo Maturation In Carica Papaya L. Proteome Science, 12(1), 1-18. https://doi.org/10.1186/1477-5956-12-37

Elhiti, M., Stasolla, C., Wang, A. 2013. Molecular regulation of plant somatic embryogenesis. *In Vitro* Cellular & Developmental Biology-Plant, 49(6), 631-642. https://doi.org/10.1007/s11627-013-9547-3

Etienne, H., Montoro, P., Michaux-Ferrière, N., Carron, M. P., 1993. Effects Of Desiccation, Medium Osmolarity And Abscisic Acid On The Maturation Of Hevea Brasiliensis Somatic Embryos. Journal Of Experimental Botany, 44(10), 1613-1619. https://doi.org/10.1093/jxb/44.10.1613

Fehér, A., 2008. The Initiation Phase of Somatic Embryogenesis: What We Know And What We Don't. Acta Biologica Szegediensis, 52(1), 53-56.

Fei, L., & Weathers, P., 2014. From Cells To Embryos To Rooted Plantlets In A Mist Bioreactor. Plant Cell Tissue And Organ Culture, 116, 37–46. https://doi.org/10.1007/S11240-013-0380-5

Gulzar, B., Mujib, A., Malik, M. Q., Sayeed, R., Mamgain, J., Ejaz, B., 2020. Genes, Proteins And Other Networks Regulating Somatic Embryogenesis in Plants. Journal Of Genetic Engineering And Biotechnology, 18(1), 1-15. https://doi.org/10.1186/s43141-020-00047-5

Gutmann, M., Von Aderkas, P., Label, P., Lelu, M. A., 1996. Effects of Abscisic Acid on Somatic Embryo Maturation of Hybrid Larch. Journal of Experimental Botany, 47(12), 1905-1917. https://doi.org/10.1093/jxb/47.12.1905

Högberg, K. A., Bozhkov, P. V., Grönroos, R., Arnold, S. V., 2001. Critical Factors Affecting Ex Vitro Performance of Somatic Embryo Plants of Picea Abies. Scandinavian Journal Of Forest Research, 16(4), 295-304. https://doi.org/10.1080/02827580116772

Jiménez, V. M., 2001. Regulation of *In Vitro* Somatic Embryogenesis with Emphasis on The Role of Endogenous Hormones. Revista Brasileira De Fisiologia Vegetal, 13, 196-223. https://doi.org/10.1590/S0103-31312001000200008

Jiménez, V. M., 2005. Involvement of Plant Hormones and Plant Growth Regulators on *In Vitro* Somatic Embryogenesis. Plant Growth Regulation, 47(2), 91-110. https://doi.org/10.1007/s10725-005-3478-x

Kacperska, A., 2004. Sensor Types in Signal Transduction Pathways in Plant Cells Responding To Abiotic Stressors: Do They Depend On Stress Intensity? Physiologia Plantarum, 122(2), 159-168.

Loyóla-Vargas, V. M., Ochoa-Alejo, N., 2016. Somatic Embryogenesis. An Overview. In Somatic Embryogenesis: Fundamental Aspects And Applications (pp. 1-8). Springer International Publishing Switzerland. https://doi.org/10.1007/978-3-319-33705-0

Murthy, B. N. S., Murch, S. J., Saxena, P. K., 1998. Thidiazuron: A Potent Regulator of *In Vitro* Plant Morphogenesis. *In Vitro* Cellular & Developmental Biology-Plant, 34(4), 267-275. https://doi.org/10.1007/BF02822732

Nic-Can, G. I., Avilez-Montalvo, J. R., Aviles-Montalvo, R. N., Márquez-López, R. E., Mellado-Mojica, E., Galaz-Ávalos, R. M., Loyola-Vargas, V. M., 2016. The Relationship Between Stress And Somatic Embryogenesis. In Somatic Embryogenesis: Fundamental Aspects And Applications (pp. 151-170). Springer International Publishing Switzerland. https://doi.org/10.1007/978-3-319-33705-0

Patnaik, D., Mahalakshmi, A., Khurana, P., 2005. Effect of Water Stress and Heavy Metals on Induction of Somatic Embryogenesis In Wheat Leaf Base Cultures. Indian journal of experimental biology, 43(8), 740-745.

Potters, G., Pasternak, T. P., Guisez, Y., Palme, K. J., Jansen, M. A., 2007. Stress-Induced Morphogenic Responses: Growing Out of Trouble? Trends In Plant Science, 12(3), 98-105.

Rode, C., Lindhorst, K., Braun, H. P., Winkelmann, T., 2012. From Callus To Embryo: A Proteomic View on The Development And Maturation of Somatic Embryos in Cyclamen Persicum. Planta, 235(5), 995-1011. https://doi.org/10.1007/s00425-011-1554-1

Sharma, C., Kumari, T., Pant, G., Bajpai, V., Srivastava, M., Mitra, K., Arya, K. R., 2015. Plantlet Formation via Somatic Embryogenesis and LC-ESI-Q-TOF MS Determination of Secondary Metabolites In Butea Monosperma (Lam.) Kuntze. Acta Physiologiae Plantarum, 37(11), 1-10. https://doi.org/10.1007/s11738-015-1973-x

Smith, D. L., & Krikorian, A. D., 1990. Somatic Embryogenesis of Carrot In Hormone-Free Medium: External Ph Control Over Morphogenesis. American Journal Of Botany, 77, 1634–1647. https://doi.org/10.1002/j.1537-2197.1990.tb11403.x

Thomas, T. L., 1993. Gene Expression During Plant Embryogenesis And Germination: An Overview. The Plant Cell, 5(10), 1401. https://doi.org/10.1105/tpc.5.10.1401

Tütüncü, M., Özcan, M., Mendi, Y. Y. 2019. Bazı siklamen türlerinde farklı doku kültürü ortamlarının gynogenesis üzerine etkileri. Anadolu Tarım Bilimleri Dergisi, 34(3), 239-249. https://doi.org/10.7161/omuanajas.529247

Vázquez-Flota, F. A., Monforte-González, M., De Lourdes Miranda-Ham, M., 2016. Application of Somatic Embryogenesis To Secondary Metabolite-Producing Plants. In Somatic Embryogenesis: Fundamental Aspects And Applications (pp. 455-469). Springer, Cham.

Von Arnold, S., Sabala, I., Bozhkov, P., Dyachok, J., Filonova, L., 2002. Developmental Pathways of Somatic Embryogenesis. Plant Cell Tiss. Organ Cult., 69, 233–249. https://doi.org/10.1023/A:1015673200621

Zavattieri, M. A., Frederico, A. M., Lima, M., Sabino, R., Arnholdt-Schmitt, B., 2010. Induction Of Somatic Embryogenesis As An Example Of Stress-Related Plant Reactions. Electronic Journal Of Biotechnology, 13(1), 12-13. http://dx.doi.org/10.4067/S0717-34582010000100012

Zhang, B. H., Feng, R., Liu, F., Wang, Q., 2001. High Frequency Somatic Embryogenesis And Plant Regeneration of An Elite Chinese Cotton Variety. Botanical Bulletin Of Academia Sinica, 42. 9-16.

Zimmerman, J. L., 1993. Somatic Embryogenesis: A Model For Early Development In Higher Plants. The Plant Cell, 5(10), 1411. http://dx.doi.org/10.1105/tpc.5.10.1411

Bölüm 7

SENTETİK TOHUM TEKNOLOJİSİ

M. Uğur KAHRAMAN [1]

7. GİRİŞ

Sentetik tohumlar veya yapay tohumlar, sürgün tomurcukları, koltuk altı tomurcukları, somatik embriyolar, sürgün uçları, hücre kümeleri gibi enkapsüle edilmiş, *in vitro* veya *ex vitro* koşullarda tam bir bitki haline getirilebilen ve soğuk depolamadan sonra canlılıklarını koruma potansiyeline sahip yapılardır (Magray ve ark., 2017; Rihan ve ark., 2017). Önceki yıllarda sentetik tohumlar somatik embriyoların kaplanmasıyla üretilirken, son yıllarda farklı bitki dokularının kaplanmasıyla da üretilmeye başlanmıştır (Danso ve Ford-Lloyd, 2003; Rai ve ark., 2008; Sahu ve ark., 2025). Son yıllarda, özellikle düşük tohum canlılığına, çekirdeksiz meyve ve zayıf çimlenme oranlarına sahip bitkiler ve çimlenme için mikorizal-mantar simbiyozuna bağlı bitkiler için sentetik tohum teknolojisi ile yapay tohum üretimine büyük ilgi olmuştur (Rai ve ark., 2008; Gantait ve ark., 2015). Ayrıca, sentetik tohum teknolojisi genotiplerin seçiminde, seçkin bitki materyallerinin çoğaltılması, nesli tükenmekte olan, nadir ve ticari olarak önemli bitkilerin doku kültürü ortamında çoğaltılmasında kullanılır (Naik ve Chand, 2006; Gantait ve ark., 2015; Deja, 2022; Abd El-Zaher ve ark., 2024). Enkapsülasyon teknolojisi kullanımının kolay ve düşük maliyetli olması ile beraber bitki materyallerinin kısa, orta ve uzun vadeli olarak depolanmasına, aynı zamanda bitki materyalinin ulusal ve uluslararası laboratuvarlar arasında taşınmasına ve değiştirilmesine olanak sağlar (Rai ve ark., 2009; Parveen ve Shahzad, 2014). Sentetik tohum teknolojisi tıbbi aromatik bitkiler, süs bitkileri, sebze ve meyveler, tarla bitkileri ve orman ağaçları gibi farklı bitki türlerinde uygulanmaktadır. Bu teknolojinin başarılı bir şekilde uygulanabilmesi için bitki materyalinin seçimi, matris hazırlama, enkapsülasyon işlemi, kullanım alanları ve enkapsülasyon tiplerinin belirlenmesi son derece önem arz etmektedir (Şekil 7.1).

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7.6 Gelecek Yıllardaki Beklentiler

Sentetik tohumlar ülkeler arası genetik kaynakların değişimi ve muhafazası için kullanılabilecek en önemli tekniklerden biri olduğundan, son yıllarda bilim dünyasının ilgisini çekmiştir. Bununla birlikte, bu tekniğin ticarileştirilmesi için birkaç sınırlayıcı faktörün çözülmesi gerekir. Yüksek rejenerasyon kabiliyetine sahip sentetik tohumların uygun maliyette büyük ölçekli üretimi bu metodun ticarileşmesi için atılacak ilk adımlardır. Sentetik tohumların üretimi için en uygun bitki materyali somatik embriyolardır. Ancak yaşlanan kültürlerde embriyojenik potansiyelin kaybolması, erken çimlenme, kurumaya olan tolerans eksikliği ve yapısal anormallikler sentetik tohum üretimindeki sınırlamalardır (Naik ve Chand, 2006; Hung ve Trueman, 2012). Sentetik tohum üretiminin karşılaştığı sorunları çözmek için bu alanda daha fazla araştırmaya ihtiyaç vardır. Ayrıca, sentetik tohumların enkapsülasyon ve rejenerasyon yöntemlerinin otomasyonu yoluyla seri üretimi için ileriki yıllarda ek araştırmalara ihtiyaç vardır ve bu araştırmaların sentetik tohum üretim maliyetini azaltması beklenmektedir (Pintos ve ark., 2008; Güler ve Gürel, 2024). Sentetik tohumların seri ve optimize edilmiş üretimi somatik embriyo oluşturamayan bitki türlerine ait embriyojenik olmayan propagüllerin geliştirilmesine de yardımcı olabilir. Embriyojenik olmayan propagüllerin kullanımı için bir başka sınırlama, özellikle odunsu bitkilerdeki köklenme zorluğudur, bu nedenle bu konuda daha fazla araştırma ve incelemeye gerek vardır (Hung ve Trueman, 2012).

Çeşitli bitki türlerinin sentetik tohumlarının üretimi için mevcut protokollerin optimizasyonu ve iyileştirilmesi için de daha fazla araştırmaya gerek vardır. Bunun yanında, genetik kaynakların kriyoprezervasyonu sentetik tohumlar için ana potansiyel uygulamalardan biri olarak kabul edilir. Bu nedenle, bu teknik umut verici bir gelecek vadetmekle beraber bu alanda daha fazla araştırmaya ihtiyaç vardır (Sahu ve ark., 2025).

KAYNAKLAR

Abbasi, F., Majd, A., Farahvash, F., Nejadsattari, T., Tarinejad, A., 2020. Synthetic seed technology for encapsulation and regrowth of *in vitro*-derived shoot-tips and somatic embryos of *Asparagus officinalis* L. Nexo Scientific Journal, 33(02), 276–285.

Abd El-Zaher, M. H., Abd El-Wahab, S. M., Saleh, S. S., Al-Maghrabi, N. N. M., 2024. Synthetic seeds production by encapsulated somatic embryo of white olive cultivar. *J. Glob. Innov. Agric. Sci.*, 12, 1109–1114.

Adhikari, S., Bandyopadhyay, T. K., Ghosh, P., 2014. Assessment of genetic stability of *Cucumis sativus* L. regenerated from encapsulated shoot tips. *Scientia Horticulturae*, 170, 115–122.

Agbidinoukoun, A., Doussoh, A., Soussou Dangou, J., Ahanhanzo, C., Engelmann, F., 2018. Used of encapsulation-dehydration technique for short-term preservation of endangered sweet potato (*Ipomoea batatas*) cultivars. *In Vitro Cellular and Developmental Biology – Plant*, 54(Suppl. 1), S40–S41.

Ahmad, N., Anis, M., 2010. Direct plant regeneration from encapsulated nodal segments of *Vitex negundo*. *Biologia Plantarum*, 54(4), 748–752.

Ahmad, N., Faisal, M., Fatima, N., Anis, M., 2012. Encapsulation of microcuttings for propagation and short-term preservation in *Ruta graveolens* L.: a plant with high medicinal value. *Acta Physiologiae Plantarum*, 34(6), 2303–2310.

Ahmed, M. R., Anis, M., Al-Etta, H. A., 2015. Encapsulation technology for short-term storage and germplasm exchange of *Vitex trifolia* L. *Rendiconti Lincei*, 26(2), 133–139.

Ai, P. F., Lu, L. P., Song, J. J., 2012. Cryopreservation of *in vitro*-grown shoot-tips of *Rabdosia rubescens* by encapsulation-dehydration and evaluation of their genetic stability. *Plant Cell, Tissue and Organ Culture*, 108(3), 381–387.

Al-Abdallat, A. M., Shibli, R. A., Akash, M. W., Rabbaa, M., Al-Qudah, T., 2017. *In vitro* preservation of transgenic tomato (*Solanum lycopersicum* L.) plants overexpressing the stress-related *SlAREB1* transcription factor. *International Journal of Molecular Sciences*, 18(7), 1477.

Alatar, A. A., Faisal, M., 2012. Encapsulation of *Rauvolfia tetraphylla* microshoots as artificial seeds and evaluation of genetic fidelity using RAPD and ISSR markers. *Journal of Medicinal Plants Research*, 6(7), 1367–1374.

Alatar, A. A., Ahmad, N., Javed, S. B., Abdel-Salam, E. M., Basahi, R., Faisal, M., 2017. Two-way germination system of encapsulated clonal propagules of *Vitex trifolia* L.: an important medicinal plant. *The Journal of Horticultural Science and Biotechnology*, 92(2), 175–182.

Ananthan, R., Mohanraj, R., Bai, V. N., 2018. *In vitro* regeneration, production, and storage of artificial seeds in *Ceropegia barnesii*, an endangered plant. *In Vitro Cellular & Developmental Biology – Plant*, 54(5), 553–563.

Aquea, F., Poupin, M. J., Matus, J. T., Gebauer, M., Medina, C., Arce-Johnson, P., 2008. Synthetic seed production from somatic embryos of *Pinus radiata*. *Biotechnology Letters*, 30(10), 1847–1852.

Arias-Peez, E., Lecona-Guzmán, C. A., Gutiérrez-Miceli, F. A., Montes-Molina, J. A., Ruiz-Lau, N., 2021. Encapsulation of immature somatic embryos of *Coffea arabica* L. for *in vitro* preservation. *Phyton*, 90(6), 1741.

Asadi, R., Abdollahi, M. R., Moosavi, S. S., Mirzaie-Asl, A., 2022. Alginate encapsulation of micro-cuttings in endangered *Satureja khuzistanica* species: a promising method for obtaining genetically stable plants with high rosmarinic acid content. *Plant Cell, Tissue and Organ Culture*, 151(2), 307–320.

Attia, A. O., Ismail, I. A., Dessoky, E. D. S., Alotaibi, S. S., 2018. Synthetic seeds as *in vitro* conservation method for Al-Taif rose plant (*Rosa damascena* trigintipetala Dieck). *Bioscience Research*, 15(2), 1113–1119.

Azad, M. Z., Kaviani, B., Sedaghathoor, S., 2024. Cold storage and cryopreservation by encapsulation-dehydration of *Cephalanthera rubra* (L.) Rich., a threatened orchid species. *Acta Agrobotanica*, 77, 189312.

Badrelden, A., 2018. New approaches for reducing the cost of the synthetic seeds storage using sugarcane bagasse and different additives to the gel matrix for sugarcane plant: in vitro. *Egyptian Journal of Botany*, 58(1), 1–10.

Baskaran, P., Kumari, A., Van Staden, J., 2015. Embryogenesis and synthetic seed production in *Mondia whitei*. *Plant Cell, Tissue and Organ Culture*, 121(1), 205–214.

Baskaran, P., Kumari, A., Van Staden, J., 2018. *In vitro* propagation via organogenesis and synthetic seeds of *Urginea altissima* (Lf) Baker: a threatened medicinal plant. *3 Biotech*, 8(1), 18.

Behera, B., Behera, S., 2021. Regeneration of plants from alginate-encapsulated axenic nodal segments of *Paederia foetida* L. – a medicinally important and vulnerable plant species. *Journal of Plant Biotechnology*, 48(4), 255–263.

Bertsouklis, K., Tsopela, S., 2023. *In vitro* propagation of three populations of the endangered, Greek endemic *Cerastium candidissimum* and short-term storability of alginate-encapsulated shoot explants for exploitation and conservation. *Horticulturae*, 9(2), 273.

Bi, W. L., Pan, C., Hao, X. Y., Cui, Z. H., Kher, M. M., Marković, Z., da Silva, J. A. T., 2017. Cryopreservation of grapevine (*Vitis* spp.)—a review. *In Vitro Cellular & Developmental Biology – Plant*, 53(5), 449–460.

Bonnart, R., Volk, G. M., 2010. Increased efficiency using the encapsulation-dehydration cryopreservation technique for *Arabidopsis thaliana*. *CryoLetters*, 31(2), 95–100.

Bruňáková, K., Čellárová, E., 2016. Conservation strategies in the genus *Hypericum* via cryogenic treatment. *Frontiers in Plant Science*, 7, 558.

Cartes, P., Castellanos, H., Ríos, D., Sáez, K., Spierccolli, S., Sánchez, M., 2009. Encapsulated somatic embryos and zygotic embryos for obtaining artificial seeds of rauli-beech (*Nothofagus alpina* (Poepp. & Endl.) Oerst.). *Chilean Journal of Agricultural Research*, 69(1), 112–118.

Charoensub, R., Hirai, D., Sakai, A., 2004. Cryopreservation of *in vitro*-grown shoot tips of cassava by encapsulation-vitrification method. *CryoLetters*, 25(1), 51–58.

Cheruvathur, M. K., Kumar, G. K., Thomas, T. D., 2013. Somatic embryogenesis and synthetic seed production in *Rhinacanthus nasutus* (L.) Kurz. *Plant Cell, Tissue and Organ Culture*, 113(1), 63–71.

Chithra, M., Martin, K. P., Sunandakumari, C., Madhusoodanan, P. V., 2005. Somatic embryogenesis, encapsulation, and plant regeneration of *Rotula aquatica* Lour., a rare rheophytic woody medicinal plant. *In Vitro Cellular & Developmental Biology – Plant*, 41(1), 28–31.

Choudhary, R., Malik, S. K., Chaudhury, R., 2018. Development of an efficient cryoconservation protocol for Himalayan mulberry (*Morus laevigata* Wall. ex Brandis) using dormant axillary buds as explants.

Ciringer, T., Martín, C., Šajna, N., Kaligarič, M., Ambrožič-Dolinšek, J., 2018. Cryopreservation of an endangered *Hladnikia pastinacifolia* Rchb. by shoot tip encapsulation-dehydration and encapsulation-vitrification. *In Vitro Cellular & Developmental Biology – Plant*, 54(6), 565–575.

Clavero-Ramirez, I., Galvez-Farfan, J., Lopez-Aranda, J. M., Gonzalez-Benito, M. E., 2005. Apex cryopreservation of several strawberry genotypes by two encapsulation-dehydration methods. *CryoLetters*, 26(1), 17–24.

Coelho, N., González-Benito, M. E., Romano, A., 2014. Approaches for the cryopreservation of *Plantago algarbiensis*, a rare endemic species of the Algarve. *CryoLetters*, 35(6), 521–529.

Cordeiro, S. Z., Simas, N. K., Henriques, A. B., Sato, A., 2014. *In vitro* conservation of *Mandevilla moricandiana* (Apocynaceae): short-term storage and encapsulation–dehydration of nodal segments. *In Vitro Cellular & Developmental Biology – Plant*, 50(3), 326–336.

Cuko, B., Sota, V., Bekheet, S., Kongjika, E., 2021. *In vitro* propagation and encapsulation of *Galatella albanica* Degen shoot tips. *Journal of Applied Biological Sciences*, 15(2), 157–168.

da Silva, J. A. T., 2012. Production of synseed for hybrid *Cymbidium* using protocorm-like bodies. *Journal of Fruit and Ornamental Plant Research*, 20(2), 135–146.

Danso, K. E., Ford-Lloyd, B. V., 2003. Encapsulation of nodal cuttings and shoot tips for storage and exchange of cassava germplasm. *Plant Cell Reports*, 21(8), 718–725.

de Oliveira Prudente, D., de Souza, L. B., Paiva, R., 2019. Synthetic seeds: prospects and advances in cryopreservation. In *Synthetic Seeds*, 417–437. Springer, Cham.

Deja, A. K., 2022. Synthetic seeds technology of *Salvia officinalis* as a method for short-term storage and *in vitro* propagation of valuable genotypes. *Herba Polonica*, 68(4), 25–29.

Devi, P. S., Satyanarayana, B., Arundhati, A., Rao, T. R., 2014. Plant regeneration from encapsulated nodal segments of *Sterculia urens* Roxb., an endangered gum-yielding tree. *Journal of Crop Science and Biotechnology*, 17(1), 21–25.

Dhir, R., Shekhawat, G. S., 2013. Production, storability and morphogenic response of alginate encapsulated axillary meristems and genetic fidelity evaluation of *in vitro* regenerated *Ceropegia bulbosa*: a pharmaceutically important threatened plant species. *Industrial Crops and Products*, 47, 139–144.

Dönmez, D., 2022. Regeneration of plants from alginate-encapsulated shoot tips of myrtle (*Myrtus communis* L.). *Erwerbs-Obstbau*, 64(2), 307–314.

Ekinci, H., Çiftçi, Y. Ö., Nadarajan, J., 2019. Medium- and long-term conservation of ornamental plants using synthetic seed technology. In *Synthetic Seeds*, 259–281. Springer, Cham.

Engelmann, F., Takagi, H., 2000. Cryopreservation of tropical plant germplasm: current research progress and applications. JIRCAS, Tsukuba and IPGRI, Rome.

Faisal, M., Anis, M., 2007. Regeneration of plants from alginate-encapsulated shoots of *Tylophora indica* (Burm. f.) Merrill, an endangered medicinal plant. *The Journal of Horticultural Science and Biotechnology*, 82(3), 351–354.

Faisal, M., Alatar, A. A., Ahmad, N., Anis, M., Hegazy, A. K., 2012. Assessment of genetic fidelity in *Rauvolfia serpentina* plantlets grown from synthetic (encapsulated) seeds following *in vitro* storage at 4 °C. *Molecules*, 17(5), 5050–5061.

Fatima, N., Ahmad, N., Anis, M., Ahmad, I., 2013. An improved *in vitro* encapsulation protocol, biochemical analysis and genetic integrity using DNA-based molecular markers in regenerated plants of *Withania somnifera* L. *Industrial Crops and Products*, 50, 468–477.

Gamez-Pastrana, R., Martinez-Ocampo, Y., Beristain, C. I., Gonzalez-Arnao, M. T., 2004. An improved cryopreservation protocol for pineapple apices using encapsulation-vitrification. *Cryo-Letters*, 25(6), 405–414.

Gantait, S., Kundu, S., Ali, N., Sahu, N. C., 2015. Synthetic seed production of medicinal plants: a review on influence of explants, encapsulation agent and matrix. *Acta Physiologiae Plantarum*, 37(5), 98.

Gantait, S., Kundu, S., Yeasmin, L., Ali, M. N., 2017a. Impact of differential levels of sodium alginate, calcium chloride and basal media on germination frequency of genetically true artificial seeds of *Rauvolfia serpentina* (L.) Benth. ex Kurz. *Journal of Applied Research on Medicinal and Aromatic Plants*, 4, 75–81.

Gantait, S., Vijayan, J., Majee, A., 2017b. Artificial seed production of *Tylophora indica* for interim storing and swapping of germplasm. *Horticultural Plant Journal*, 3(1), 41–46.

Ghanbarali, S., Abdollahi, M. R., Zolnorian, H., Moosavi, S. S., Seguí-Simarro, J. M., 2016. Optimization of the conditions for production of synthetic seeds by encapsulation of axillary buds derived from minituber sprouts in potato (*Solanum tuberosum*). *Plant Cell, Tissue and Organ Culture*, 126(3), 449–458.

González-Arnao, M. T., Ravelo, M., Villavicencio, C., Martínez-Montero, M. E., Engelmann, F., 1998. Cryopreservation of pineapple (*Ananas comosus*) apices. *CryoLetters*, 19, 375–382.

González-Benito, M. E., Kremer, C., Ibáñez, M. A., Martín, C., 2016. Effect of antioxidants on the genetic stability of cryopreserved mint shoot tips by encapsulation–dehydration. *Plant Cell, Tissue and Organ Culture*, 127(2), 359–368.

Grzegorczyk, I., Wysokinska, H., 2011. A protocol for synthetic seeds from *Salvia officinalis* L. shoot tips. *Acta Biologica Cracoviensia Series Botanica*, 53(1), 80–85.

Gupta, A. K., Rai, M. K., Phulwaria, M., Agarwal, T., Shekhawat, N. S., 2014. *In vitro* propagation, encapsulation, and genetic fidelity analysis of *Terminalia arjuna*: a cardioprotective medicinal tree. *Applied Biochemistry and Biotechnology*, 173(6), 1481–1494.

Güler, B., Gürel, A., 2023. A research on the production, storage and germination of synthetic seeds in tea plant (*Camellia sinensis* [L.] O. Kuntze). *Journal of Scientific Reports-A*, 57, 68–80.

Halmagyi, A., Deliu, C., 2011. Cryopreservation of redwood (*Sequoia sempervirens* (D. Don.) Endl.) shoot apices by encapsulation-dehydration. *Contributii Botanice*, 46.

Haque, S. M., Ghosh, B., 2016. High-frequency somatic embryogenesis and artificial seeds for mass production of true-to-type plants in *Ledebouria revoluta*: an important cardioprotective plant. *Plant Cell, Tissue and Organ Culture*, 127(1), 71–83.

Haque, S. M., Ghosh, B., 2017. Regeneration of cytologically stable plants through dedifferentiation, redifferentiation, and artificial seeds in *Spathoglottis plicata* Blume (Orchidaceae). *Horticultural Plant Journal*, 3(5), 199–208.

Hatzilazarou, S., Kostas, S., Nendou, T., Economou, A., 2021. Conservation, regeneration and genetic stability of regenerants from alginate-encapsulated shoot explants of *Gardenia jasminoides* Ellis. *Polymers*, 13(10), 1666.

Hirai, D., Sakai, A., 1999. Cryopreservation of *in vitro*-grown meristems of potato (*Solanum tuberosum* L.) by encapsulation-vitrification. *Potato Research*, 42(1), 153–160.
Hu, G., Dong, N., Zhou, Y., Ye, W., Yu, S., 2015. *In vitro* regeneration protocol for synthetic seed production in upland cotton (*Gossypium hirsutum* L.). *Plant Cell, Tissue and Organ Culture*, 123(3), 673–679.
Hung, C. D., Trueman, S. J., 2011. Encapsulation technology for short-term preservation and germplasm distribution of the African mahogany *Khaya senegalensis*. *Plant Cell, Tissue and Organ Culture*, 107(3), 397–405.
Hung, C. D., Trueman, S. J., 2012. Alginate encapsulation of shoot tips and nodal segments for short-term storage and distribution of the eucalypt *Corymbia torelliana* × *C. citriodora*. *Acta Physiologiae Plantarum*, 34(1), 117–128.
Ipekci, Z., Gozukirmizi, N., 2003. Direct somatic embryogenesis and synthetic seed production from *Paulownia elongata*. *Plant Cell Reports*, 22(1), 16–24.
Javed, S. B., Alatar, A. A., Anis, M., Faisal, M., 2017. Synthetic seeds production and germination studies for short-term storage and long-distance transport of *Erythrina variegata* L.: a multipurpose tree legume. *Industrial Crops and Products*, 105, 41–46.
Jeon, S. M., Arun, M., Lee, S. Y., Kim, C. K., 2015. Application of encapsulation-vitrification in combination with air dehydration enhances cryotolerance of *Chrysanthemum morifolium* shoot tips. *Scientia Horticulturae*, 194, 91–99.
Kami, D., Kikuchi, T., Sugiyama, K., Suzuki, T., 2009. Cryopreservation of shoot apices of cranberry and highbush blueberry *in vitro* cultures. *Cryobiology*, 59(3), 411–412.
Kamińska, M., Gołębiewski, M., Tretyn, A., Trejgell, A., 2018. Efficient long-term conservation of *Taraxacum pieninicum* synthetic seeds in slow growth conditions. *Plant Cell, Tissue and Organ Culture*, 132(3), 469–478.
Kaya, E., Souza, F. V. D., 2017. Comparison of two PVS2-based procedures for cryopreservation of commercial sugarcane (*Saccharum* spp.) germplasm and confirmation of genetic stability after cryopreservation using ISSR markers. *In Vitro Cellular & Developmental Biology – Plant*, 53(4), 410–417.
Khabbazi, S. D., Özmen, C. Y., Ergül, A., 2019. Synthetic seeds of wild beet: basic concepts and related methodologies. In *Synthetic Seeds*, 377–396. Springer, Cham.
Khan, M. I., Ahmad, N., Anis, M., Alatar, A. A., Faisal, M., 2018. *In vitro* conservation strategies for the Indian willow (*Salix tetrasperma* Roxb.), a vulnerable tree species via propagation through synthetic seeds. *Biocatalysis and Agricultural Biotechnology*, 16, 17–21.
Khanam, M. N., Javed, S. B., Ahmad, N., Anis, M., 2021. Encapsulation of nodal segments of *Allamanda cathartica* for short-term storage and germplasm exchange. *Plant Cell, Tissue and Organ Culture*, 145(2), 435–443.
Kim, M. A., Park, J. K., 2002. High-frequency plant regeneration of garlic (*Allium sativum* L.) calli immobilized in calcium alginate gel. *Biotechnology and Bioprocess Engineering*, 7(4), 206.
Kitto, S. L., Janick, J., 1985. Hardening increases survival of synthetically coated asexual embryos of carrot. *Journal of the American Society for Horticultural Science*, 110, 283–286.
Kocak, M., Sevindik, B., Izgu, T., Tutuncu, M., Mendi, Y. Y., 2019. Synthetic seed production of flower bulbs. In *Synthetic Seeds*, 283–299. Springer, Cham.
Kulus, D., Abratowska, A., 2017. (CRYO) conservation of *Ajania pacifica* (Nakai) Bremer et Humphries shoot tips via encapsulation-dehydration technique. *CryoLetters*, 38(5), 387–398.
Kulus, D., Serocka, M., Mikuła, A., 2018. Effect of various preculture and osmotic dehydration conditions on cryopreservation efficiency and morphogenetic response of chrysanthemum shoot tips. *Acta Scientiarum Polonorum Hortorum Cultus*, 17, 17.
Kulus, D., 2020. Shoot tip cryopreservation of *Lamprocapnos spectabilis* (L.) Fukuhara using different approaches and evaluation of stability on the molecular, biochemical, and plant architecture levels. *International Journal of Molecular Sciences*, 21(11), 3901.

Kumar, M. A., Vakeswaran, V., Krishnasamy, V., 2005. Enhancement of synthetic seed conversion to seedlings in hybrid rice. *Plant Cell, Tissue and Organ Culture*, 81(1), 97–100.

Kumar, G. K., Thomas, T. D., 2012. High-frequency somatic embryogenesis and synthetic seed production in *Clitoria ternatea* Linn. *Plant Cell, Tissue and Organ Culture*, 110(1), 141–151.

Kundu, S., Sutradhar, M., Salma, U., 2019. Synthetic seed technology in forest trees: a promising technology for conservation and germplasm exchange. In *Synthetic Seeds*, 241–258. Springer, Cham.

Gantait, S., Mitra, M., 2019. Applications of synthetic seed technology for propagation, storage, and conservation of orchid germplasms. In *Synthetic Seeds*, 301–321. Springer, Cham.

Li, J. W., Hosokawa, M., Nabeshima, T., Motoki, K., Yamada, H., Wang, Q. C., 2019. Cryopreservation of viroid-infected chrysanthemum shoot tips. *Scientia Horticulturae*, 244, 1–9.

Lynch, P. T., Souch, G. R., Zámeník, J., Harding, K., 2016. Optimization of water content for the cryopreservation of *Allium sativum in vitro* cultures by encapsulation-dehydration. *CryoLetters*, 37(5), 308–317.

Magray, M. M., Wani, K. P., Chatto, M. A., Ummyiah, H. M., 2017. Synthetic seed technology. *Int J Curr Microbiol App Sci*, 6(11), 662–674.

Mahfeli, M., Minaei, S., Fadavi, A., Daylami, S. D., 2022. Precision measurement of physical properties of orchid synthetic seeds produced under various encapsulation conditions using Image J platform. *Industrial Crops and Products*, 187, 115364.

Malabadi, R. B., Van Staden, J., 2005. Storability and germination of sodium alginate encapsulated somatic embryos derived from the vegetative shoot apices of mature *Pinus patula* trees. *Plant Cell, Tissue and Organ Culture*, 82(3), 259–265.

Mandal, J., Pattnaik, S., Chand, P. K., 2000. Alginate encapsulation of axillary buds of *Ocimum americanum* L. (hoary basil), *O. basilicum* L. (sweet basil), *O. gratissimum* L. (shrubby basil), and *O. sanctum* L. (sacred basil). *In Vitro Cellular & Developmental Biology – Plant*, 36(4), 287–292.

Manjkhola, S., Dhar, U., Joshi, M., 2005. Organogenesis, embryogenesis, and synthetic seed production in *Arnebia euchroma*—a critically endangered medicinal plant of the Himalaya. *In Vitro Cellular & Developmental Biology – Plant*, 41(3), 244–248.

Maslanka, M., Szewczyk, A., 2021. Droplet-vitrification cryopreservation of *Tulipa tarda* Stapf. apical meristems. *Plant Cell, Tissue and Organ Culture*, 144(1), 91–95.

Matsumoto, T., Sakai, A., Takahashi, C., Yamada, K., 1995. Cryopreservation of *in vitro*-grown apical meristems of wasabi (*Wasabia japonica*) by encapsulation-vitrification method. *CryoLetters* (United Kingdom).

Merhy, T. S. M., Vianna, M. G., Garcia, R. O., Pacheco, G., Mansur, E., 2014. Cryopreservation of *Passiflora pohlii* nodal segments and assessment of genetic stability of regenerated plants. *CryoLetters*, 35(3), 204–215.

Micheli, M., Benelli, C., 2019. Synthetic seeds of two aquatic plants. In *Synthetic Seeds*, 233–239. Springer, Cham.

Micheli, M., Hafiz, I. A., Standardi, A., 2007. Encapsulation of *in vitro*-derived explants of olive (*Olea europaea* L. cv. Moraiolo): II. Effects of storage on capsule and derived shoots performance. *Scientia Horticulturae*, 113(3), 286–292.

Micheli, M., Regni, L., da Silva, D. F., 2022. Encapsulation in calcium alginate of nodes from stolons of *Mentha spicata* L. *Horticulturae*, 8(5), 456.

Ming-Hua, Y., Sen-Rong, H., 2010. A simple cryopreservation protocol of *Dioscorea bulbifera* L. embryogenic calli by encapsulation-vitrification. *Plant Cell, Tissue and Organ Culture*, 101(3), 349–358.

Mishra, N., Supe, U., Sahu, H., 2025. Callus formation from encapsulated explants of plant *Diplocyclos palmatus* (Shivlingi). *African Journal of Biomedical Research*, 28, 42–47.

Moges, A. D., Shibli, R. A., Karam, N. S., 2004. Cryopreservation of African violet (*Saintpaulia ionantha* Wendl.) shoot tips. *In Vitro Cellular & Developmental Biology – Plant*, 40(4), 389–395.

Mohamed, A. E. S., Gomaa, A. H., Abd-El-Zaher, M. H., Reda, A. E. S. A. E. H., Girgis, N. D., 2023. Protocol for plant regeneration from encapsulated somatic embryos of *Citrus sinensis* L. *Bulletin of the National Research Centre*, 47(1), 101.

Mohanty, P., Nongkling, P., Das, M. C., Kumaria, S., Tandon, P., 2013. Short-term storage of alginate-encapsulated protocorm-like bodies of *Dendrobium nobile* Lindl.: an endangered medicinal orchid from North-east India. *3 Biotech*, 3(3), 235–239.

Naik, S. K., Chand, P. K., 2006. Nutrient-alginate encapsulation of *in vitro* nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange. *Scientia Horticulturae*, 108(3), 247–252.

Negahdar, N., Hashemabadi, D., Kaviani, B., 2021. *In vitro* conservation and cryopreservation of *Buxus sempervirens* L., a critically endangered ornamental shrub. *Russian Journal of Plant Physiology*, 68(4), 661–668.

Nishitha, I. K., Martix, K. P., Beegum, A. S., Madhusoodanan, P. V., 2006. Micropropagation and encapsulation of medicinally important *Chonemorpha grandiflora*. *In Vitro Cellular & Developmental Biology – Plant*, 42(5), 385–388.

Ouzhand, B., Mohayeji, M., Pourseyedi, S., Abdolshahi, R., 2023. Dormancy breaking, indirect somatic embryogenesis, and encapsulation of somatic embryos in Black Zira. *Plant Cell, Tissue and Organ Culture*, 155(1), 197–208.

Ozudogru, E. A., Kaya, E., 2012. Cryopreservation of *Thymus cariensis* and *T. vulgaris* shoot tips: comparison of three vitrification-based methods. *CryoLetters*, 33(5), 363–375.

Özdemir, M. Ş., 2021. Bazı *Colchicum* türlerinde somatik embriyogenesis, histolojik analizler ve sentetik tohum üretiminin araştırılması [Yüksek lisans tezi, Çukurova Üniversitesi]. YÖK Ulusal Tez Merkezi. https://tez.yok.gov.tr/

Özkaya, A., 2021. Bazı *Colchicum* türlerinde somatik embriyogenesis ve sentetik tohum üretim protokollerinin oluşturulması [Doktora tezi, Çukurova Üniversitesi]. YÖK Ulusal Tez Merkezi. https://tez.yok.gov.tr/

Panis, B., Totte, N., Van Nimmen, K., Withers, L. A., Swennen, R., 1996. Cryopreservation of banana (*Musa* spp.) meristem cultures after preculture on sucrose. *Plant Science*, 121(1), 95–106.

Parveen, S., Shahzad, A., 2014. Encapsulation of nodal segments of *Cassia angustifolia* Vahl. for short-term storage and germplasm exchange. *Acta Physiologiae Plantarum*, 36(3), 635–640.

Pence, V. C., Philpott, M., Culley, T. M., Plair, B., Yorke, S. R., Lindsey, K., Ballesteros, D., 2017. Survival and genetic stability of shoot tips of *Hedeoma todsenii* R.S. Irving after long-term cryostorage. *In Vitro Cellular & Developmental Biology – Plant*, 53(4), 328–338.

Phanomchai, S., Bodhipadma, K., Noichinda, S., Leung, D. W., 2022. Short-term storability of alginate-encapsulated Persian violet microshoots for germplasm exchange. *Plants*, 11(2), 185.

Phunchindawan, M., Hirata, K., Sakai, A., Miyamoto, K., 1997. Cryopreservation of encapsulated shoot primordia induced in horseradish (*Armoracia rusticana*) hairy root cultures. *Plant Cell Reports*, 16(7), 469–473.

Pintos, B., Bueno, M. A., Cuenca, B., Manzanera, J. A., 2008. Synthetic seed production from encapsulated somatic embryos of cork oak (*Quercus suber* L.) and automated growth monitoring. *Plant Cell, Tissue and Organ Culture*, 95(2), 217.

Prakash, A. V., Nair, D. S., Alex, S., Soni, K. B., Viji, M. M., Reghunath, B. R., 2018. Calcium alginate encapsulated synthetic seed production in *Plumbago rosea* L. for germplasm exchange and distribution. *Physiology and Molecular Biology of Plants*, 24(5), 963–971.

Preetha, T. S., Hemanthakumar, A. S., Krishnan, P. N., 2021. Cryopreservation of aromatic ginger *Kaempferia galanga* L. by encapsulation-dehydration. *Notulae Scientia Biologicae*, 13(4), 11024–11024.

Qahtan, A. A., Abdel-Salam, E. M., Alatar, A. A., Wang, Q. C., Faisal, M., 2019. An introduction to synthetic seeds: production, techniques, and applications. In *Synthetic Seeds*, 1–20. Springer, Cham.

Rabba'a, M. M., Shibli, R. A., Shatnawi, M. A., 2012. Cryopreservation of *Teucrium polium* L. shoot-tips by vitrification and encapsulation-dehydration. *Plant Cell, Tissue and Organ Culture*, 110(3), 371–382.

Rafique, T., Yamamoto, S. I., Fukui, K., Tanaka, D., Arizaga, M. V., Abbas, M., Niino, T., 2016. Cryopreservation of shoot-tips from different sugarcane varieties using D cryo-plate technique. *Pakistan Journal of Agricultural Sciences*, 53(1), 151–158.

Rai, M. K., Jaiswal, V. S., Jaiswal, U., 2008. Encapsulation of shoot tips of guava (*Psidium guajava* L.) for short-term storage and germplasm exchange. *Scientia Horticulturae*, 118(1), 33–38.

Rai, M. K., Asthana, P., Singh, S. K., Jaiswal, V. S., Jaiswal, U., 2009. The encapsulation technology in fruit plants—a review. *Biotechnology Advances*, 27(6), 671–679.

Raju, C. S., Aslam, A., Shajahan, A., 2016. Germination and storability of calcium-alginate coated somatic embryos of mango ginger (*Curcuma amada* Roxb.). *Horticulture, Environment, and Biotechnology*, 57(1), 88–96.

Ramírez-Mosqueda, M. A., de la Cruz Díaz-Sánchez, M., Gómez-Merino, F. C., Hernandez-Rosas, F., Rodríguez-Lagunes, D. A., Bello-Bello, J. J., 2023. Nodal segment size and encapsulation matrix affect sugarcane bud emergence from synthetic seeds. *Sugar Tech*, 25(4), 827–833.

Ray, A., Bhattacharya, S., 2008. Storage and plant regeneration from encapsulated shoot tips of *Rauvolfia serpentina*—an effective way of conservation and mass propagation. *South African Journal of Botany*, 74(4), 776–779.

Redenbaugh, K., Nichol, J., Kossler, M. E., Paasch, B., 1984. Encapsulation of somatic embryos for artificial seed production. *In Vitro Cellular & Developmental Biology*, 20, 256–257.

Redenbaugh, K., Paasch, B. D., Nichol, J. W., Kossler, M. E., Viss, P. R., Walker, K. A., 1986. Somatic seeds: encapsulation of asexual plant embryos. *Bio/technology*, 4(9), 797–801.

Remya, M., Bai, V. N., Mutharaian, V. N., 2013. *In vitro* regeneration of *Aristolochia tagala* and production of artificial seeds. *Biologia Plantarum*, 57(2), 210–218.

Rihan, H. Z., Kareem, F., El-Mahrouk, M. E., Fuller, M. P., 2017. Artificial seeds (principle, aspects and applications). *Agronomy*, 7(4), 71.

Rohini, M. R., Malik, S. K., Choudhary, R., Kaur, S., Uchoi, A., Chaudhury, R., 2016. Storage behavior and cryopreservation studies in Indian rough lemon (*Citrus jambhiri*): a promising rootstock for long-term conservation. *Turkish Journal of Agriculture and Forestry*, 40(6), 865–873.

Saha, S., Sengupta, C., Ghosh, P., 2014. Molecular and phytochemical analyses to assess genetic stability in alginate-encapsulated microshoots of *Ocimum gratissimum* L. following *in vitro* storage. *The Nucleus*, 57(1), 33–43.

Saha, S., Sengupta, C., Ghosh, P., 2015. Encapsulation, short-term storage, conservation and molecular analysis to assess genetic stability in alginate-encapsulated microshoots of *Ocimum kilimandscharicum* Guerke. *Plant Cell, Tissue and Organ Culture*, 120(2), 519–530.

Sahu, S., Monalisa, S. P., Jigile, S., Kar, S., Parida, S. S., Swain, S. K., 2025. Exploring the potential of synthetic seeds: influence of explant, encapsulating agent and matrix, advantages and challenges. *Plant Science Today*, 2348–1900.

Saifan, S., Shibli, R. A., Al Qudah, T. S., Tahtamouni, R. W., Al–Qudah, T., 2024. Cryopreservation of *Arum palaestinum* plant callus as a strategy for mitigating extinction risks. *Current Plant Biology*, 40, 100402.

Saiprasad, G. V. S., 2001. Artificial seeds and their applications. *Resonance*, 6(5), 39–47.

Saxena, A., Shukla, M., Saxena, P., 2019. Synthetic seeds: relevance to endangered germplasm conservation *in vitro*. In *Synthetic Seeds*, 21–60. Springer, Cham.

Sgueglia, A., Frattarelli, A., Gentile, A., Urbinati, G., Lucioli, S., Germanà, M. A., Caboni, E., 2021. Cryopreservation of hazelnut (*Corylus avellana* L.) axillary buds from *in vitro* shoots using the droplet vitrification method. *Horticulturae*, 7(11), 494.

Sharaf, S. A., Shibli, R. A., Kasrawi, M. A., Baghdadi, S. H., 2012. Cryopreservation of wild Shih (*Artemisia herba-alba* Asso.) shoot-tips by encapsulation-dehydration and encapsulation-vitrification. *Plant Cell, Tissue and Organ Culture*, 108(3), 437–444.

Sharma, S., Shahzad, A., 2012. Encapsulation technology for short-term storage and conservation of a woody climber, *Decalepis hamiltonii* Wight and Arn. *Plant Cell, Tissue and Organ Culture*, 111(2), 191–198.

Sharma, S., Shahzad, A., da Silva, J. A. T., 2013. Synseed technology—a complete synthesis. *Biotechnology Advances*, 31(2), 186–207.

Sharma, P., Roy, B., 2021. Impact of encapsulation on plantlet regeneration from *in vitro* grown shoot tips of *Citrus aurantifolia* (lime). *Plant Tissue Culture and Biotechnology*, 31(1), 43–49.

Shibli, R. A., Al-Juboory, K. H., 2000. Cryopreservation of 'Nabali' olive (*Olea europaea* L.) somatic embryos by encapsulation-dehydration and encapsulation-vitrification. *CryoLetters*, 21(6), 357–366.

Shubha, P., Rashmishree, S., Hamalton, T., Khannam, A., Jyothsna, B. S., 2025. Storage of encapsulated somatic embryos of *Santalum album* Linn.: progressing towards *in vitro* conservation and mass production. *Journal of Advances in Biology & Biotechnology*, 28, 19–26.

Siddique, I., Bukhari, N. A. W., 2018. Synthetic seed production by encapsulating nodal segment of *Capparis decidua* (Forsk.), *in vitro* regrowth of plantlets and their physio biochemical studies. *Agroforestry Systems*, 92(6), 1711–1719.

Singh, S. K., Rai, M. K., Asthana, P., Sahoo, L., 2010. Alginate-encapsulation of nodal segments for propagation, short-term conservation and germplasm exchange and distribution of *Eclipta alba* (L.). *Acta Physiologiae Plantarum*, 32(3), 607–610.

Singh, S., Tripathi, M. K., Tiwari, S., Tripathi, N., Tejovathi, G., Ahuja, A., 2021. Encapsulation of nodal segments for propagation and short-term storage of giloe (*Tinospora cordifolia* Willd.): a medicinally important plant species. *Curr J Appl Sci Technol*, 40(30), 15–24.

Subrahmanyeswari, T., Laha, S., Kamble, S. N., Singh, S., Bhattacharyya, S., Gantait, S., 2023. Alginate encapsulation of shoot tips and their regeneration for enhanced mass propagation and germplasm exchange of genetically stable *Stevia rebaudiana* Bert. *Sugar Tech*, 25(3), 542–551.

Sundararaj, S. G., Agrawal, A., Tyagi, R. K., 2010. Encapsulation for *in vitro* short-term storage and exchange of ginger (*Zingiber officinale* Rosc.) germplasm. *Scientia Horticulturae*, 125(4), 761–766.

Tadda, S. A., Kui, X., Yang, H., Li, M., Huang, Z., Chen, X., Qiu, D., 2021. The response of vegetable sweet potato (*Ipomoea batatas* Lam) nodes to different concentrations of encapsulation agent and MS salts. *Agronomy*, 12(1), 19.

Tanaka, D., Niino, T., Isuzugawa, K., Hikage, T., Uemura, M., 2004. Cryopreservation of shoot apices of *in vitro* grown gentian plants: comparison of vitrification and encapsulation-vitrification protocols. *CryoLetters*, 25(3), 167–176.

Tomiczak, K., Grzyb, M., Rybczyński, J. J., Mikuła, A., 2018. Somatic embryogenesis and somatic embryo cryopreservation of the tree-fern *Cyathea delgadii* Sternb. In *Step Wise Protocols for Somatic Embryogenesis of Important Woody Plants: Volume II*, 291–303. Cham: Springer International Publishing.

Trejo-Tapia, G., León-Romero, Y., Montoya-Medina, E. B., López-Laredo, A. R., Trejo-Espino, J., 2019. Perspectives of synthetic seed technology for conservation and mass propagation of the medicinal plant *Castilleja tenuiflora* Benth. In *Synthetic Seeds*, 335–345. Springer, Cham.

Uchendu, E. E., Shukla, M. R., Reed, B. M., Saxena, P. K., 2013. An efficient method for cryopreservation of St John's wort and tobacco: role of melatonin. In *II International Symposium on Plant Cryopreservation* 1039, 233–241.

Upadhyay, R., Kashyap, S. P., Singh, C. S., Tiwari, K. N., Singh, K., Singh, M., 2014. Ex situ conservation of *Phyllanthus fraternus* Webster and evaluation of genetic fidelity in regenerates using DNA-based molecular marker. *Applied Biochemistry and Biotechnology*, 174(6), 2195–2208.

Varshney, A., Anis, M., 2014. Synseed conception for short-term storage, germplasm exchange and potentialities of regeneration genetically stable plantlets of desert date tree (*Balanites aegyptiaca*

Del.). *Agroforestry Systems*, 88(2), 321–329.

Verma, S. K., Rai, M. K., Asthana, P., Jaiswal, V. S., Jaiswal, U., 2010. *In vitro* plantlets from alginate-encapsulated shoot tips of *Solanum nigrum* L. *Scientia Horticulturae*, 124(4), 517–521.

Wang, W. G., Wang, S. H., Wu, X. A., Jin, X. Y., Chen, F., 2007. High frequency plantlet regeneration from callus and artificial seed production of rock plant *Pogonatherum paniceum* (Lam.) Hack. (Poaceae). *Scientia Horticulturae*, 113(2), 196–201.

Wang, M. R., Chen, L., da Silva, J. A. T., Volk, G. M., Wang, Q. C., 2018. Cryobiotechnology of apple (*Malus* spp.): development, progress and future prospects. *Plant Cell Reports*, 37(5), 689–709.

West, T. P., Ravindra, M. B., Preece, J. E., 2006. Encapsulation, cold storage, and growth of *Hibiscus moscheutos* nodal segments. *Plant Cell, Tissue and Organ Culture*, 87(3), 223–231.

Yücesan, B., 2019. Synseed: a new trend in seed technology. In *Synthetic Seeds*, 61–75. Springer, Cham.

Zabicki, P., Mikuła, A., Sliwinska, E., Migdałek, G., Nobis, A., Żabicka, J., Kuta, E., 2021. Cryopreservation and post-thaw genetic integrity of *Viola stagnina* Kit., an endangered species of wet habitats—a useful tool in ex situ conservation. *Scientia Horticulturae*, 284, 110056.

Zarghami, R., Pirseyedi, M., Hasrak, S., Sardrood, B. P., 2008. Evaluation of genetic stability in cryopreserved *Solanum tuberosum*. *African Journal of Biotechnology*, 7(16), 2798–2802.

Zych, M., Furmanowa, M., Krajewska-Patan, A., Łowicka, A., Dreger, M., Mendlewska, S., 2005. Micropropagation of *Rhodiola kirilowii* plants using encapsulated axillary buds and callus. *Acta Biologica Cracoviensia Series Botanica*, 47(2), 83–87.

Bölüm 8

EMBRİYO KÜLTÜRÜ

Hatıra TAŞKIN [1]

8.1 GİRİŞ

Geleneksel ıslah çalışmalarında, populasyondaki mevcut varyasyonu kullanarak veya farklı yöntemlerle varyasyonu artırarak çeşit geliştirmek hedeflenmektedir. Bu amaçla, yani varyasyon oluşturmak amacıyla, istenilen özellikleri taşıyan ebeveynler öncelikle farklı yollarla homozigotlaştırılmakta, daha sonra melezlemeler yapılmakta ve seleksiyonla yeni çeşitler geliştirilmektedir. Bu prosedür sadece tür içinde uygulanmamakta, türler veya cinsler arasında da faydalı melezlemelerin yapılması istenmektedir. Ancak, bu her zaman klasik yöntemlerle mümkün olamayabilmektedir. Döllenme öncesi meydana gelen; polenlerin stigma üzerinde çimlenememesi, polenlerin anormal çimlenmesi, polen tüpünün kısa kalması sonucu yumurtalığa ulaşamaması, yumurtalığa ulaşmadan önce polen tüpünün kaybolması ve döllenmenin gerçekleşememesi ve döllenme sonrası meydana gelen; döllenmenin gerçekleşmesi ancak zigotun bölünememesi, zigotun birkaç hücreli embriyo oluşturmak üzere bölünmesi ve daha ileri gelişme gösterememesi veya ölmesi, endospermin embriyonun gelişimini destekleyecek yapıda olmaması ve embriyonun olgunlaşamaması gibi sorunlarla verimli bir döllenme gerçekleşemeyebilmektedir (Bajaj, 1990; Uysal ve ark., 2007).

Biyoteknolojik tekniklerden birisi olan embriyo kültürü, canlı bir bitki elde etmek amacıyla steril koşullar altında aseptik bir besin ortamında olgunlaşmamış veya olgun bir zigotik embriyonun izole edilip büyütülmesini içermektedir. Teknik, embriyonun zarar görmeden izole edilmesine, uygun bir besin ortamı protokolü oluşturulmasına ve embriyonik büyüme ve gelişmenin sağlanması ile bitki oluşumuna dayanır (Bridgen, 1994). Olgunlaşmamış embriyoların kültürü ve olgun embriyoların kültürü olarak birbirinden

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8.6 SONUÇ

Bu kitap bölümünde embriyo kültürünün tanımı yapılmış, kullanım alanları, embriyo kültürü teknikleri, uygulanışı ve kültür esnasında başarıyı etkileyen faktörler ayrıntılı olarak incelenmiştir. Bitki doku kültürü yöntemleri klasik bitkisel üretimde ve bitki ıslahında çözülemeyen problemlere çözüm sunmak, süreci hızlandırmak ve klasik sistemleri tamamlamak adına önemli teknikler barındırmaktadır. Embriyo kültürü de bitkisel üretim ve ıslahta önemli avantajlar sunmaktadır. Çimlenme sorunu olan bitki türlerinin çimlendirilebilmesi, nadir bitkilerin çoğaltılması ve geleceğe aktarılması, embriyo gelişim fizyolojisinin incelenmesi, bazı bitki tohumlarında görülen dormansinin kırılması ve üretim sürecinin kısaltılması, ıslahta seçilimin hızlandırılması embriyo kültürünün bilinen kullanım alanlarındandır. Yaygın kullanım alanları arasında ise erken olgunlaşan sert çekirdekli meyvelerde olgunlaşmamış embriyonun kurtarılması, haploidizasyon tekniklerinden olan "ışınlanmış polenle tozlama" yönteminde partenogenesis ile elde edilen embriyoların yaşatılması ve bunlardan bitki elde edilmesi, türler arası ve cinsler arası melezlemelerde uyuşmazlık durumunda embriyonun yaşatılması gelmektedir. Daha farklı amaçlarla da embriyo kültüründen faydalanılmaktadır. Özetle embriyo kültürü hem bitkisel yetiştiricilikte hem de bitki ıslahında yaygın kullanım alanı bulmakta ve faydalı çözümler sunmaktadır. Embriyo kültüründe prosedürler geliştirilirken genotip etkisinin önemi unutulmamalıdır. Embriyonun gelişme dönemi de hayati öneme sahiptir. Olgun embriyonun izolasyonu ve kültürü ile olgun olmayan embriyonun izolasyonu ve kültürü birbirinden tamamen farklı yöntemler ve prosedürler içermektedir. Olgun olmayan embriyoların kültürü daha titiz ve detaylı teknikler içermektedir. Besin ortamı bileşimi de olgun ve olgun olmayan embriyolarda farklılaşmaktadır. Olgun olmayan embriyoların besin ortamı içeriği daha farklı, detaylı ve bazı durumlarda belli dönemlerde farklı besin ortamlarına transfer etme şeklinde ilerlemektedir. Tüm bu sonuçlar bize göstermektedir ki embriyo kültürü üzerine olan araştırmalar güncelliğini korumakta ve sunduğu avantajlar göz önünde bulundurulduğunda gelecekte de artarak devam edeceği öngörülmektedir.

KAYNAKLAR

Ata, A., 2019. Patlıcanda *Solanum melongena* × *Solanum torvum* Melez Populasyonlarının Oluşturulma Olanaklarının Araştırılması ve Patlıcan Anaçlarında Androgenesis Yoluyla Dihaploidizasyon. Doktora Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Adana, s. 147.

Bajaj, Y. P. S., 1990. Wide Hybridization in Legumes and Oilseed Crops Through Embryo, Ovule and Ovary Culture. In: Bajaj Y. P. S. (ed.) Biotechnology in Agriculture and Foresty, vol 10, Legumes and Oilseed Crops I. Springer, Berlin, Heidelberg, New York, pp. 3–37.

Baktemur, G., Taşkın, H., Büyükalaca, S., 2013. Comparison of Different Methods for Separation of Haploid Embryo Induced Through Irradiated Pollen and Their Economic Analysis in Melon (*Cucumis melo* var. *inodorus*). The Scientific World Journal. https://doi.org/10.1155/2013/529502

Baktemur, G., Yücel, N. K., Taşkın, H., Çömlekçioğlu, S., Büyükalaca, S., 2014. Effects of Different Genotypes and Gamma Doses on Haploidization Using Irradiated Pollen Technique in Squash. Turkish Journal of Biology, 38(3), 318–327. https://doi.org/10.3906/biy-1309-5

Barsberg, S., Rasmussen, H. N., Kodahl, N., 2013. Composition of *Cypripedium calceolus* (Orchidaceae) seeds analyzed by attenuated total reflectance IR spectroscopy: In search of understanding longevity in the ground. Am. J. Bot., 100, 2066–2073. https://doi.org/10.3732/ajb.120064

Bhojwani, S. S., Razdan, M. K., 1983. Plant Tissue Culture: Theory and Practice. Elsevier, Amsterdam.

Blake, M. A., 1939. Some results of crosses of early ripening varieties of peaches. Proceedings American Society of Horticultural Science, 37, 232–241.

Bridgen, M. P., 1994. A Review of Plant Embryo Culture. HortScience, 29(11).

Brown, H. T., 1906. On the culture of the exised embryos of barley on nutrient solutions containing nitrogen in different forms. Trans. Guinness Res. Lab., 1, 288–299.

Bürün, B., Gürel, A., 2002. Embriyo Kültürü. In: Babaoğlu, M., Gürel, E., Özcan, S. (eds.). Bitki Biyoteknolojisi I, Doku Kültürü ve Uygulamaları. Selçuk Üniversitesi Yayınları, Konya.

Chée, R. P., Leskovar, D. I., Cantliffe, D. J., 1992. Optimizing embryogenic callus and embryo growth of a synthetic seed system for sweet potato by varying media nutrient concentrations. J. Amer. Soc. Hortic. Sci., 117, 663–667. https://doi.org/10.21273/JASHS.117.4.663

Coble, C. J., Schapaugh,, W. T., 1990. Nutrient culture medium components affecting plant recovery from immature embryos of three *Glycine* genotypes and an interspecific hybrid grown *in vitro*. Euphytica, 50, 127–133. https://doi.org/10.1007/BF00023635

Collins, G. B., Grosser, J. W., 1984. Culture of embryos. In: Vasil, I. K. (ed.). Cell culture and somatic cell genetics of plants. vol. 1. Laboratory procedures and their applications. Academic, New York. pp. 241–257.

De Jeu, M. J., 2000. *In vitro* techniques for ornamental breeding. Acta Hortic., 508, 55–60. https://doi.org/10.17660/ActaHortic.2000.508.6

De Verna, J. W., Myers, J. R., Collins, G. B. 1987. Bypassing prefertilization barriers to hybridization in *Nicotiana* using *in vitro* pollination and fertilization. Theor. Appl. Genet., 73, 665–671. https://doi.org/10.1007/BF00260773

Denli, N., 2019. Türler Arası Melezleme ile Biberde (*Capsicum annuum*) Genetik Tabanın Genişletilmesi ve Androgenesisin Kalıtımının Belirlenmesi. Doktora Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Adana, s. 165.

Devreux, M., 1963. Effets de l'irradiation gamma cronique sur l'embryogénèse de Capsella bursa-pastoris Moench. In L'Energia Nucleare in Agricultura; 6th Congresso Nucleare di Roma; Vallecchi: Firenze, Italy, pp. 119–217.

Dietrich, K., 1924. Über Kultur von Embryonen ausserhalb des Samens. Flora (Jena), 117, 379–417.

Ellialtığlu, Ş. Ş., Taşkın, H., 2021. Süs Bitkileri Islahında Kullanılan Doku Kültürü, genetik Mühendisliği ve Moleküler Markır Yöntemleri. Embriyo Kültürü. In: Yalçın Mendi, N. Y., Kazaz, S. (eds.). Süs Bitkileri Islahı (Klasik ve Biyoteknolojik Yöntemler). Gece Kitaplığı, Ankara, Türkiye.

Ellialtıoğlu, Ş., Abak, K. 1986. Domates ıslahında embriyo kültüründen yararlanma. Bitki Islahı Sempozyumu; İzmir, Türkiye. S. 42–52.

Ellialtıoğlu, Ş., Öztürk, S., 1998. Patlıcanda embriyo kültürü üzerinde çalışmalar. III. Ulusal Biyoteknoloji Simpozyumu, Biyoteknolojide Üniversite-Sanayi İşbirliği; Eskişehir, Türkiye. s. 33-38.

Erol, M. H., 2018. Hıyarlarda ovül-ovaryum kültürleri ve ışınlanmış polen tekniği ile spermidine ve putrescine uygulamalarının haploid embriyo uyartımına etkileri. Yüksek Lisans Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Adana.

Espinasse, A., Volin, J., Dybing, C. D., Lay, C., 1991. Embryo rescue through in ovolo culture in *Helianthus*. Crop. Sci., 31, 102–108. https://doi.org/10.2135/cropsci1991.0011183X00003100010026x

Gamborg, O. L., Miller, R. A., Ojima, K., 1968. Nutrient requirements of suspension cultures of soybean root cells. Expt. Cell Res., 50, 151–158. https://doi.org/10.1016/0014-4827(68)90403-5

Goodspeed, T. H., Avery, P., 1929. The occurrence of a *Nicotiana glutinosa* haplont. Proc. Natl. Acad. Sci. USA, 15, 502–504. https://doi.org/10.1073/pnas.15.6.502

Grigolo, C. R., Citadin, I., De Cacia Oliveira, M., Scariotto, S., Pertille, R. H., Santos, E. P., 2020. Culture media and bap concentrations in the embryo culture of 'BRS Kampai' peach. Rev. Bras. Frutic., 42(6). https://doi.org/10.1590/0100-29452020661

Grout, B. W. W. 1986. Embryo culture and cryopreservation for the conservation of genetic resources of species with recalcitrant seed. In: Withers L. A., Alderson P. G. (eds.). Plant tissue culture and its agricultural applications. Butterworths, London. pp. 303–309.

Guha, S., Maheshwari, S., 1964. *In vitro* production of embryos from anthers of *Datura*. Nature, 204, 497. https://doi.org/10.1038/204497a0

Hanning, E., 1904. Zur Physiologie pflanzlicher Embryonen. I. Über die Kultur von Cruciferen-embryonen ausserhalb des Embryosacks. Bot. Ztg., 62, 45–80.

Hu C. Y., Wang, P. J., 1986. Embryo culture: Technique and Application. In: Evans, D. A., Shaprp, W. R., Ammirato, P. V. (eds.). Hadbook of Plant Cell Culture, vo. 4. New York: MacMillan. pp. 43–96.

Hu, C., Wang, P., 1986. Embryo Culture: Technique and Applications, In: Evans, D. A., Sharp, W. R., Ammirato, P. V. (eds.). Handbook of Plant Cell Culture. vol. 4. Macmillan, New York. p. 43–96.

Jeengool, N., Boonprakob, U., 2004. Rescue of Peach Embryo in Culture Media with Additional of 6-benzylademine and Gibberellic Acid. Kasetsart J. (Nat. Sci.), 38, 468–474.

Joergensen, C. A. 1928. The experimental formation of heteroploidy plants in the genus *Solanum*. J. Genet., 19, 133–211. https://doi.org/10.1007/BF02984237

Kara, B., 2019. Sakız ve siyah kabaklarda ovül kültüründe spermidine ve putrescine uygulamalarının haploid bitki elde edilmesine etkileri. Yüksek Lisans Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Adana.

Keleş, D., Özcan, C., Pınar, H., Ata, A., Denli, N., Yücel, N. K., Taşkın, H., Büyükalaca, S., 2016. First Report of Obtaining Haploid Plants Using Tissue Culture Techniques in Spinach. HortScience, 51(6), 742–749. https://doi.org/10.21273/HORTSCI.51.6.742

Kinoshita, T., 2007. Reproductive barrier and genomic imprinting in the endosperm of flowering plants. Genes Genet. Syst., 82, 177–186. https://doi.org/10.1266/ggs.82.177.

Küden, A. B., Tanriver, E., Gülen, H. Büyükalaca, S., 1998. Embryo Rescue of Peach Hybrids. Acta Hortic., 484, 531–534. https://doi.org/10.17660/ActaHortic.1998.484.90

Laibach, F., 1925. Das Taubwerden von Bastardsamen und die künstliche Aufzucht früh absterbender Bastardembryonen. Z. Bot., 17, 417–459.

Matsubara, S., 1964. Effect of nitrogen compounds on the growth of isolated young embryos of *Datura*. Bot. Mag. (Tokyo), 77, 253–259.

Mehetre, S. S., Aher, A. R., 2004. Embryo rescue: A tool to overcome incompatible interspecific hybridization in *Gossypium* Linn. A review. Ind. J. Biotechnol., 3, 29–36.

Merritt, D. J., Hay, F. R., Swarts, N. D., Sommerville, K. D., Dixon, K.W., Herendeen, P. S., 2014. *Ex situ* conservation and cryopreservation of orchid germplasm. Int. J. Plant Sci. 175, 46–58.

Momotaz, A., Kato, M., Kakihara, F. 1998. Production of intergeneric hybrids between *Brassica* and *Sinapis* species by means of embryo rescue techniques. Euphytica, 103, 123–130. https://doi.org/10.1023/A:1018331528368

Monnier, M. 1990. Culture of zygotic embryos of higher plants. In: Pollard, J. W., Walker, J. M. (eds.). Plant Cell and Tissue Culture. Methods in Molecular Biology, Volume 6, Humana Press. pp. 129–139.

Monnier, M., 1978. Culture of zygotic embryos. In: Thorpe T. A. (ed.). Frontiers of Plant Tissue Culture. Univ. of Calgary Press, Canada. p. 277–286.

Monnier, M., 1995. Culture of zygotic embryos. In: Thorpe, T.A. (ed.). *In Vitro* Embryogenesis in Plants. Kluwer: Dordrecht, The Netherlands, pp. 117–153.

Moyle, L. C., Jewell, C. P., Kostyun, J. L. 2014. Fertile approaches to dissecting mechanisms of premating and postmating prezygotic reproductive isolation. Curr. Opin. Plant Biol., 18, 16–23. https://doi.org/10.1016/j.pbi.2013.12.005

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15, 473–497.

Narayanaswamy, S., Norstog, K., 1964. Plant Embryo Culture. Bot. Rev., 30, 587–628.

Norstog, K., 1979. Embryo culture as a tool in the study of comparative and developmental morphology In: Sharp, W. R., Larsen, P. O., Paddock, E.F., Raghavan, V. (eds.). Plant Cell and Tissue Culture. Ohio State Univ. Press, Columbus. p. 179–202.

Ochatt, S. J., Sangwan, R. S., Marget, P., Ndong, Y. A., Rancillac, M., Perney, P., Röbbelen, G., 2002. New approaches towards the shortening of generation cycles for faster breeding of protein legumes. Plant Breed., 121, 436–440. https://doi.org/10.1046/j.1439-0523.2002.746803.x

Oh, S., Yun, B., Kim, S. H., Cho, S. Y., Jung, N., Do, K. R., Cho, K. H., Kim, H. R., 2024. Embryo Rescue Efficiency Affected by Developmental Stages of Embryo and Medium Composition in Early-Ripening Peach (*Prunus persica*). Korean J. Plant Res., 37(3), 263–269. https://doi.org/10.7732/kjpr.2024.37.3.263

Pickersgill, B., 1992. Barriers to interspecific gene exchange in *Capsicum*. VIIIth Meeting "Genetics and Breeding on Capsicum and Eggplant" Rome, Italy, 7-10 September 1992, pp. 57–60.

Pickersgill, B., 1997. Genetic resources and breeding of *Capsicum* spp. Euphytica, 96, 129–133. https://doi.org/10.1023/A:1002913228101

Pierik, R. L. M., 1987. *In vitro* culture of higher plants. Martinus Nijhoff, Dordrecht, Netherlands.

Portemer, V., Renne, C., Guillebaux, A., Mercier, R. 2015. Large genetic screens for gynogenesis and androgenesis haploid inducers in *Arabidopsis thaliana* failed to identify mutants. Front. Plant Sci., 6, 147. https://doi.org/10.3389/fpls.2015.00147

Prasanth, C., Baddigam, K. R., Kailas, K. A., 2023. Embryo Culture and its Application in Biotechnology. In: Bhati, J., Vaghasiya, K. P., Chavan, R. M., Beese, S., Gajjar, K., Jogender (eds.). Plant Sciences at a Glance. Integrated Publications, New Delhi. pp. 269–280.

Raghavan, V., 1966. Nutrition, growth and morphogenesis of plant embryos. Biol. Rev., 41, 1–58. https://doi.org/10.1111/j.1469-185X.1966.tb01536.x

Raghavan, V., 1980. Embryo culture. In: Vasil, I. K. (ed.). Perspectives in Plant Cell and Tissue Culture. Intl. Rev. Cytol., Suppl. 11B. Academic, New York. p. 209–240.

Raghavan, V., 2003. One hundred years of zygotic embryo culture investigations. Vitr. Cell Dev. Biol. Plant, 39, 437–442. https://doi.org/10.1079/IVP2003436

Ramming, D. W., 1986. Embryo Rescue Technıques in Nectarine and Peach Breeding. Dsir Plant Breeding Symposium. N.Z. Agronomy Society.

Rangan, T. S., 1984. Culture of ovules. In: Vasil, I. K. (ed.). Cell Culture and somatic Cell Genetics of Plants. vol. 1. Laboratory Procedures and their Applications. Academic, New York. p. 227–231.

Reed, S. M., Collins, G .B. 1978. Interspecific hybrids in *Nicotiana* through *in vitro* culture of fertilized ovule. J. Hered. 69, 311–315.

Rogo, U., Fambrini, M., Pugliesi, C. 2023. Embryo Rescue in Plant Breeding. Plants, 12, 3106. https://doi.org/10.3390/plants12173106

San Noeum, L. H., 1976. Haploides d'*Hordeum vulgare* L. par culture *in vitro* d'ovaries non fecondes. Ann. Amelior. Plant., 26, 751–754.

Sanders, M. E., Burkholder, P. R., 1948. Influence of amino acids on growth of *Datura* embryos in culture. Proc. Natl. Acad. Sci. USA, 34, 516–526. https://doi.org/10.1073/pnas.34.11.51

Sarı, N., Abak, K., Pitrat, M., Rode, J. C., Dumas De Vaulx, R., 1994. Induction of parthenogenetic haploid embryos after pollination by irradiated pollen in watermelon. HortScience 29(10), 1189–1190.

Sharma, D. R., Kaur, R., Kumar, K. 1996. Embryo rescue in plants: A review. Euphytica, 89, 325–337. https://doi.org/10.1007/BF00022289

Sharma, H. C., 2004. Recent Research Developments in Genetics and Breeding. Vol. 1 Part II. India: Research Signpost, Trivandrum.

Stebbins, G. L., 1958. The inviability, weakness and sterility of interspecific hybrids. Adv. Genet. 9, 147–215. https://doi.org/10.1016/S0065-2660(08)60162-5

Taşkın, H., Yücel, N. K., Baktemur, G., Çömlekçioğlu, S., Büyükalaca, S., 2013. Effects of Different Genotypes and Gamma Ray Doses on Haploidization with Irradiated Pollen Technique in Watermelon (*Citrullus lanatus* L.). Canadian Journal of Plant Science, 93(6), 1165–1168. https://doi.org/10.4141/CJPS2013-059

Tukey, H. B., 1933. Artificial culture of sweet cherry embryos. Journal of Heredity, 24, 7–12. https://doi.org/10.1093/oxfordjournals.jhered.a103677

Umbeck, P. F. Norstog, K., 1979. Effects of abscisic acid and ammonium ion on morphogenesis of cultured barley embryos. Bul. Torrey Bot. Club, 106, 110–116. https://doi.org/10.2307/2484285

Unek, C., Tanrıver, E., Kuden, A.B., 2017. Embryo rescue for breeding of early peach cultivar 'Flored' and determination of Sharka resistant genotypes with marker-assisted selection. Acta Hortic., 1187, 285–290. https://doi.org/10.17660/ActaHortic.2017.1187.29

Uysal, H., Seyis, F., Kurt, O., 2007. Tarla Bitkilerinde Melezleme Bariyerlerinin Aşılmasında Alternatif Bir Yöntem: Embriyo Kültürü. OMÜ Zir. Fak. Dergisi, 22(1), 116–122.

Van Overbeek, J., Conklin, M. E., Blakeslee, A. F., 1941. Factors in coconut milk essential for growth and development of very young *Datura* embryos. Science, 94, 350–351. https://doi.org/10.1126/science.94.2441.350

Van Overbeek, J., Conklin, M. E., Blakeslee, A. F., 1942. Cultivation *in vitro* of small *Datura* embryos. Amer. J. Bot., 29, 472–477. https://doi.org/10.2307/2437313

Veen, H., 1963. The effect of various growth-regulators on embryos of *Capsella bursapastoris* growing *in vitro*. Acta Bot. Neerl., 12, 129–171.

Wilcock, C., Neiland, R., 2002. Pollination failure in plants: Why it happens and when it matters. Trends Plant Sci., 7, 270–277. https://doi.org/10.1016/S1360-1385(02)02258-6

Williams, E. G., Verry, I. M., Williams, W. M. 1982. Use of embryo culture in interspecific hybridization. In: Vasil, I. K., Scowcroft, W. R., Frey, K. J. (eds.). Plant Improvement and Somatic Cell Genetics, Academic Press, New York, NY, USA,. pp. 119–128.

Yeung, E. C., Thorpe, T. A., Jensen, C. J., 1981. *In vitro* fertilization and embryo culture. In: Thorpe T. A. (ed.). Plant Tissue Culture: Methods and Applications in Agriculture. Academic, New York. p. 253–271.

Yıldız, Ç., Koruk, M., Doğan, A., Ellialtıoğlu, Ş. Ş. 2019. Farklı hıyar genotiplerinin ışınlanmış polenle tozlama ile partenogenetik embriyo oluşturma frekansları ve haploid embriyo gelişimi. 12. Sebze Tarımı Sempozyumu; Kayseri, Türkiye.

Zenkteler, M. E., Nitzsche, W., 1985. *In vitro* culture of ovules of *Triticum aestivum* at early stages of embryogenesis. Plant Cell Rep., 4, 168–171. https://doi.org/10.1007/BF00571309

Bölüm 9

HAPLOİD VE KATLANMIŞ HAPLOİD BİTKİLERİN ÜRETİMİ

Halit YETİŞİR [1]
Nebahat SARI [2]
Şebnem ELLİALTIOĞLU [3]
Hasan PINAR [4]

9.1 GİRİŞ

Çoğu organizma, her iki ebeveynin de genetik katkıları ile yeni nesiller oluşturur. Ancak, bu kuralın önemli istisnaları da vardır. Somatik hücrelerinde gametik hücreler kadar kromozom bulunduran bitkilere 'haploid bitkiler' denir. Doğada spontan olarak oluşan 'haploid bitkileri' izole etmek veya uyartımla oluşturmak mümkündür. Haploid bitkiler sadece bir set kromozom içerimesi ve resesif allellerin ortaya çıkartılmasına imkân sağlaması ıslah ve genetik çalışmaları için benzersiz fırsatlar sunar. Bu amaçla bir haploid birey üretildikten sonra 'dihaploidizasyon' yolu ile kromozom sayısının ikiye katlanması gereklidir. Bu yolla üretilen birey %100 homozigot hale gelir. Bu yöntemi kullanılarak ıslahçılar, saf hatları klasik yönteme göre daha hızlı bir şekilde üreterek kombinasyon ve F_1 çeşit ıslahı programlarında zaman yönünden önemli kazançlar sağlayabilmektedirler. Haploid bitkilerin doğada oluşum şekilleri Şekil 9.1'de verilmiştir.

- **Ginogenezis:** Yumurta hücresinin döllenme olmadan partenogenetik olarak bölünerek embriyo oluşturmasıdır. Generatif çekirdek ile yumurta hücresi birleşmediği hal-

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asitleri ve amino asitlerin biyosentezinde yer alan çok sayıda genin ifadesinin arttığını bildirmişlerdir. Özetle, ABA'nın ısı şokunu takiben sporofitik haploid uyartımında önemli bir rol oynayabileceğini göstermişlerdir. Androjenik yanıtta farklılık gösteren genotipler arasındaki transkriptomik, proteomik ve metabolomik farklılıkların belirlenmesi ve bu bilgi ve metabolomiklerin (proteinler, enzimler, hormonlar, amino asitler vb.) androjenez yoluyla haploid bitki üretimine sunulması bu alanı daha da güçlendirecektir. Mevcut haploid teknolojisinin sınırlamaları nedeniyle, haploidizasyonun yeni ilkelerinin yanı sıra metodolojik gelişmeler de ıslahçılar tarafından memnuniyetle karşılanacaktır.

KAYNAKLAR

Aalders, K.O., 1958. Monoploidy in Cucumber. J. Hered., 49, 41-44.

Abak, K., Comlekcioglu, N., Buyukalaca, S., Sari, N., 1998. Use of Stomatal Characteristics to Estimate Ploidy Level of Haploid And Dihaploid Pepper Plants. Xth EUCARPIA Meeting Capsicum and Eggplant, 7-11 September 1998, Avignon-France, 179-182.

Awan, M. J. A., Farooq, M. A., Buzdar, M. I., Zia, A., Ehsan, A., Waqas, M. A. B., & Mansoor, S., 2025. Advances in gene editing-led route for hybrid breeding in crops. Biotechnology Advances, 108569.

Alan, A.R., Martha, A.M., Aigars, B., Cobb, E., Elizabeth, D., 2003. Early Production of Gynogenic Plants From Hybrids of *Allium cepa* L. and *A. roylei* Stearn. Plant Sci., 165, 1201-1211.

Alan, A.R., Brants, A., Cobb, E., Goldschnied, P.A., 2004. Fecund Gynogenic Lines From Onion (*Allium cepa* L.) Breeding Materials. Plant Sci., 167, 1055-1066.

Alan, A.R., Toprak, F.C., Kaska, A., 2016. Production and Evaluation of Gynogenic Leek (*Allium ampeloprasum* L.) Plants. Plant Cell Tissue Organ Cult., 125(2), 249-259.

Aleza, P., Juárez, J., Hernández, M., Pina, J.A., Ollitrault, P., Navarro, L., 2009. Recovery and Characterization of a *Citrus clementina* Hort. ex Tan.'Clemenules' Haploid Plant Selected to Establish the Reference Whole Citrus Genome Sequence. BMC Plant Biol. 9: 110.

Arabzai, M. G., Huang, D., Mohammadi, N. K., Gao, J., Wang, X., Zheng, P., & Wang, L., 2025. Techniques and advantages of microspore culture for crop improvement. *Plant Growth Regulation*, 1-16. https://doi.org/10.1007/s10725-025-01312-8

Asif, M., 2013. Progress and Opportunities of Doubled Haploid Production. Springer Cham Heidelberg New York Dordrecht London.

Bajaj, Y.P.S., 1980. Enhancement of the *In-vitro* Development of Triticale Embryos by the Endosperm of Durum Wheat *Triticum durum*. Cereal Research Communications, 8(2), 359–364.

Bajaj, Y.P.S., 1990. *In vitro* Production of Haploids and Their Use in Cell Genetics and Plant Breeding. Bajaj Y.P.S. Haploids in crop improvement (ss.1-44), Springer, Berlin.

Baktemur, G., Yücel, N. K., Taşkın, H., Çömlekçioğlu, S., & Büyükalaca, S. 2014. Effects of different genotypes and gamma ray doses on haploidization using irradiated pollen technique in squash. Turkish Journal of Biology, 38(3), 318-327.

Ball, S.T., Zhou, H.P., Konzak, C.F., 1993. Influence of 2,4-D, IAA, and Duration of Callus İnduction in Anther Cultures of Spring Wheat. Plant Sci., 90(2), 195-200. doi: 10.1016/0168-9452(93)90240-z

Barany, I., Testillano, P.S., Mityko, J., Risueno, M.C., 2001. The Switch of The Microspore Developmental Programme in *Capsicum* Involves HSP70 Expression and Leads to The Production of Haploid Plants. The International Journal of Developmental Biology, 45, 39–40.

Başay, S., Ellialtioglu, Ş.Ş., 2013. Effect of Genotypical Factors on the Effectiveness of Anther Culture in Eggplant (*Solanum melongena* L.). Turkish Journal Biology, 37, 499-505.

Bat, H., 2019. Patlıcan ve Biberde Androgenezis Yoluyla Dihaploidizasyon Yönteminin Optimizasyonu ve Hibrit Adaylarının Geliştirilmesi (Tübitak Teydeb 3170610 No'lu proje, United Genetics Turkey).

Batzenschlager, M., Lermontova, I., Schubert, V., 2015. *Arabidopsis* MZT1 homologs GIP1and GIP2 are Essential for Centromere Architecture. Proc. Natl. Acad. Sci. USA, 112, 8656–8660.

Bayat, M., Zargar, M., Murtazova, K.M.-S., Nakhaev, M.R., Shkurkin, S.I., 2022. Ameliorating Seed Germination and Seedling Growth of Nano-Primed Wheat and Flax Seeds Using Seven Biogenic Metal-Based Nanoparticles. Agronomy, 12, 811.

Bennett, M.D., Bhandol, P., Leitch, I.J., 2000. Nuclear DNA Amounts in Angiosperms and their Modern Uses-807 New Estimates. Ann Bot-London, 86, 859-909.

Berenguer, E., Bárány, I., Solís, M. T., Pérez-Pérez, Y., Risueño, M. C., and Testillano, P. S., 2017. Inhibition of Histone H3K9 Methylation by BIX-01294 Promotes Stress-Induced Microspore Totipotency and Enhances Embryogenesis Initiation. Front. Plant Sci., 8:1161. doi: 10.3389/fpls.2017.01161

Bhagyalakshmi, N., 1999. Factors Influencing Direct Shoot Regeneration from Ovary Explants of Saffron. Plant Cell Tissue Organ Cult., 58, 205–211

Bhat, J.G., Murthy, H.N., 2007. Factors Affecting *In vitro* Gynogenic Haploid Production in Niger (*Guizotia abyssinica* (L. f.) Cass.). Plant Growth Regul., 52, 241–248

Binarova, P., Hause, G., Cenklova, V., 1997. A Short Severe Heat Shock is Required to Induce Embryogenesis in Late Bicellular Pollen of *Brassica Napus* L. Sexual Plant Reproduction, 10(4), 200–208.

Bishnoi, U., Jain, R.K., Rohilla. J.S., Chowdhury, V.K., Gupta, K.R., Chowdhury, J.B., 2000. Anther Culture of *Recalcitrant indica* × Basmati Rice Hybrids. Euphytica, 114(2), 93–101. doi: 10.1023/A:1003915331143

Blakeslee, A.F., Belling, J., Farnham, M.E., Bernger, D., 1922. A Haploid Mutant in the Jimson Weed, *Datura stramonium*. Science, 55(143), 646-647.

Balasco, M., Badenes, M.L., del Mar Naval, M., 2016. Induced Parthenogenesis by Gamma-Irradiated Pollen in Loquat for Haploid Production. *Breeding Science,* 66, 606–612.

Bohanec, B., 2003. Ploidy Determination Using Flow Cytometry. Maluszynski M., Kasha K.J., Forster B.P. ve Szarejko I. Doubled Haploid Production in Crop Plants (ss. 397-403). Springer.

Bohanec, B., Jakse, M., 1999. Variation of Gynogenic Response Among Long-Day Onion (*Allium cepa* L.) Accessions. Plant Cell Rep., 18, 737–742

Bohanec, B., Marijana, J., Alojz, I. ve Branka, J., 1995. Studies of Gynogenesis in Onion (*Allium cepa* L.) Induction Procedures and Genetic Analysis of Regenerents. Plant Sci., 104, 215–224

Boutilier, K., Offringa, R., Sharma, V.K., 2002. Ectopic Expression of BABY BOOM Triggers a Conversion from Vegetative to Embryonic Growth. Plant Cell, 14, 1737–1749.

Bouvier, L., Zhang, Y.X., Lespinasse, Y., 1993. Two Methods of Haploidization in Pear, *Pyrus communis* L.: Greenhouse Seedling Selection and *In situ* Parthenogenesis Induced by Irradiated Pollen. Theor Appl Genet., 87, 229–232.

Branch, W.D., & Kvien, C.K., 1992. Cytoplasmically İnherited Albinism in Peanut Seedlings. Journal of Heredity, 83(6), 455–457.

Brazauskas, G., Pasakinskiene, I., Jahoor, A., 2004. AFLP Analysis Indicates No Introgression of Maize DNA in Wheat Maize Crosses. Plant Breed., 123, 117-120.

Broughton, S., 2008. Ovary Co-Culture Improves Embryo and Green Plant Production in Anther Culture of Australian Spring Wheat (*Triticum aestivum* L.). Plant Cell Tissue Organ Cult., 95(2), 185–195.

Büyükalaca, S., Comlekcioglu, N., Abak, K., Ekbic, E., Kilic, N., 2004. Effects of Silver Nitrate and Donor Plant Growing Conditions on Production of Pepper (*Capsicum annuum* L.) Haploid Embryos via Anther Culture. European Journal of Horticultural Science, 69(5), 206–209.

Caglar, G., & Abak, K., 1997. *In vitro* Colchicine Application of Haploid Cucumber Plants. Plant Cell

Tiss Organ Cult 48, 111-119.
Calleberg, E.K., Johansson, L.B., 1996. Effect of Gelling Agents on Anther Culture. Ed. S.M. Jain, S.K. Sopory, and R.E. Veilleux., *In vitro* Haploid Production in Higher Plants, Fundamental Aspects and Methods (ss. 189-203). Kluwer Academic Publishers, Dordrecht, the Netherlands.
Caranta, C., 1992. Essai d'induction de la Partenogenese Haploide par du Pollen Irradié chez le Fraisier cultivé *Fargaria ananassa* Duch. et etude du Gametophyte Femelle. Memorie (D.E.A.) Option Science Agronomiques: Amélioration des Plantes, Faculte des Science de Luminy, Marseille, 15 p.
Castillo, A.M., Valero-Rubira, I., Burrell, M.Á., Allué, S., Costar, M.A., and Vallés, M.P., 2020. Trichostatin A Affects Developmental Reprogramming of Bread Wheat Microspores Towards an Embryogenic Route. Plan. Theory, 9:1442. doi: 10.3390/plants9111442
Chalyk, S.T. 1999. Use of Maternal Haploids for Improving Maize Inbred Lines. MNL, 73, 54–56.
Chand, S., & Sahrawat, A.K., 2007. Embryogenesis and Plant Regeneration from Unpollinated Ovary Culture of *Psorea corylifolia*. Biologia Plantarum, 51(2), 223-228.
Chase, S.S., 1952. Production of Homozygous Diploids of Maize from Monoploids. Agron. J., 44, 263–267.
Chen, J.F., Cui, L., Malik, A.A., Mbira, K.G., 2010. *In vitro* Haploid and Dihaploid Production via Unfertilized Ovule Culture. Plant Cell Tiss Organ Cult., 104, 311-319.
Cheng, Y., Ma, R.L., Jiao, Y.S., Qiao, N., Li, T.T., 2013. Impact of Genotype, Plant Growth Regulators and Activated Charcoal on Embryogenesis İnduction İn Microspore Culture of Pepper (*Capsicum annuum* L.). S. Afr. J. Bot., 88:306-9.
Chen, J., Strieder, N., Krohn, N.G., Cyprys, P., Sprunck, S., Engelmann, J.C., Dresselhaus, T., 2017. Zygotic Genome Activation Occurs Shortly After Fertilization in Maize. Plant Cell, 29, 2106–2125.
Chen, X., Li, Y., Ai, G., Chen, J., Guo, D., Zhu, Z., Zhu, X., Tian, S., Wang, J., Liu, M., Yuan, L., 2023. Creation of a Watermelon Haploid Inducer Line via *ClDMP3*-mediated Single Fertilization of the Central Cell, Horticulture Research, 10: 6, uhad081.
Cherkaoui, S., Lamsaouri, O., Chlyah, A., Chlyah, H., 2000. Durum Wheat x Maize Crosses for Haploid Wheat Production: Influence of Parental Genotypes and Various Experimental Factors. Plant Breed., 119, 31–36.
Chiancone, B., Tassoni, A., Bagni, N., Germana, M.A., 2006. Effect of Polyamines on *In vitro* Anther Culture of *Citrus clementina* Hort. ex Tan. Plant Cell Tissue Organ Cult., 87(2), 145-153.
Chu, C.C., 1981. The N6 Medium and Its Applications to Anther Culture of Cereal Crops. Proceedings of symposium on plant tissue culture, *Beijing*, 43–50.
Chu, C.G., Xu, S.S., Friesen, T.L., Faris, J.D., 2008. Whole Genome Mapping in a Wheat Doubled Haploid Population Using SSRs and Traps and the Identification of QTL for Agronomic Traits. Mol. Breed., 22, 251-266.
Cistué, L., Vallés, M.P., Echavarri, B., Sanz, J., Castillo, A., 2003. Barley Anther Culture. Maluszynski M, Kasha KJ, Forster BP, Szarejko I (eds) Doubled Haploid Production in Crop Plants: A Manual (ss. 29-34). Kluwer Academic, Dordrecht.
Clausen, R.E., Mann, M.C., 1924. Inheritance in *Nicotiana tabacum*. V. the Occurrence of Haploid Plant in Interspecific Progenies. PNAS, 10, 121–124.
Conner, J.A., Mookkan, M., Huo, H., Chae, K., Ozias-Akins, P.A. 2015. Parthenogenesis Gene of Apomict Origin Elicits Embryo Formation from Unfertilized Eggs in a Sexual Plant. Proc. Natl. Acad. Sci. USA, 112, 11205–11210.
Conner, J.A., Podio, M., Ozias-Akins, P., 2017. Haploid Embryo Production in Rice and Maize Induced by PsASGR-BBML Transgenes. Plant Reprod., 30, 41-52.
Cuny, F., Grotte, M., Dumas de Vaulx, R., Rieu, A., 1993. Effects of Gamma Irradiation of Pollen on Parthenogenetic Haploid Production in Muskmelon (*Cucumis melo* L.). Environ Exp Bot., 33(2), 301-312.

Çaglar, G., & Abak, K., 1996a. Efficiency of Haploid Production in Cucumber. Cucurbit Genetics Cooperative Report, 19, 36-37.

Çağlar, G., Abak, K., 1999b. Obtention of *In vitro* Haploid Plants from *In situ* Induced Haploid Embryos in Cucumber (*Cucumis sativus* L.). Turk J Agric For., 23(3), 283-290

Çömlekçioğlu, N., & Ellialtıoğlu, Ş.Ş., 2018. Review on the Research Carried out on *In vitro* Androgenesis of Peppers (*Capsicum annuum* L.) in Turkey. *Research Journal of Biotechnology*,13(6), 75-84.

Chun, C., Park, H., Na, H., 2011. Microspore-derived Embryo Formation in Radish (*Raphanus Sativus* L.) According to Nutritional and Environmental Conditions. Hortic. Environ. Biotechnol., 10:530-5.

Dahleen, L.S., 1999. Donor-plant Environment Effects on Regeneration from Barley Embryo-Derived Callus. Crop Science*,* 39(3), 682–685.

Dal, B., Sarı, N., Solmaz, İ., 2016. Effect of Different Irradiation Sources and Doses on Haploid Embryo Induction in Altinbas (*Cucumis melo* L. var. *inodorus*) Melons. Turk J Agric For., 40, 552-559.

De La Fuente, G.N., Frei, U.K., Trampe, B., Nettleton, D., Zhang, W., Lübberstedt, T., 2018. A Diallel Analysis of a Maize Donor Population Response to *In Vivo* Maternal Haploid Induction: I. Inducibility. Crop Sci., 58, 1830-1837.

De Maine, M.J., 2003. Potato Haploid Technologies. Maluszynski M, Kasha K.J., Forster B.P. ve Szarejko I., Doubled Haploid Production in Crop Plants-A Manual (ss. 241-247). Kluwer, Dordrecht/ Boston/London.

Deng, Y., Tang, B., Zhou, X., 2020. Direct regeneration of Haploid or Doubled Haploid Plantlets in Cucumber (*Cucumis sativus* L.) Through Ovary Culture. Plant Cell Tiss Organ Cult., 142, 253–268. https://doi.org/10.1007/s11240-020-01839-w

Deimling, S., & Fleihinghaus-Roux, T., 1996. Haploids in Rye. Mohan-Jain S., Spory S.K., and Veilleux R.E., *In vitro* Haploid Production in Higher Plants (ss. 181-204), Kluwer, Dordrecht.

Denli, N., 2019. Türler Arası Melezleme ile Biberde (*Capsicum annuum*) Genetik Tabanın Genişletilmesi ve Androgenesisin Kalıtımının Belirlenmesi. Doktora Tezi, Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Bahçe Bitkileri Anabilim Dalı, Adana.

Devaux, P., & Pickering, R., 2005. Haploids in the İmprovement of Poaceae. Palmer C.E., Keller, W.E. and Kasha, K.J., Biotechnology in Agriculture and Forestry: Haploids in Crop Improvement II (ss. 215-242). Berlin, Heidelberg, Springer.

Devaux, P., Zivy, M., Kilian, A., Kleinhofs, A., 1996. Doubled Haploids in Barley. Scoles, G. ve Rossnagel B., Proc. V. International Oat Conference and VII International Barley Genetics Symposium (ss. 213-222). Univ. of Saskatchewen, Saskatoon.

Diao, W.P., Jia, Y.Y., Song, H., Zhang, X.Q., Lou, Q.F., Chen, J.F., 2009. Efficient Embryo Induction in Cucumber Ovary Culture and Homozygous Identification Of the Regenerates Using SSR Markers. Sci Hortic., 119, 246-251.

Doi, H., Hoshi, N., Yamada, E., Yokoi, S., Nishihara, M., Hikage T., Takahata, Y., 2013. Efficient Haploid and Doubled Haploid Production from Unfertilized Ovule Culture of Gentians (*Gentiana* spp.). Breeding Science, 63, 400–406.

Dolcet-Sanjuan, R., Claveria, E., Garcia-Mas, J., 2006. Cucumber (*Cucumis sativus* L.) Dihaploid Line Production Using *In vitro* Rescue of In vivo Induced Parthenogenic Embryos. Acta Hortic., 725(2), 837-844

Dong, X., Xu, X., Miao, J., Li, L., Zhang, D., Mi, X., Liu, C., Tian, X., Melchinger, A.E., Chen, S., 2013. Fine Mapping of qhir1 Influencing *In vivo* Haploid Induction in Maize. Theor. Appl. Genet., 126, 1713–1720.

Dong, Y., Zhao, W., Li, X., Hui X.L., Liu, C.X., Gao, N.N., Huang, J.H., Wang, W.Y., Xu, X.L., Tang, Z.H., 2016. Androgenesis, Gynogenesis, and Parthenogenesis Haploids in Cucurbit Species. Plant Cell Rep., 35, 1991–2019

Doré, C., 1989. Obtention de Plantes Haploides de Chou Cabus (*Brasssica oleracea* L. spp *capitata*) Apres Culture *In vitro* d'ovules Polinises par du Pollen Irradié. C.R. Academic Science.

Doré, C., Prigent, J., Desprez, B., 1996. *In situ* Gynogenetic Haploid Plants of Chicory (*Cichorium inthybus* L.) after Intergenic Hybridisation with *Cicerbita albina* Wallbr. Plant cell rep., 15: 758-761.

Doruk, N., Kandemir, N., Gökmen, S., 2020. Doubled Haploid Production in Cereals Using Microspore Culture. Selcuk Journal of Agriculture and Food Sciences, 34, 2, 169–176.

Dumas de Vaulx, R., 1979. Obtention des Plantes Haplöides chez le Melon (*Cucumis melo* L.) Après Pollinisation par *Cucumis ficifolius* A. Rich. C.R. Academic Science.

Dumas de Vaulx, R., Chambonnet, D., Pochard, E., 1981. Culture *In vitro* d'anthères de Piment (*Capsicum annuum* L.): Amélioration des taux D'obtention de Plantes chez Différents Génotypes par des traitements à+ 35° C. Agronomie, 1(10), 859-864.

Duncan, E.J., Heberle, E., 1976. Effect of Temperature Shock on Nuclear Phenomena in Microspores of *Nicotiana tabacum* and Consequently on Plantlet Production. Protoplasma, 90(1–2), 173-177.

Dunwell, J., 1996. Microspore culture. In: Jaın, S. Soporys. K. M., ve Veilleuxr. E. (eds.): *In Vitro* Haploid Production in Higher Plants 1, pp. 205-216. Kluwer Academic Publishers, Dordrecht.

Dunwell, J.M., 2010. Haploids in Flowering Plants: Origins and Exploitation. Plant Biotech.Journal, 8, 377-424.

Dwivedi, S.L., Nigam, S.N., Pandey, S.K., Gibbons, R.W., 1984. Inheritance of Albinism in Certain Interspecific and Intersubspecific Crosses in Groundnut (*Arachis hypogaea* L). Euphytica,33(3), 705–708.

Ebrahimi, R., & Zamani, Z., 2009. Effect of Polyamines on *In vitro* Gynogenesis of Onion (*Allium cepa* L.). Am Eurasian J Sustain Agric*,* 3(1), 71–74

Ellialtıoğlu, Ş., Başay, S., Kuşvuran, Ş., 2012. Patlıcanda Polen Dimorfizmi ve Anter Kültürü İlişkisinin İncelenmesi. TABAD (Research Journal of Agricultural Sciences), 5(1), 149-152.

Ellialtıoğlu, Ş., Sarı, N., Abak, K., 2002. Haploid Bitki Üretimi. Babaoğlu, M., Gürel E., ve Özcan S., (ss. 137-189). Selçuk Üniversitesi Basım Evi.

Ellialtıoğlu, Ş.Ş., Sönmez, K., Evcen, F., 2015. The Effect of Growth Regulator and Carbon Source Combinations on the Formation of Haploid Embryos in Eggplant Anther Cultures. Green Biotechnology Workshop, 21-23 September 2015, Darıca, Kocaeli.

Eudes, F., & Chugh, A., 2009. An overview of Triticale Doubled Haploids. Touraev A, Forster B.P., and Jain S.M., Advances in Haploid Production in Higher Plants (ss. 87-96). Springer, Dordrecht.

Evans, M.M., 2007. The Indeterminate Gametophyte1 Gene of Maize Encodes a LOB Domain Protein Required for Embryo Sac and Leaf Development. Plant Cell, 19, 46–62.

Falque, M., Kodia, A.A., Sounigo, O., Eskes, A.B., Charrier, A., 1992. Gamma-Irradiation of Cacao (*Theobroma cacao*) Pollen: Effect on Pollen Grain Viability, Germination Mitosis and Fruit Set. Euphytica, 64, 167–172.

Fan, Z., Armstrong, K.C., Keller, W.A., 1988. Development of Microspores *In vivo* and *In vitro* in *Brassica napus* L. Protoplasma, 147(2–3), 191-199.

Forster, B.P., & Thomas, W.T.B., 2005. Doubled Haploids in Genetics and Plant Breeding. Plant Breed Rev., 25, 57-88.

Foster, B.P., Heberle-Bors, E., Kasha, K.J., Touraev, A., 2007. The Resurgence of Haploids in Higher Plants. Trends Plant Sci., 12(8), 368-375

Froelicher, Y., Bassene, J.B., Jedidi-Neji, E., Dambier, D., Morillon, R., Bernardini, G.G., Costantino, G., Ollitrault, P., 2007. Induced Parthenogenesis in Mandarin for Haploid Production: Induction Procedures and Genetic Analysis of Plantlets. Plant Cell Rep., 26, 937-944.

Fu, S., Yin, L., Xu, M., Li, Y., Wang, M., Yang, J., Fu, T., Wang, J., Shen, J., Ali, A., 2018. Maternal Doubled Haploid Production in Interploidy Hybridization Between *Brassica napus* and *Brassi-*

ca Allooctaploids. Planta, 247, 113–125.
Furusho, M., Suenaga, K., Nakajima, K., 1991. Production of Haploid Barley Plants from Barley x Maize and Barley x Italian Ryegrass Crosses. Jpn J Breed., 41, 175-179
Gains, E.F., & Aase, H.C., 1926. A Haploid Wheat Plant. Am J Bot., 13, 373-385.
Gamborg, O.L., Miller, R.A., Ojima, K., 1968. Nutrient Requirements of Suspension Cultures of Soybean Root Cells. Experimental Cell Research, 50, 157-158.
Gémés-Juhász, A., Balogh, P., Ferenczy, A., Kristof, Z., 2002. Effect of Optimal Stage of Female Gametophyte and Heat Treatment on *İn Vitro* Gynogenesis Induction in Cucumber (*Cucumis sativus* L.). Plant Cell Reports, 21(2), 105-111.
Geoffriau, E., Kahane, R., Rancillac, M., 1997. Variation of Gynogenesis Ability in Onion (*Allium cepa* L.). Euphytica, 94, 37-44.
Germana, M.A., 2007. Haploidy. Khan I.A., Citrus genetics Breeding and Biotechnology (ss. 167-197). Cabi.
Germana, M.A., 2011a. Anther Culture for Haploid and Doubled Haploid Production. Plant Cell Tissue Organ Cult., 104(3), 283-300. doi: 10.1007/s11240-010-9852-z
Germana, M.A., & Chiancone, B., 2003. Improvement of *Citrus clementina* Hort. ex Tan. Microspore Derived Embryoid Induction and Regeneration. Plant Cell Reports, 22(3), 181-187. doi: 10.1007/ s00299-003-0669-7
Germana, M.A., Crescimanno, F.G., Motisi, A., 2000. Factors Affecting Androgenesis in *Citrus clementina* Hort. ex Tan. Advanced Horticultural Science, 14(2), 43-51.
Ghadamkheir, M., Vladimirovich, K.P., Orujov, E., Bayat, M., Madumarov, M.M., Avdotyin, V., Zargar, M., 2020. Influence of Sulfur Fertilization on Infection of Wheat Take-All Disease Caused by The Fungus *Gaeumannomyces graminis* var. *tritici*. Res. Crops, 21, 627–633.
Ghalagi C., 2022. *In Vitro* Production of Doubled Haploids from KRH-4 Rice Hybrid and Development of Strategies to Identify Hploids at Early Developmental Stages. Ph, D. Thesis. University of Agricultural Sciences, GKVK; Bangalore, India.
Gilles, L.M., Khaled, A., Laffaire, J.B., Chaignon, S., Gendrot, G., Laplaige, J., Bergès, H., Beydon, G., Bayle, V., Barret, P., 2017a. Loss of Pollen-Specific Phospholipase NOT LIKE DAD Triggers Gynogenesis in Maize. EMBO J., 36, 707–717.
Gilles, L.M., Martin, J.P., Rogowsky, P.M., 2017b. Haploid Induction in Plants. Curr. Biol., 27, 1095–1097.
Gland, A., Lichter, R., Schweiger, H.G., 1988. Genetic and Exogenous Factors Affecting Embryogenesis in Isolated Microspore Cultures of *Brassica napus* L. Journal of Plant Physiology, 132(5), 613-617.
Gok-Guler, G.P., Sari, N., Yetisir, H., Yegul, M., Kantoglu, K., Kunter, B., 2017. The Effects of Genotypes and Irradiation Doses on Haploid Embryo Induction and Plant Production in Bottle Gourd [*Lagenaria siceraria* (Malign) Stanley. Acta Hortic., 1151, 135-142.
Gong, S., Tang, B., Dai, Y., Sun, X., Song, H., Xiong, C., Zou, T., Sun, L., Liu, G., Yang, H., 2025. Transcriptome and Proteome Reveal Heat Shock Promotes Haploid Induction Rate via Activating ABA Signal Transduction in Watermelon. Agronomy, *15*, 1063. https://doi.org/10.3390/agronomy15051063
Guha, S., & Maheshwari, S.C., 1964. *In vitro* Production of Embryos from Anthers of Datura. Nature, 204(495), 497. doi: 10.1038/204497a0
Guha, S., & Maheshwari, S.C., 1966. Cell Division and Differentiation of Embryos in The Pollen Grains of *Datura In vitro*. Nature, 212, 97-98.
Guo, F., Huang, B.Q., Han, Y. Zee, S.Y., 2004. Fertilization in Maize Indeterminate Gametophyte1 Mutant. Protoplasma, 223, 111–120.
Gürel, S., Gürel, E., Kaya, Z., 2000. Doubled Haploid Plant Production from Unpollinated Ovules of Sugar Beet (*Beta vulgaris* L.). Plant Cell Rep., 19, 1155–1159.
Gürsöz, N., Abak, K., Pitrat, M., Rode, J.C., Dumas de Vaulx, R., 1991. Obtention of Haploid Plants

Induced by Irradiated Pollen in Watermelon (*Citrullus lanatus*). Cucurbit Genetic Coop., 14, 109-110.

Gürsöz-Sarı, N. 1990. Kavun (*Cucumis melo* var. *inodorus* ve *reticulatus*) ve Karpuzda (*Citrullus lanatus* (Thunb.) Mansf.) Işınlanmış Polenle Uyartılan *In situ* Partenogenetik Embriyolardan *In vitro* Kültürü ile Haploid Bitki Eldesi. Ç.Ü. Fen Bilimleri Enstitüsü Bahçe Bitkileri Anabilim Dalı Yüksek Lisans Tezi, 60 s.

Hale, B., Ferrie, A.M.R., Chellamma, S., Samuel, J.P., Phillips, G.C., 2022. Androgenesis-Based Doubled Haploidy: Past, Present, and Future Perspectives. Front Plant Sci. 7(12):751230.

Hansen, M., 2003. Protocol for Microspore Culture in Brassica. Maluszymski M., Kasha K.J., Forster B.P., ve Szarejko I.,) Doubled Haploid Production in Crop Plants: A Manual (ss. 217-222). Kluwer Academic.

Hayase, H., 1954. *Cucurbita* Crosses. Occurrence of a Haploid Twin Pair from a F1 Progeny of *C. maxima* × *C. moschata*. Jpn. J. Breed., 4, 55.

Heberle-Bors, E., 1989. Isolated Pollen Culture in Tobacco: Plant Reproductive Development in a Nutshell. *Sexual* Plant Reproduction, 2(1), 1-10.

Ho, I., Wan, J., Widholm, M., Rayburn, A.L., 1990. The Use of Stomatal Chloroplast Number for Rapid Determination of Ploidy Level in Maize. Plant Breeding, 105, 203-210.

Hofinger, B.J., Huynh, O.A., Jankowicz-Cieslak, J., Müller, A., Otto, I., Kumlehn, J., Till, B.J., 2013. Validation of Doubled Haploid Plants by Enzymatic Mismatch Cleavage. Plant Methods, **9**, 43.

Hoekstra, S., Vanzijderveld, M.H., Louwerse, J.D., Heidekamp, F., Vandermark, F., 1992. Anther and Microspore Culture of *Hordeum vulgare* L cv Igri. *Plant Science*, 86(1), 89-96. doi: 10.1016/0168-9452(92)90182-l

Hooghvorst, I., Torrico, O., Hooghvorst, S., Nogués, S., 2020. *In situ* Parthenogenetic Doubled Haploid Production in Melon "Piel De Sapo" for Breeding Purposes. Frontiers in Plant Science, 11, 378.

Hoseman, D., & Bossoutrot, D., 1983. Induction of Haploid Plants From *In vitro* Culture of Unpollinated Beet (*Beta vulgaris* L.). Z Pflanzenzuchtg, 91, 74-77.

Hu, H., Schrag, T.A., Peis, R., Unterseer, S., Schipprack, W., Chen, S., Lai, J., Yan, J., Prasanna, B.M., Nair, S.K., 2016. The genetic Basis of Haploid induction in Maize Identified with a Novel Genome-Wide Association Method. Genetics, 202, 1267-1276.

Hu, T., & Kasha, K.J., 1997. Improvement of Isolated Microspore Culture of Wheat (*Triticum Aestivum* L.) Through Ovary Co-Culture. Plant Cell Reports, 16(8), 520-525.

Hu, T.C., & Kasha, K.J., 1999. A cytological Study of Pretreatments Used to Improve Isolated Microspore Cultures of Wheat (*Triticum aestivum* L.) cv. Chris. Genome, 42(3), 432-441. doi: 10.1139/gen-42-3-432

Huang, B., Bird, S., Kemble, R., Simmonds, D., Keller, W., Miki, B., 1990. Effects of Culture Density, Conditioned Medium and Feeder Cultures on Microspore Embryogenesis in *Brassica napus* L cv Topas. Plant Cell Reports, 8(10), 594-597.

Huang, B.Q., & Sheridan, W.F., 1996. Embryo Sac Development in the Maize Indeterminate Gametophyte1 Mutant: Abnormal Nuclear Behavior and Defective Microtubule Organization. Plant Cell, 8, 1391–1407.

Husbands, A., Bell, E.M., Shuai, B., Smith, H.M., Springer, P.S., 2007. Lateral Organ Boundaries Defines a New Family of DNA Binding Transcription Factors and can Interact With Specific bHLH Proteins. Nucleic Acids Res., 35, 6663–6671.

Immonen, S., & Anttila, H., 2000. Media Composition and Anther Plating Fandor Production Of Androgenetic Green Plants From Cultivated Rye (*Secale cereale* L.). Journal of Plant Physiology, 156(2), 204-210.

Inagaki, M., 1985. Chromosome Doubling of the Wheat Haploids Obtained from Crosses with *Hordeum bulbosum* L. Jpn J Breed., 35, 193-195.

Indrianto, A., Heberle-Bors, E., Ouraev, A., 1999. Assessment of Various Stresses and Carbohyd-

rates for their Effect on the Induction of Embryogenesis in Isolated Wheat Microspores. Plant Science, 143(1), 71-79.
Ingouff, M., Rademacher, S., Holec, S., Šolji´c, L., Xin, N., Readshaw, A., Foo, S.H., Lahouze, B., Sprunck, S., Berger, F., 2010. Zygotic Resetting of the Histone 3 Variant Repertoire Participates in Epigenetic Reprogramming in *Arabidopsis*. Curr. Biol., 20, 2137–2143.
Irikova, T., Grozeva, S., Rodeva, V., 2011. Anther Culture in Pepper (*Capsicum annuum* L.) *In vitro*. Acta Physiol Plant., 33(5), 1559-1570. doi: 10.1007/s11738-011-0736-6
Ishii, T., Karimi-Ashtiyani, R., Houben, A., 2016. Haploidization via Chromosome Elimination: Means and Mechanisms. Annu. Rev. Plant Biol., 67, 421–438.
Ishizaka, H., & Uematsu, J., 1993. Production of Plants from Pollen in *Cyclamen persicum* Mill. through Anther Culture. Japanese Journal of Breeding, 43, 207-218.
Jiang, J., Gibbons, J.W., Moldenhauer, K.A.K., 2002. Developing an Efficient Haploid Rice Breeding System. BR Wells Rice Res Ser Stud., 495, 31-38
Jiang, F., Ryabova, D., Diedhiou, J., Hucl, P., Randhawa, H., Marillia, E. F., 2017. Trichostatin a Increases Embryo and Green Plant Regeneration In Wheat. Plant Cell Rep., 36, 1701-1706.
Jiaojiao, R., Wu, P., Trampe, B., Tian, X., Lübberstedt, T., Chen, S., 2017. Novel Technologies in Doubled Haploid Line Development. Plant Biotechnology Journal, 15, 1361-1370
Juðkeviéienë, D., Stanys, V., Bobinas, E., 2005. Gynogenesis Pecularities of *Allium* L. Vegetables Grown in Lithuania. Biologija, 3, 6-9.
Kószegi, D., Johnston, A.J., Rutten, T., Czihal, A., Altschmied, L., Kumlehn, J., Wüst, S.E.J., Kirioukhova, O., Gheyselinck, J., Grossniklaus, U., 2011. Members of the RKD Transcription Factor Family Induce an Egg Cell-Like Gene Expression Program. Plant J., 67, 280-291.
Kao, K.N., Saleem, M., Abrams, S., Pedras, M., Horn, D., Mallard, C., 1991. Culture Conditions for Induction of Green Plants From Barley Microspores by Anther Culture Methods. Plant Cell Reports, 9(11), 595-601.
Kandemir, N.& Saygılı İ., 2015. Apomixis: New Horizons in Plant Breeding. Turkish Journal of Agriculture and Forestry. Turk J. Agric. For., 39: 549-556
Kara, B., & Sarı, N., 2019. Sakız ve Siyah Kabak (*Cucurbita pepo* L.)'larda Ovül Kültüründe Spermidine ve Putrescine Uygulamalarının Haploid Bitki Elde Edilmesine Etkileri. Türkiye Tarımsal Araştırmalar Dergisi, 6 (2), 206-213.
Kara, E., 2024. Karpuzda (*Citrullus lanatus* L.) Ovaryum Kültürü ile Saf Hatların Geliştirilmesi. Doktora tezi. Sivas Bilim ve Teknoloji Üniversitesi Lisansüstü Eğitim Enstitüsü Fen Bilimleri Enstitütsü, Sivas. p. 149.
Karakullukçu, Ş., 1991. Değişik Patlıcan Genotiplerinde *İn Vitro* Androgenesis ve Haploid Bitki Oluşumunu Uyartıcı Bazı Etmenler Üzerinde Araştırmalar. Doktora Tezi, Ankara Üniversitesi, Fen Bilimleri Enstitüsü.
Karimi-Ashtiyani, R., Ishii, T., Niessen, M., Stein, N., Heckmann, S., Gurushidze, M., Banaei-Moghaddam, A.M., Fuchs, J., Schubert, V. Koch, K., 2015. Point Mutation Impairs Centromeric CENH3 Loading and Induces Haploid Plants. Proc. Natl. Acad. Sci. USA, 112, 11211-11216.
Kasha, K.J., & Kao, K.N., 1970. High Frequency Haploid Production in Barley (*Hordeum vulgare* L.). Nature, 225, 874-876
Kasha, K.J., & Maluszynski, M., 2003. Production of Doubled Haploids in Crop Plants. An Introduction. Maluszynski M., Kasha K.J., Forster B.P. ve Szarejko I., Doubled Haploid Production in Crop Plants-A Manual (ss. 1-4). Kluwer.
Kasha, K.J., Simion, E., Oro, R., Yao, Q.A., Hu, T.C., Carlson, A.R., 2001. An Improved *In vitro* Technique for Isolated Microspore Culture of Barley. Euphytica, 120(3), 379-385. doi: 10.1023/a:1017564100823
Keleş, D., Özcan, C., Pınar, H., Ata, A., Denli, N., Yücel, N. K., Büyükalaca, S., 2016. First Report of Obtaining Haploid Plants Using Tissue Culture Techniques in Spinach. HortScience, 51(6), 742-749.

Keller, W.A., & Armstrong, K.C., 1979. Stimulation of Embryogenesis and Haploid Production in *Brassica Campestris* Anther Cultures by Elevated Temperature Treatments. Theoret. Appl. Genetics, 55, 65–67.

Keller, W., Fan, Z., Pechan, P., Long, N., Grainger, J., 1987. An Efficient Method for Culture of Isolated Microspores in *B. napus* (Poster). In: 7th International Rapeseed Congress, Poznan, 11-14 May 1987, p 71.

Kelliher, T., Starr, D., Richbourg, L., Chintamanani, S., Delzer, B., Nuccio, M.L., Green, J., Chen, Z., McCuiston, J., Wang, W., 2017. MATRILINEAL, a Sperm-Specific Phospholipase, Triggers Maize Haploid Induction. Nature, 542, 105-109.

Kendall, J., 1934. A Parthenogenetic Aberrant Tobacco Plant. J. Hered., 21, 363-366.

Kermicle, J.L., 1969. Androgenesis Conditioned by a Mutation in Maize. Science, 166, 1422–1424.

Khanday, I., Skinner, D., Yang, B., Mercier, R., Sundaresan, V., 2019. A Male-Expressed Rice Embrygenic Trigger Redirected for Asexual Propagation Through Seeds. Nature, 565, 91–95.

Kielkowska, A., & Adamus, A. 2010. *In vitro* Culture of Unfertilized Ovules in Carrot (*Daucus carota* L.). Plant Cell Tiss Org Cult., 102, 309-319

Kim, M., Jang, I.C., Kim, J.A., Park E.J., Yoon, M., Lee, Y., 2008. Embryogenesis and Plant Regeneration of Hot Pepper (*Capsicum annuum* L.) through Isolated Microspore Culture. Plant Cell Reports, 27(3), 425-434.

Knox, R.E., Clarke, J.M., De Pauw, R.M., 2000. Dicamba and Growth Condition Effects on Doubled Haploid Production in Durum Wheat Crossed with Maize. Plant Breed., 119, 289-298.

Kobayashi, R.S., Sinden, S.L., Bouwkamp, J.C., 1993. Ovule Culture of Sweet Potato (*Ipomoea batatas*) and Closely Related Species. Plant Cell Tiss. Organ Cult., 32, 77-82.

Koi, S., Hisanaga, T., Sato, K., Shimamura, M., Yamato, K.T., Ishizaki, K., Kohchi, T., Nakajima, K., 2016. An Evolutionarily Conserved Plant RKD Factor Controls Germ Cell Differentiation. Curr. Biol., 26, 1-7.

Konieczny, R., Pawłowski, T.A., Surman, M., 2025. Identification of Proteins Associated with Gum Arabic-Enhancement of Androgenesis from Barley Anthers. Plant Cell Tiss Organ Cult., 160, 77.

Konzak, C.F., Polle, E.A., Liu, W., Zheng, Y., 1999. Methods for Generating Doubled-Haploid Plants. US Patent US6362393. PCT/US99/19498

Kostoff, D., 1929. An Androgenic Nicotina Haploid. Z. Zellforsch Mikrosk. Anat., 9, 640-642.

Krohn, N.G., Lausser, A., Juranic, M., Dresselhaus, T., 2012. Egg Cell Signaling by the Secreted Peptide ZmEAL1 Controls Antipodal Cellfate. Dev. Cell, 23, 219-225.

Kumar, H.G.A., Ravishankar, B.V., Murthy, H.N., 2004. The Influence of Polyamines on Androgenesis of *Cucumis sativus* L. European Journal of Horticultural Science, 69(5), 201-205.

Kumlehn, J., Kirik, V., Czihal, A., Altschmied, L., Matzk, F., Lörz, H., Bäumlein, H., 2001. Parthenogenetic Egg Cells of Wheat: Cellular and Molecular Studies. Sex. Plant Reprod., 14, 239–243.

Kundu, M., Dubey, A., Srivasta, M., Malik, S., 2017. Induction of Haploid Plants in Citrus Through Gamma-Irradiated Pollen and Ascertainment of Ovule Age for Maximum Recovery of Haploid Plantlets. Turk., J., Biol., 41, 469-483.

Kuppu, S., Tan, E.H., Nguyen, H., Rodgers, A., Comai, L., Chan, S.W., Britt, A.B., 2015. Point Mutations İn Centromeric Histone Induce Post-Zygotic Incompatibility and Uniparental Inheritance. PLoS Genet., 11, e1005494.

Kurtar, E.S., 1999. Kabakta (*Cucurbita pepo* L.) Haploid Embriyo Uyartımı ve Bitki Oluşturma Üzerine Araştırmalar. Dokora tezi, Ç.Ü. Fen Bilimleri Enstitüsü.

Kurtar, E.S., Sari, N., Abak, K. 2002. Obtention of Haploid Embryos and Plants Through Irradiated Pollen Technique in Squash (*Cucurbita pepo* L.). Euphytica, 127(3), 335-344.

Kurtar, E.S., Balkaya, A., Özbakır, MÖ., Ofluoğlu, T., 2009. Induction of Haploid Embryo and Plant Regeneration via Irradiated Pollen Technique in Pumpkin (*Cucurbita moschata* Duchesne ex. Poir). African Journal of Biotechnology, 8(21), 5944-5951

Kurtar, E.S., & Balkaya A., 2010. Production of *In vitro* Haploid Plants From *In situ* Induced Haploid Embryos in Winter Squash (*C. maxima* Duch. ex Lam.) via Irradiated Pollen. Plant Cell Tiss Org Cult., 102(3), 267-277.

Kurtar, E. S., Metin, D., Atakul, Z., Kal, Ü., Seymen, M., Takcı, E., & Toprak, F. Ç., 2025. Effects of gamma ray sources and irradiation doses on the haploidization frequency of citron watermelon (*Citrullus lanatus* var. citroides). Plant Cell, Tissue and Organ Culture (PCTOC), 160(3), 62.

Kuzuya, M., Hosoya, K., Yashiro, K., Tomita, K., Ezura, H., 2003. Powdery Mildew (*Sphaerotheca fuliginea*) Resistance in Melon is Selectable at the Haploid Level. J. Exp. Bot., 54, 1069-1074.

Lakshmi, T.V.R., Lakshmi, K.A., Isola, R.N., 2002. Culture of Unfertilized Embryo Sacs of Pearl Millet: *Pennisetum glaucum* (L.). R Br. Plant Tissue Cult., 12(1),19-25.

Lantos, C., Juhasz, A.G., Somogyi, G., Otvos, K., Vagi, P., Mihaly, R., Kristof, Z., Somogyi, N., Pauk, J., 2009. Improvement of Isolated Microspore Culture of Pepper (*Capsicum annuum* L.) via Co-culture with Ovary Tissues of Pepper or Wheat. Plant Cell Tissue Organ Cult., 97(3), 285-293.

Lantos, C., Paricsi, S., Zofajova, A., Weyen, J., Pauk, J., 2006. Isolated Microspore Culture of Wheat (*Triticum aestivum* L.) with Hungarian Cultivars. Acta Biologica Szegediensis, 50(1-2), 31-35.

Larsen, E.T., Tuvesson, I.K.D., Andersen, S.B., 1991. Nuclear Genes Affecting Percentage of Green Plants İn Barley (*Hordeum vulgare* L) Anther Culture. Theoretical and Applied Genetetics, 82(4), 417-420.

Laurie, D.A., & Bennett, M.D., 1986. Wheat-maize Hybridization. Can J Genet Cytol., 28, 313-316.

Laurie, D.A., & Bennett, M.D., 1989. The Timing of Chromosome Elimination in Hexaploid Wheat-Maize Crosses. Genome, 32, 953-961.

Lee, S.Y, Lee, J.H., Kwon, T.O., 2003. Selection of Salt-Tolerant Doubled Haploids in Rice Anther Culture. Plant Cell Tiss Organ Cult., 74(2), 143-149.

Lei, C., Chen, J.F., Qian, C.T., Zhang, Y.B., Song, H. 2004. Recovery of Cucumber (*Cucumis sativus* L.) Haploid Plants Through Pollination by Irradiated Pollens and Embryo Culture. Acta Bot Boreali- Occident Sin., 24(9),1739-1743.

Lentini, Z., Gonzalez, A., Tabares, E., Buitrago, M.E., Wędzony, M., 2020. Studies on Gynogenesis Induction in Cassava (*Manihot esculenta* Crantz) Unpollinated Ovule Culture. Frontiers in Plant Science, 11, 365

Lermontova, I., Kuhlmann, M., Friedel, S., Rutten, T., Heckmann, S., Sandmann, M., Demidov, D., Schubert, V., Schubert, I., 2013. *Arabidopsis* KINETOCHORE NULL2 is an Upstream Component for Centromerichistone H3 Variant cenH3 Deposition at Centromeres. Plant Cell, 25, 3389-3404.

Letarte, J., Simion, E., Miner, M., Kasha, K.J., 2006. Arabinogalactans and Arabinogalactan-Proteins Induce Embryogenesis in Wheat (*Triticum aestivum* L.) Microspore Culture. Plant Cell Reports, 24(12), 691-698.

Li, L., Xu, X., Jin, W., Chen, S., 2009. Morphological and Molecular Evidences for DNA Introgression in Haploid Induction via a High Oilinducer CAUHOI in Maize. Planta, 230, 367-376.

Li, H., Soriano, M., Cordewener, J., Muiño, J. M., Riksen, T., Fukuoka, H., 2014. The Histone Deacetylase Inhibitor Trichostatin a Promotes Totipotency in the Male Gametophyte. Plant Cell, 26, 195–209.

Li, F., Cheng, Y., Zhao, X., Yu, R., Li, H., Wang, L., Li, S., Shan, Q., 2020. Haploid Induction via Unpollinated Ovule Culture in *Gerbera hybrida*. Scientific Reports, 10(1), 1-10.

Li, Y., Lin, Z., Yue, Y., Zhao, H., Fei, X., Liu, C., Chen, S., Lai, J., Song, W., 2021. Loss-of-Function Alleles of ZmPLD3 Cause Haploid Induction in Maize. Nat. Plants, 7, 1579-1588.

Liang, G.H., Xu, A., Tang-Hoang 1987. Direct Generation of Wheat Haploids via Anther Culture. Crop Science, 27(2), 336-339.

Lichter, R., 1982. Induction of Haploid Plants From Isolated Pollen of *Brassica napus*. Zeitschrift für Pflanzenphysiologie, 105(5), 427-434.

Likyelesh, G., Ashok, K.S., Horst, L., Jochen, K., 2006. Gynogenic Plant Regeneration from Unpollinated Flower Explants of Eragrostis Tef (Zuccagni) Trotter. Plant Cell Rep., 25, 1287-1293.

Lim, W., & Earle, E.D., 2008. Effect of *In vitro* and *In vivo* Colchicine Treatments on Pollen Production and Fruit Recovery on Melon Plants Obtained after Pollination with Irradiated Pollen. Plant Cell Tiss Org Cult., 95(1), 115-124.

Liu, C., Li, W., Zhong, Y., Dong, X., Hu, H., Tian, X., Wang, L., 2015. Fine Mapping of qhir8 Affecting *In vivo* Haploid Induction in Maize. Theor. Appl. Genet., 128, 2507-2515.

Liu, C., Li, X., Meng, D., Zhong, Y., Chen, C., Dong, X., Xu, X., Chen, B., Li, W., Li, L., 2017. A 4-bp Insertion at ZmPLA1 Encoding A Putative Phospholipase a Generates Haploid Induction in Maize. Mol. Plant, 10, 520–522.

Liu, C., Zhong, Y., Qi, X., Chen, M., Liu, Z., Chen, C., Tian, X., Li, J., Jiao, Y., Wang, D., 2020. Extension of The *İn vivo* Haploid Induction System from Diploid Maize to Hexaploid Wheat. Plant Biotechnol. J., 18, 316–318.

Luk, Y.S.F., Ignatova, S.A., Sozinov, A.A., 1983. Use of *In vitro* Techniques for Producing Haploids in Barley and Triticale. Tagungsbericht, Akademie der Landwirtschaftswissenschaften der Deutschen Demokratischen Republik, 207, 41-48.

Luthar, Z., & Bohanec, B., 1999. Induction of Direct Somatic Embryogenesis in Onion (*Allium cepa* L.) Using a Two Step Flower or Ovary Culture. Plant Cell Rep.,18, 797-802.

Lux, H., Herrmann, L., Wetzel, C., 1990. Production of Haploid Sugarbeet (*Beta vulgaris* L.) by Culturing Unpollinated Ovules. Plant Breed., 104, 177-183

Maheshwari, S., Tan, E.H., West, A., Franklin, F.C., Comai, L., Chan, S.W., 2015. Naturally Occurring Differences in CENH3 Affect Chromosome Segregation in Zygotic Mitosis of Hybrids. PLoS Genet., 11, e1004970.

Maluszynska, J., 2003. Cytogenetic Tests for Ploidy Level Analyses Chromosome Counting. Maluszynski M., Kasha K.J., Forster B.P. ve Szarejko I., Doubled Haploid Production in Crop Plants (ss. 391-395). Springer.

Manzoor, A., Ahmad, T., Bashir, M.A., Hafiz, I.A., Silvestri, C., 2019. Studies on Colchicine-Induced Chromosome Doubling for Enhancement of Quality Traits in Ornamental Plants. Plants (Basel), 8(7), 194.

Marciniak, K., Kaczmarek, Z., Adamski, T., Surma, M., 2003. The Anther-Culture Response of Triticale Line X Tester Progenies. Cellular and Molecular Biology Letters, 8 (2), 343-351.

Martı´nez, L.E., Aguero, C.B., Lopez, M.E., Galmarini, C.R., 2000. Improvement of *In vitro* Gynogenesis Induction in Onion (*Allium cepa* L.) Using Polyamines. Plant Sci., 156, 221-226.

Mathur, D.S., Aman, M., Sarkar, K.R., 1980. Induction of Maternal Haploids in Maize Through Heat Treatment of Pollen. Curr. Sci., 49, 744-746.

Matzk, F., Meyer, H.M., Bäumlein, H., Balzer, H.J., Schubert, I., 1995. A Novel Approach to the Analysis of The Initiation of Embryo Development in Gramineae. Sex. Plant Reprod., 8, 266-272.

McCormick, S., 2004. Control of Male Gametophyte Development. The Plant Cell 16 Suppl., 142-53.

Metwally, E.I., Moustafa, S.A., El-Sawy, B.I., Haroun, S.A., Shalaby, T.A., 1998. Production of Haploid Plants from *In vitro* Culture of Unpollinated Ovules of *Cucurbita pepo*. Plant Cell Tiss Organ Cult., 52, 117-121.

Miah, M.A.A., Earle, E.D., Khush, G.S., 1985. Inheritance of Callus Formation Ability in Anther Cultures of Rice, *Oryza sativa* L. Theoretical and Applied Genetics, 70(2),113-116.

Monakhos, S.G., Nguen, M.L., Bezbozhnaya, A.V., Monakhos, G.F., 2014. A Relationship Between Ploidy Level and The Number of Chloroplasts in Stomatal Guard Cells in Diploid and Amphidiploid Brassica Species. Sel'skokhozyaistvennaya Biologiya (Agricultural Biology), 5, 44-54.

Montelongo-Escobedo, H., Rowe, P.R., 1969. Haploid Induction in Potato: Cytological Basis for the Pollinator Effect. Euphytica, 18, 116-123.

Mujeeb-Kazi, A., Riera-Lizarazu, O., 1997. Polyhaploid Production in the Triticeae by Sexual Hybridization. Mohan-Jain S., Spory S.K. and Veilleux R.E., *In vitro* Haploid Production in Higher

Plants (ss. 276-295). Kluwer.
Mukhambetzhanov, S.K., 1997. Culture of Nonfertilized Female Gametophytes *In vitro*. Plant Cell Tiss Org Cult, 48, 111-119.
Murashige, T., & Skoog, F., 1962. A Revised Medium for Rapid Growth and Bio Bioassays with Tobacco Tissue Cultures. Physiol Plantarum, 15(3), 473-497.
Murovec, J., & Bohanec, B., 2012. Haploids and Dihaploid in Plant Breeding. Abdurrakhamonov I.Y., Plant breeding (ss. 87-106). Intech.
Musial, K., & Przywara, L., 1998. Influence of Irradiated Pollen on Embryo and Endosperm Development in Kiwifruit. Ann Bot., 82, 747–756.
Nakajima, I., Kobayashi, S., Nakamura, Y., 2000. Embryogenic Callus Induction and Plant Regeneration from Unfertilized Ovule of 'Kyoho' Grape. J Japan Soc Hort Sci., 69(2), 186-188.
Neuffer, M.G., Coe, E.H., Wessler, S.R., 1997. Mutants of Maize. Cold Spring Harbor Laboratory Press.
Niemirowicz-Szczytt, K., Dumas de Vaulx, R., 1989. Preliminary Data of Haploid Cucumber (*Cucumis sativus* L.) Induction. Cucurbit Genet Coop Rpt., 12, 24-25.
Nitsch, C., 1974. Pollen Culture a New Technique for Mass Production of Haploid and Homozygous Plants. Kasha K.J., Haploids in Higher Plants, Advances And Potential. II. Methods of Producing Haploids (ss. 123-135). University of Guelph.
Nitsch, J.P., Nitsch, C. 1969. Haploid Plants from Pollen Grains. *Science*, 163, 85-87.
Noga, A., Skrzypek., E., Warchoł. M., Czyczyło-Mysza, I., Dziurka, K., Marcińska, I., Juzoń, K., Warzecha, T., Sutkowska, A., Nita, Z., Werwińska, K., 2016. Conversion of Oat (*Avena sativa* L.) Haploid Embryos into Plants in Relation to Embryo Developmental Stage and Regeneration Media. *In Vitro* Cell. Dev. Biol. Plant., 52, 590-597.
Nowicka, A., Juzoń, K., Krzewska, M., Dziurka, M., Dubas, E., Kopeć, P., 2019. Chemically Induced DNA Demethylation Alters the Effectiveness of Microspore Embryogenesis in Triticale. Plant Sci. 287:110189.
Obert, B., & Barnabas, B., 2004. Colchicine Induced Embryogenesis in Maize. Plant Cell Tissue Organ Cult., 77(3), 283-285.
Ochatt, S.J., Patat-Ochatt, E.M., Moessner, A., 2011. Ploidy Level Determination within the Context of *In Vitro* Breeding. Plant Cell Tiss Organ Cult., 104, 329-341.
Olesen, A., Andersen, S.B., Due, I.K., 1988. Anther Culture Response in Perennial Ryegrass (*Lolium perenne* L.). Plant Breed 101:60-65.
Osolnik, B., Bohanec, B., Jelaska, S., 1993. Stimulation of Androgenesis in White Cabbage (*Brassica oleracea* var capitata) Anthers by Low-Temperature and Anther Dissection. Plant Cell Tiss. Org. Cult., 32(2), 241-246.
Otani, M., & Shimada, T., 1994. Pollen Embryo Formation and Plant Regeneration from Cultured Anthers of Tetraploid Wheat. Journal of Genetics and Breeding, 48(1),103-106.
Özdemir-Çelik, B., 2018. The Effects of Different Applications to Induce Haploid Embryo and Plant Formation on Eggplant (*Solanum melongena* L.) Microspore Culture, Doktora tezi, Akdeniz Üniv., Fen Bilimleri Entitüsü.
Paksoy, M., Ellialtıoğlu, Ş., Sarı, N., Abak, K. 1995. Bazı Brassica sp. türlerinde anter kültürüne etki eden faktörler üzerinde araştırmalar. DOĞA TU Botanik 19, 281-286.
Palmer, C.E., Keller, W.A., Kasha, K.J., 2005. Haploids in Crop Improvement II, Biotechnology in Agriculture and Forestry Vol. 56 (Palmer, C.E., Keller, W.A. and Kasha, K.J., eds) Heidelberg, Germany: Springer. p. 318.
Pamuk, S., Sari, N., Solmaz, I., Mancak, I., 2020. Development of Double-Haploid Lines in Galia-Type Melons (*Cucumis melo* var. *cantalupesis*) Using *In vitro* Colchicine Treatments. Acta Hortic., 1282, 149-156.
Pandey, K.K., Przywara, L., Sanders, P.M., 1990. Induced Parthenogenesis in Kiwifruit (*Actinidia deliciosa*) through the Use of Lethally irradiated Pollen. Euphytica, 51, 1-9.

Park, D.S., Park, S.K., Lee, B.C., Song, S.Y., Jun, N.S., Manigbas, N.L., Hee Nam, M., Seong Jeon, J., Deok Han, C., 2009. Molecular Characterization and Physico-Chemical Analysis of a New Giant Embryo Mutant Allele (ge) in Rice (*Oryza sativa* L.). Genes Genomics, 31, 277-282.

Pechan, P.M., & Smykal, P. 2001. Androgenesis: Affecting the Fate of the Male Gametophyte. Physiologia Plantarum, 111(1),1-8. doi: 10.1034/j.1399-3054.2001.1110101.x

Perera, P.I.P., Hocher, V., Verdeil, J.L., Doulbeau, S., Yakandawala, D.M.D., Weerakoon, L.K., 2007. Unfertilized Ovary: a Novel Explants for Coconut (*Cocos nucifera* L.). Plant Cell Rep., 26, 21-28.

Perera, P.I.P., Perera, L., Hocher, V., Verdeil, J-L., Yakandawala, D.M.D., Weerakoon, L.K. 2008. Use of SSR Markers to Determine the Anther-Derived Homozygous Lines in Coconut. Plant Cell Rep 27:1697-1703

Perera, P.I.P., Yakandawala, D.M.D., Hocher, V., Verdeil, J.L., Weerakoon, L.K., 2009. Effect of Growth Regulators on Microspore Embryogenesis in Coconut Anthers. Plant Cell Tissue Organ Cult., 96, 171-180.

Piosik, Ł., Zenkteler, E., Zenkteler, M., 2016. Development of Haploid Embryos and Plants of *Lactuca sativa* Induced by Distant Pollination with *Helianthus annuus* and *H. tuberosus*. Euphytica, 208, 439-451.

Powell, W. 1990. Environmental and Genetical Aspects of Pollen Embryogenesis. Bajaj Y.P.S., Biotechnology in agriculture and forestry, vol. 12: Haploids in Crop Improvement (ss. 45-65). Springer-Verlag.

Prem, D., Gupta, K., Sarkar, G., Agnihotri, A., 2008. Activated Charcoal Induced High Frequency Microspore Embryogenesis and Efficient Doubled Haploid Production in *B. juncea*. Plant Cell Tissue Org. Cult., 93(3), 269-282.

Prigge, V., Xu, X., Li, L., Babu, R., Chen, S., Atlin, G.N., Melchinger, A.E., 2012. New Insights into the Genetics of *In vivo* Induction of Maternal Haploids, the Backbone of Doubled Haploid Technology in Maize. Genetics, 190, 781-793.

Przyborowski, J.A., & Niemirowicz-Szczytt, K., 1994. Main Factors Affecting Cucumber (*Cucumis sativus* L.) Haploid Embryo Development and Haploid Plant Characteristics. Plant Breed, 112(1), 70-75

Puddephat, I.J., Robinson, H.T., Smith, B.M., 1999. Influence of Stock Plant Pretreatment on Gynogenic Embryo Induction From Buds of Onion. Plant Cell Tissue Organ Cult., 57, 145-148.

Pulli, S., & Guo, J.D., 2003. Microspore Culture of Rye. Maluszymski M., Kasha K.J., Forster B.P. and Szarejko I.,) Doubled Haploid Production in Crop Plants: A Manual (ss. 151–154). Kluwer Academic.

Raina, S.K., & Irfan, S.T., 1998. High-frequency Embryogenesis and Plantlet Regeneration from Isolated Microspores of Indica Rice. Plant Cell Reports, 17(12), 957-962.

Raquin, C., 1985. Induction of Haploid Plants by *In vitro* Culture of Petunia Ovaries Pollinated with Irradiated Pollen. Z Pflanzenzucht, 94, 166-169.

Ravi, M., & Chan, S.W., 2010. Haploid Plants Produced by Centromere-Mediated Genome Elimination. Nature, 464, 615-618.

Ravi, M., Marimuthu, M.P.A., Tan, E.H., Maheshwari, S., Henry, I.M., Marin-Rodriguez, B., Urtecho, G., Tan, J., Thornhill, K., Zhu, F., 2014. A Haploid Genetics Toolbox for *A. thaliana*. Nat. Commun., 5, 5334.

Redha, A., & Talaat, A., 2008. Improvement of Green Plant Regeneration by Manipulation of Anther Culture Induction Medium of Hexaploid Wheat. Plant Cell Tissue Organ Culture, 92, 141-146.

Reed, S.M., 2005. Haploid Cultures. Gray D.J., Plant development and Biotechnology (ss. 225-234). CRC Press.

Reinbothe, S., Pollmann, S., Reinbothe, C., 2003. *In situ* Conversion of Protochlorophyllide to Protochlorophyllide A in Barley Evidence for a Novel Role of 7-formyl Reductase in the Prolamellar Body of Etioplasts. J Biol Chem., 278(2), 800-806. doi: 10.1074/jbc.M209737200

Ren, J., Wu, P., Trampe, B., Tian, X., Lubberstedt, T., Chen, S., 2017. Noveltechnologies in Doubled Haploid Line Development. Plant Biotechnol. J., 15, 1361-1370.

Riera-Lizarazu, O., Mujeeb-Kazi, A., William, M.D.H.M., 1992. Maize (*Zea mays* L.) Mediated Polyhaploid Production in Some Triticeae Using a Detached Tiller Method. J Genet Breed, 46, 335-346

Rietz, S., Dermendjiev, G., Oppermann, E., Tafesse, F.G., Effendi, Y., Holk, A., Parker, J.E., Teige, M., Scherer, G.F., 2010. Roles of *Arabidopsis* Patatin-Related Phospholipases a in Root Developent are Related to Auxin Responses and Phosphate Deficiency. Mol. Plant, 3, 524–538.

Rode, J.C., & Dumas de Vaulx, R., 1987. Obtention des Plantes Haploides de Carotte (*Daucus carota* L.) İssues de Parthénogenese Induite *In situ* par du Pollen Irradié et Culture *In vitro* des Graines Immatures. CR Acad Sci Paris, 305, 225-229.

Rogers, O.M., & Ellis, J.H., 1966. Pollen Nuclear Division Preveneted with Toludine Blue in *Vinca rosea* L. HortScience, 1 (2), 62-63.

Röber, F.K., Gordillo, G.A., Geiger, H.H., 2005. *In vivo* Haploid Induction in Maize: Performance of New Inducers and Significance of Doubled Haploid Line in Hybrid Breeding. Maydica, 50, 275-284

Rövekamp, M., Bowman, J.L., Grossniklaus, U., 2016. Marchantia MpRKD Regulates the Gametophyte-Sporophyte Transition by Keeping Egg Cells Quiescent in the Absence of Fertilization. Curr. Biol., 26, 1–8.

Ruth, S.K., Stephen, L.S., John, C.B., 1993. Ovule Culture of Sweet Potato (*Ipomoea batatas*) and Closely Related Species. *Plant Cell Tiss Organ Cult.,* 32, 77-82.

Sabehat, A., Lurie, S., Weiss, D., 1998. Expression of Small Heat-Shock Proteins at Low Temperatures a Possible Role in Protecting Against Chilling Injuries. Plant Physiology, 117(2), 651-658. doi: 10.1104/pp. 117.2.651

San Noeum, L.H., 1976. Haploides d'*Hordeum vulgare* L. par Culture *In vitro* non Fecondes. Ann Amelior Plant, 26, 751-754

San Noeum, L.H., & Gelebart, P., 1986. Production of Gynogenic Haploids. Vasil I.K., Cell culture and Somatic Cell Genetics of Plants vol 3 (ss. 305-322). Academic.

Sanei, M., Pickering, R., Kumke, K., Nasuda, S., Houben, A., 2011. Loss of Centromeric Histone H3 (CENH3) from Centromeres Precedes Uniparental Chromosome Elimination in Interspecific Barley Hybrids. Proc. Natl. Acad. Sci. USA, 108, E498–E505.

Sangwan, R.S., & Sangwannorreel, B.S., 1987. Ultrastructural Cytology of Plastids in Pollen Grains of Certain Androgenic and Nonandrogenic Plants. Protoplasma, 138(1),11-22. doi: 10.1007/bf01281180

Santos, E.K., & Zanettini, M.H.B., 2002. Androgênese: Uma Rota Alternativa no Desenvolvimento do Pólen. Ciencia Rural, 32(1), 165-173.

Sarı, N., Abak, K., Pitrat, M., Dumas de Vaulx, R., 1992. Induction of Parthenogenetic Haploid Embryos and Plant Obtention in Melon (*Cucumis melo* L. var. *inodorus* Naud. and *C. melo* L. var. *reticulatus* Naud.). Turk J Agr For., 16, 302-314.

Sarı, N., Ellialtıoğlu, Ş., Solmaz, İ., 2014. Haploidi ve Katlanmış Haploidi Tekniğinin Sebze Islahında Kullanımı. 10. Sebze Tarımı Sempozyumu, 2-4 Eylül 2014, Tekirdağ, Bahçe Bilimi Yayın No: 4, 76-88.

Sari, N., & Abak, K. 1996. Effect of Colchicine Treatment with Different Doses and Periods on *In vitro* Chromosome Duplication in Haploid Watermelon. Turk J Agric Forestry, 20 (6), 555-559.

Sari, N., Abak, K., Pitrat, M., 1999. Comparison of Ploidy Level Screening Methods in Watermelon: *Citrullus lanatus* (Thunb.) Matsum. and Nakai. Sci Hortic., 82 (3), 265-277.

Sari, N., Abak, K., Pitrat, M., Rode, J.C., Dumas de Vaulx, R., 1994. Induction of Parthenogenetic Haploid Embryos after Pollination by Irradiated Pollen in Watermelon. HortScience, 29, 1189-119

Sato, S., Katoh, N., Iwai, S., Hagimori, M., 2002. Effect of Low Temperature Pretreatment of Buds or

Inflorescence on Isolated Microspore Culture in *B. rapa* (syn. *B. campestris*). Breeding Science, 52, 23-26.

Sato, S., Katoh, N., Yoshida, H., Iwail, S., Hagimori, M., 2000. Production of Doubled Haploid Plants of Carnation (*Dianthus caryophyllus* L.) by Pseudofertilized Ovule Culture. Sci Hortic., 83, 301-310.

Sauton, A., 1989. Haploid Gynogenesis in *C. sativus* by İrradiated Pollen. Cucurbit Genet Coop., 12, 22-23.

Sauton, A., & Dumas de Vaulx, R., 1987. Induction of Gynogenetic Haploid Plants in Muskmelon (*Cucumis melo* L.) by Use of irradiated Pollen. Agronomie, 7, 141-148.

Sauton, A., 1988. Effects of Season and Genotype on Gynogenic Haploid Production in Musk Melon, *Cucumis melo* L., Scientia Hortic., 35, 71-75.

Sayed Tabatabaei, B. E., Saeidi, G. and Sabzalian, M.R., 2007. Genetic Analysis of Androgenetic Traits in Wheat (*Triticum aestivum* L.). Iranian Journal of Biotechnology, *5*(1), 34-41.

Scott, P., Lyne, R.L., Aprees T., 1995. Metabolism of Maltose and Sucrose by Microspores Isolated from Barley (*Hordeum vulgare* L). Planta, 197(3), 435-441.

Shalaby, T.A., 2007. Factors Affecting Haploid Induction Through *In vitro* Gynogenesis in Summer Squash (*Cucurbita pepo* L.). Sci Hortic., 115, 1-6.

Shariatpanahi, M.E., Bal, U., Heberle-Bors, E., Touraev, A., 2006. Stresses Applied for the Re-programming of Plant Microspores Towards *In vitro* Embryogenesis. Physiol Plantarum, 127(4), 519-534.

Shatskaya, O.A., Zabirova, E.R., Shcherbak, V.S., Chumak, M.V., 1994. Mass Induction of Maternal Haploids in Corn. MNL, 68, 51.

Sheng, H., Gao, P., Yang, C., Quilichini, T.D., Kochian, L.V., Datla, R., Xiang, D., 2025. Advances in Genome Editing Through Haploid Induction Systems. Int J Mol Sci., 16;26(10):4779.

Sibi, M.L., Kobbaissi, A., Shekafandeh, A., 2001. Green Haploid Plants from Unpollinated Ovary Culture in Tetraploid Wheat (*Triticum durum* Defs.). Euphytica, 122, 351-359.

Sitch, L.A., & Snape, J.W., 1986. Doubled Haploid Production in Winter Wheat and Triticale Genotypes Using the *Hordeum bulbosum* System. Euphytica, 35, 1045-1051

Solís, M.T., El-Tantawy, A.A., Cano, V., Risueño, M.C., and Testillano, P.S., 2015. 5-Azacytidine Promotes Microspore Embryogenesis Initiation By Decreasing Global DNA Methylation, but Prevents Subsequent Embryo Development in Rapeseed and Barley. Front. Plant Sci., 6:472. doi: 10.3389/ fpls.2015.00472

Sopory, S.K., & Munshi, M., 1997. Anther Culture. Jain S.M., Sopory S.K., ve Veilleux R.E., *In vitro* haploid Production in Higher Plants (ss. 145-176). Kluwer Academic.

Sulistyaningsih, E., Aoyagi, Y., Tashiro, Y., 2006. Flower bud Culture of Shallot (*Allium cepa* L. *aggregatum* group) with Cytogenetic Analysis of Resulting Gynogenic Plants and Somaclones. Plant Cell Tissue Organ Cult., 86, 249-255.

Sunderland, N., & Dunwell, J.M., 1977. Anther and Pollen Culture. Street H.E., Plant Tissue and Cell Culture (ss. 223-265). Blackwell.

Sunderland, N., & Roberts, M. 1979. Cold-pretreatment of Excised Flower Buds in Float Culture of Tobacco Anthers. Annals of Botany, 43(4), 405-414.

Sunderland, N., & Xu, ZH., 1982. Shed Pollen Culture in *Hordeum vulgare*. Journal of Experimental Botany, 33(136),1086-1095. doi: 10.1093/jxb/33.5.1086

Szarejko, I., & Forster, B.P., 2007. Doubled Haploidy and iIduced Mutation. Euphytica, 158, 359-370.

Şeker, E., 2015. Anter Kültüründe Sitolojik Yöntemlerin Kullanımı. Yüksek Lisans Tezi, Ankara Üniv. Fen Bilimeri Enstitüsü.

Takahata, Y., Brown, D.C.W., Keller, W.A., 1991. Effect of Donor Plant-Age and Inflorescence Age on Microspore Culture of *Brassica napus* L. *Euphytica*, 58(1), 51-55. doi: 10.1007/bf00035339

Tanaka, I., 1996. Haploid Protoplasts: Pollen Protoplasts. Jain S.M., Sopory S.K. ve Veilleux R.E., *In vitro* haploid Production in Higher Plants. Current Plant Science and Biotechnology in Agri-

culture, vol 24. Springer.
Tang, F., Tao, Y., Zhao, T., Wang, G., 2006. *In vitro* Production of Haploid and Double Haploid Plants from Pollinated Ovaries of Maize (*Zea mays*). Plant Cell Tissue Organ Cult., 84, 233-237.
Telmer, C.A., Newcomb, W., Simmonds, D.H., 1993. Microspore Development in *Brassica napus* and the Effect of High-Temperature on Division *In vivo* and *In vitro*. Protoplasma, 172(2-4),154-165.
Terzioğlu, Ş., Ellialtıoğlu, Ş., Abak, K., 2000. İnkübasyon Koşullarının Biber Anter Kültüründe Embriyo Oluşumu Üzerine Etkisi. III. Sebze Tarımı Simpozyumu, 11-13 Eylül 2000, Isparta, s: 233-237.
Thomas, D.T., Bhatnagar, A.K., Razdan, M.K., Bhojwani, S.S., 1999. A Reproducible Protocol for the Production of Gynogenic Haploids of Mulberry, *Morus alba* L. Euphytica, 110, 169-173.
Thomas, W.T.B., Newton, A.C., Wilson, A., Booth, A., Macaulay, M., Keith, R., 2000. Development of Recombinant Chromosome Substitution Lines: A Barley Resource. SCRI Annual Report, 1999/2000, 99-100.
Tian, S., Zhang, J., Zhao, H., Zong, M., Li, M., Wang, J., Zhang, J., Ren, Y., Zhang, H., Li, S., 2023. Production of Double Haploid Watermelon via Maternal Haploid Induction. Plant Biotechnology Journal, 21, 1308-1310.
Tian, X., Qin, Y., Chen, B., Liu, C., Wang, L., Li, X., Dong, X., Liu, L., Chen, S., 2018. Hetero-Fertilization along With Failed Egg-Sperm Cell Fusion Supports Single Fertilization Involved in *In vivo* Haploidinduction in Maize. J. Exp. Bot., 69, 4689–4701.
Torp, A.M., Hansen, A.L., Andersen, S.B., 2001. Chromosomal Regions Associated with Green Plant Regeneration in Wheat (*Triticum aestivum* L.) Anther Culture. Euphytica, 119(3), 377-387. doi: 10.1023/a:1017554129904
Tosca, A., Pandolfi, R., Citterio, S., Fasoli, A., Sgorbati, S., 1995. Determination by Flow Cytometry of the Chromosome Doubling Capacity of Colchicine and Oryzalin in Gynogenetic Haploids of Gerbera. Plant Cell Reports, 14, 455-458.
Touraev, A., Ilham, A., Vicente, O., Heberle-Bors, E., 1996a. Stress-induced Microspore Embryogenesis in Tobacco: An Optimized System for Molecular Studies. Plant Cell Reports, 15(8), 561-565.
Touraev, A., Indrianto, A., Wratschko, I., Vicente, O., HeberleBors, E., 1996b. Efficient Microspore Embryogenesis in Wheat (*Triticum aestivum* L) Induced by Starvation at High Temperature. Sexual Plant Reproduction, 9(4), 209-215. doi: 10.1007/bf02173100
Touraev, A., Pfosser, M., Vicente, O., HeberleBors, E., 1996c. Stress as the Major Signal Controlling the Developmental Fate of Tobacco Microspores: Towards a Unified Model of Induction of Microspore/Pollen Embryogenesis. Planta, 200(1), 144-152.
Touraev, A., Pfosser, M., Heberle-Bors, E., 2001. The Microspore: A Haploid Multipurpose Cell. Advanced Botanical Research, 35, 53-109. doi: 10.1016/s0065-2296(01)35004-8
Truong Andre, I., Demarly, A., 1983. Otaining Plants by *In vitro* Culture of Unfertilized Maize Ovaries (*Zea mays* L.) and Preliminary Studies on progeny of a Gynogetic Plants. Z. Für Phlanzenzücht, 92, 309-320.
Tsay, H.S., 1982. The Microspore Development and Haploid Embryogenesis of Anther Culture with Five Nitrogen Doses to the Donor Tobacco Plants. Journal of Agricultural Research of China, 31(1), 1-13.
Tsunewaki, K., & Mukai, Y., 1990. Wheat Haploids through the Salmon Method. In Biotechnology Agriculture Forestry; Bajaj, Y.P.S., Ed.; Springer: Berlin/Heidelberg, Germany, 13, 460–478.
Tulukoğlu, K.S., & Sarı, N., 2014. Karpuzlarda Anter ve Ovül Kültüründe Soğuk Uygulaması, Thidiazuron (TDZ) ve 2,4 D Uygulamalarının Haploid Embriyo Uyartımına Etkileri. 10. Sebze Tarımı Sempozyumu, 2-4 Eylül 2014, Tekirdağ, Bahçe Bilimi Yayın No: 4, 289-296.
Tuncer, B., & Yanmaz, R., 2007. Haploid Bitki Elde Etme Yollarından Biri: Mikrospor Kültürü. Alatarım 6 (2), 1-8.

Ushiyama, T., Shimizu, T., Kuwabara, T., 1991. High Frequency of Haploid Production of Wheat through Intergeneric Cross with Teosinte. Jpn J Breed, 41, 353-357.

Vagera, J., Novotny, J., Ohnoutkova, L., 2004. Induced Androgenesis *In vitro* in Mutated Populations of Barley, *Hordeum vulgare*. Plant Cell Tissue Organ Cult.,, 77(1), 55-61. doi: 10.1023/B:TICU.0000016504.82810.a4

van Leeuwen, H., Monfort, A., Zhang, H.B., Puigdomenech, P., 2003. Identification and Characterisation of a Melon Genomic Region Containing a Resistance Gene Cluster from a Constructed BAC Library. Microcolinearity Between *Cucumis melo* and *Arabidopsis thaliana*. Plant Mol. Biol., 51, 703-718.

Van-Creij, M.G.M., Kerckhoffs, D.M.F.J., De Bruijn, S.M., Vreugdenhil, D., Van Tuyl, J.M., 2000. The Effect of Medium Composition on Ovary-Slice Culture and Ovule Culture in interspecific *Tulipa gesneriana* Crosses. Plant Cell Tiss Organ Cult., 60, 61-67.

Verde, I., E. Vendramin, Micali, S., Dettori, M.T., Sosinski, B., 2012. Genome Sequencing Initiative. Kole, C. and Abbott A.G., Genetics, Genomics and Breeding of Stone Fruits: Genetics, Genomics and Breeding of Crop Plants (ss. 244-269). CRC Press, Taylor & Francis.

Vural, G.E., Arı, E., Zengin, S., Ellialtıoğlu, Ş.Ş., 2019. Development of Androgenesis Studies on Eggplant (*Solanum melongena* L.) in Turkey from Past to Present. Hasanuzzaman, M., Sustainable Crop Production (ss.1-27). Intechopen.

Wang, S., Jin, W., Wang, K., 2019a. Centromere Histone H3- and Phospholipase-Mediated Haploid Induction in Plants. Plant Methods, 15, 42.

Wang, H.M., Enns, J.L., Nelson, K.L., Brost, J.M., Orr, T.D., and Ferrie, A.M.R. 2019b. Improving the Efficiency of Wheat Microspore Culture Methodology-Evaluation of Pretreatments, Gradients, and Epigenetic Chemicals. Plant Cell Tissue Organ Cult., 139, 589–599. doi: 10.1007/s11240-019-01704-5

Wang, N., Gent, J.I., Dawe, R.K., 2021. Haploid Induction by a Maize cenh3 Null Mutant. Sci. Adv., 7, eabe2299.

Weber, S., Ünker, F., Friedt, W., 2005. Improved Doubled Haploid Production Protocol for *Brassica napus* Using Microspore Colchicine Treatment *In vitro* and Ploidy Determination by Flow Cytometry. Plant Breeding, 124, 511-513.

Wędzony, M., Marciňska, I., Ponitka, A., Ślusarkiewicz-Jarzina, A., Woźna, J., 1998. Factors Influencing Triticale Doubled Haploid Production by Means of Crosses with Maize. Juskiw P., Proceedings of the Fourth International Triticale Symposium Vol. 1. Lectures. Red Deer, Canada, July 26-30, 45-52.

Wędzony, M., 2003. Protocol for Anther Culture in Hexaploid Triticale (*Triticosecale wittm*). Maluszynski M., Kasha K.J., Forster B.P. ve Szarejko I., Doubled Haploid Production in Crop Plants-A Manual (ss. 123-128). Kluwer.

Wędzony, M., Forster, B.P., Zur, I., Golemiec, E., Szechynska-Hebda, M., Dubas, E., Gotebiowska, G., 2009. Progress in Doubled Haploid Technology in Higher Plants. Touraev A., Forster B.P. ve Jain S.M., Advances in Haploid Production in Higher Plants (ss. 1-33). Springer.

Weyen J., 2021. Doubled Haploid Technology: Volume 1: General Topics, Alliaceae, Cereals. Humana; New York, NY, USA: Applications of Doubled Haploids in Plant Breeding and Applied Research; pp. 23–39.

Winton, L.L., & Einspahr, D.W., 1968. The Use of Heat-Treated Pollen for Aspen Haploid Production. For. Sci., 14, 406-407.

Wolyn, D.J., & Nichols, B., 2003. Asparagus Microspore and Anther Culture. Maluszynski M, Kasha K.J., Forster B.P. ve Szarejko L., Doubled Haploid Production in Crop Plants: A Manual (ss. 265-273). Kluwer Academic.

Wu, B.J. & Chen, K.C., 1982. Cytological and Embryological Studies on Haploid Plant Production from Cultured Unpollinated Ovaries of *Nicotiana tabacum* L. Act Bot Sin., 24, 125-129.

Wu, P., Li, H., Ren, J., Chen, S., 2014. Mapping of Maternal QTLs for *In vivo* Haploid İnduction Rate in Maize (*Zea mays* L.). Euphytica, 196, 413-421.

Yao, L., Zhang, Y., Liu, C., Liu, Y., 2018. OsMATL Mutation Induces Haploid Seed Formation in Indica Rice. Nat. Plants, 4, 530–533.

Yetisir, H., & Sari, N., 2003. A New Method for Haploid Muskmelon (*Cucumis melo* L.) Dihaploidization. Sci Hortic., 98, 277-283.

Yuan, S.X., Liu, Y.M., Fang, Z.Y., Yang, L.M., Zhuang, M., Zhang, Y.Y., Sun, P.T., 2009. Study on the Relationship Between the Ploidy Level of Microspore-Derived Plants and the Number of Chloroplasts in Stomatal Guard Cells in *Brassica oleracea*. Agricultural Sciences in China, 8(8), 939-946.

Zagorcheva, L., Alexandrova, M., Kichukova, C., 1987. Pollen Mother Cell Meiosis in the Haploid of *Cucumis ficifolius* A. Rich. Cucurbite Genet. Coop. 10, 37-38.

Zargar, M., Zavarykina, T., Voronov, S., Pronina, I., Bayat, M., 2022. The Recent Development in Technologies for Attaining Doubled Haploid Plants *In vivo*. *Agriculture, 12,* 1595.

Zenkteler, M., & Nitzsche, W., 1984. Wide Hybridization Experiments in Cereals. Theor Appl Gent., 68, 311-315.

Zhang, Y.X., & Lespinasse, Y., 1991. Pollination with Gamma-Irradiated Pollen and Development of Fruits, Seeds and Partenogenetic Plants in Apple. *Euphytica*, 4, 101-109.

Zhang, K., Tian, J., Zhao, L., Wang, S., 2008a. Mapping QTLs with Epistatic Effects and QTL × Environment Interactions for Plant Height Using A Doubled Haploid Population in Cultivated Wheat. J. Genet. Genomics, 35, 119-127.

Zhang, Z., Qiu, F., Liu, Y., Ma, K., Li, Z., Xu, S., 2008b Chromosome Elimination and *In vivo* Haploid Production Induced by Stock6-derived Inducer Line in Maize (*Zea mays* L.). Plant Cell Rep. 2008, 27, 1851–1860.

Zhang, J., Tang, W., Huang, Y., Niu, X., Zhao, Y., Han, Y., Liu, Y., 2015. Down-regulation of an LBD-Like Gene, OsIG1, Leads to the Occurrence of Unusual Double Ovules and Developmental Abnormalities of Various Floral Organs and Megagametophyte in Rice. J. Exp. Bot., 66, 99–112.

Zhang, L., Zhang, Y., Gao, Y., Jiang, X., Zhang, M., Wu, H., 2016. Effects of Histone Deacetylase İnhibitors on Microspore Embryogenesis and Plant Regeneration in Pakchoi (*Brassica rapa* ssp. *chinensis* L.). Sci Hort. 209, 61–66. doi: 10.1016/j.scienta.2016.05.001

Zhao, X., Xu, X., Xie, H., Chen, S., Jin, W., 2013. Fertilization and Uniparental Chromosome Elimination During Crosses with Maize Haploid Inducers. Plant Physiol., 163, 721–731.

Zhebentyayeva, T.N., Swire-Clark, G., Georgi, L.L., Garay, L., Jung, S., Forrest, S., Blenda, A.V., Blackmon, B., Mook, J., Horn, R., 2008. A Framework Physical Map for Peach, A Model Rosaceae Species. Tree Genet. Genomes, 4, 745-756.

Zheng, M.Y., 2003. Microspore Culture in Wheat (*Triticum aestivum*)-Doubled Haploid Production via Induced Embryogenesis. Plant Cell Tissue Organ Cult., 73(3), 213-230. doi: 1 0.1023/a:1023076213639

Zhong, Y., Liu, C., Qi, X., Jiao, Y., Wang, D., Wang, Y., Liu, Z., Chen, C., Chen, B., Tian, X., 2019. Mutation of ZmDMP Enhances Haploid Induction in Maize. Nat. Plants, 5, 575–580.

Zhong, Y., Chen, B., Wang, D., Zhu, X., Wang, Y., Li, M., Li, Y., Liu, J., Zhang, J., Chen, M., 2021. A Genotype Independent *DMP*-HI System in Dicot Crops. bioRxiv, 449224.

Zhong, Y., Chen, B., Wang, D., Zhu, X., Li, M., Zhang, J., Chen, M., Wang, M., Riksen, T., Liu, J., 2022. *In vivo* Maternal Haploid Induction in Tomato. Plant Biotechnol. J., 20, 250–252.

Zhou, H., 1996. Genetics of Green Plant Regeneration from Anther Culture in Cereals. Jain S.M., Sopory S.K. ve Veilleux R.E., *In Vitro* Haploid Production in Higher Plants. vol 2 (ss. 169-187). Kluwer Academic.

Zhou, H.P., Ball, S.T., Konzak, C.F., 1992. Functional Properties of Ficoll and their Influence on Anther Culture Responses of Wheat. I, 30(1), 77-83. doi: 10.1007/bf00040004

Zhu, Y.C., Sun, D.X., Deng, Y., An, G.L., Li, W.H., Si, W.I., Liu, J.P., Sun, X.W., 2020. Comparative

Transcriptome Analysis of the Effect of Different Heat Shock Periods on the Unfertilized Ovule in Watermelon (*Citrullus lanatus*). J. Integr. Agric., 19, 528–540.

Zoriniants, S., Tashpulatov, A.S., Heberle-Bors, E., Touraev, A., 2005. The Role of Stress in the Induction of Haploid Microspore Embryogenesis. Palmer C.E., Keller W.A. ve Kasha K.J., Biotechnology in Agriculture and Forestry, vol 56 (ss. 35-52). Springer.

Zou, T., Su, HN., Wu, Q., 2018. Haploid Induction via Unfertilized Ovary Culture in Watermelon. Plant Cell Tiss Organ Cult., 135, 179–187. https://doi.org/10.1007/s11240-018-1454-1

Zou, T., Min, Z., Song, H., Gong, H., Tong, L., Sun, L., Yang, H., Zhang, J., Sun, X., 2024. Whole-Transcriptome Sequence Analysis of Pumpkin Ovules in Response to Heat Shock During Unfertilized Ovary Culture. Sci. Hortic. 2024, 329, 113007.

Bölüm 10

SOMAKLONAL VARYASYON

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10.1 GİRİŞ

Bitki doku kültürü; bitki hücre, doku ve organlarının aseptik koşullarda ve yapay besin ortamlarında, çoğaltma ve ıslah amacıyla *in vitro* olarak yetiştirilmesini kapsayan tekniklerin tümüdür. Bu teknik, bitki biyoteknolojisinin temel taşlarından biridir ve hem temel araştırmalarda hem de tarımsal uygulamalarda geniş ölçüde kullanılmaktadır (George ve Debergh, 2008; Thorpe, 2007).

Mikroçoğaltım, genetik olarak homojen bitkilerin çoğaltılması için uygulanabilir ve ölçeklenebilir bir yöntem sunarak, bitki doku kültürünün önemli bir uygulamasıdır. Doku kültürü çalışmalarında; sürgün ucu, meristem, embriyo, spor, kök, yaprak, çiçek kısımları ve protoplast dahil olmak üzere herhangi bir bitki hücresi veya organ, eksplant olarak kullanılmaktadır. Kitlesel çoğaltımdaki rolünün yanı sıra doku kültürü; hastalıksız bitkisel materyalin çoğaltılması, genetik saflığın korunması, endemik türlerin muhafazası, kriyoprezervasyon ve sekonder metabolit üretimi gibi çok sayıda kritik uygulamaya sahiptir. Bu nedenle tarımsal üretim açısından stratejik bir yöntem olarak değerlendirilmektedir (Espinosa-Leal ve ark., 2018; Thorpe, 2007). Özellikle ticari öneme sahip bitkilerin kısa sürede büyük ölçekli üretimine olanak sağlamaktadır (Smith ve Drew, 1990). Meristem kültürü ile virüs ve bakteri gibi patojenlerden arındırılmış elit hatların çoğaltımı müm-

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Bu değişiklikler sonucunda gelişen varyantlar ise bitki doku kültürü sisteminin yapıldığı amaca bağlı olarak avantajlı ya da dezavantajlı durum olarak değerlendirilmektedir. Özellikle birörnek materyal eldesini hedefleyen ticari firmalar için somaklonal varyasyonlar istenmeyen özellikte olup; ıslah açısından olumlu görülebilecek değişiklikler avantajlı durumda görülmektedir. Bu nedenle, bu süreçlerin kontrollü bir şekilde yürütülmesi çok önemlidir. Özellikle evrimini tamamlamamış bitkilerde, uzun süre kültürlenen eksplantlarda ve kallus oluşumu gibi karmaşık ve uzun süreli kültür tiplerinde varyasyon olup olmadığının dikkatle izlenmesi gereklidir.

KAYNAKLAR

Altındal, N., 2022. Somaklonal varyasyon ve patates ıslahında kullanımı. *Doğa ve Mühendislik Bilimlerinde Güncel Tartışmalar 7*, 48–56. Ankara: Bilgin Kültür Sanat Yayınları.

Antony, J. J. J., Shamshir, R. A., Poobathy, R., Chew, B. L., Subramaniam, S., 2015. Somaclonal variations were not induced by the cryopreservation: levels of somaclonal variations of *in vitro* and thawed protocorms of *Dendrobium* Bobby Messina analysed by SCoT and TRAP DNA markers. *South African Journal of Botany*, 100, 148–157.

Bairu, M. W., Aremu, A. O., Van Staden, J., 2011. Somaclonal variation in plants: causes and detection methods. *Plant Growth Regulation*, 63(2), 147–173.

Bhojwani, S. S., Dantu, P. K., 2013. Somaclonal variation. In *Plant tissue culture: an introductory text*, 141–154. India: Springer India.

Cao, Z., Sui, S., Cai, X., Yang, Q., Deng, Z., 2016. Somaclonal variation in 'Red Flash' caladium: morphological, cytological and molecular characterization. *Plant Cell, Tissue and Organ Culture*, 126(2), 269–279.

Cassells, A. C., Curry, R. F., 2001. Oxidative stress and physiological, epigenetic and genetic variability in plant tissue culture: implications for micropropagators and genetic engineers. *Plant Cell, Tissue and Organ Culture*, 64(2), 145–157.

Chawla, H. S., 2000. *Introduction to plant biotechnology*. Science Publishers, Inc., Enfield.

Chen, W. H., Chen, T. M., Fu, Y. M., Hsieh, R. M., Chen, W. S., 1998. Studies on somaclonal variation in *Phalaenopsis*. *Plant Cell Reports*, 18(1), 7–13.

Çördük, N., Yücel, G., Akıncı, N., Tuna, M., 2017. Assessment of the genetic stability of indirect shoot organogenesis-derived plantlets of *Digitalis trojana* Ivanina by flow cytometry and cytological analyses. *Tekirdağ Ziraat Fakültesi Dergisi*, 14(1).

Deepthi, V. P., 2016. Role of plant growth regulators in grape production: a review. *Int. J. Appl. and Nat. Sci.*, 6, 21–32.

Espinosa-Leal, C. A., Puente-Garza, C. A., García-Lara, S., 2018. *In vitro* plant tissue culture: means for production of biological active compounds. *Planta*, 248(1), 1–18.

Ferreira, M. D. S., Rebouças, T. A., Rocha, A. D. J., Oliveira, W. D. D. S., Santos, A. C. L. S. D., Jesus, J. P. F. L. D., Amorim, E. P., 2024. Selection and characterization of somaclonal variants of Prata banana (AAB) resistant to Fusarium wilt. *Agronomy*, 14(8), 1740.

Gao, D. Y., Vallejo, V. A., He, B., Gai, Y. C., Sun, L. H., 2009. Detection of DNA changes in somaclonal mutants of rice using SSR markers and transposon display. *Plant Cell, Tissue and Organ Culture*, 98(2), 187–196.

George, E. F., Hall, M. A., Klerk, G. J. D., 2008. Micropropagation: uses and methods. In *Plant Propagation by Tissue Culture: Volume 1. The Background*, 29–64. Dordrecht: Springer.

Grover, A., Sharma, P. C., 2016. Development and use of molecular markers: past and present. *Critical Reviews in Biotechnology*, 36(2), 290–302.

Gülşen, O., Mutlu, N., 2005. Bitki biliminde kullanılan genetik markırlar ve kullanım alanları. *Alatarım*, 4(2), 27–37.

Isah, T., 2015. Adjustments to *in vitro* culture conditions and associated anomalies in plants. *Acta Biologica Cracoviensia Series Botanica*, 57(2).

Kaçar, Y. A., 2024. Somaclonal variation in *in vitro* culture. In *Book of Proceedings In: In vitro culture of woody crops: problem solving by new approaches*. Sota V and Werbrouck S (eds). ISBN: 978-9934-38-084-6. Proceedings of the 2nd Conference of Cost Action CA21157 - COPYTREE. Bulduri, Latvia, 22–24 April 2024, 156.

Kado, C. I., Kleinhofs, A., 1961. Genetic modification of plant cells through uptake of foreign DNA. In *International Review of Cytology*, 11, 47–80. Academic Press.

Kaeppler, S. M., Kaeppler, H. F., Rhee, Y., 2000. Epigenetic aspects of somaclonal variation in plants. *Plant Molecular Biology*, 43(2), 179–188.

Karp, A., 1991. On the current understanding of somaclonal variation. *Oxford Surveys of Plant Molecular and Cell Biology*, 7, 1–58.

Karp, A., 1994. Origins, causes and uses of variation in plant tissue cultures. In *Plant cell and tissue culture*, 139–151. Dordrecht: Springer Netherlands.

Kordrostami, M., Rahimi, M., 2015. Molecular markers in plants: concepts and applications. *Genet. 3rd Millenn*, 13, 4024–4031.

Krishna, H., Alizadeh, M., Singh, D., Singh, U., Chauhan, N., Eftekhari, M., Sadh, R. K., 2016. Somaclonal variations and their applications in horticultural crops improvement. *3 Biotech*, 6(1), 54.

Kumar, N. S., Gurusubramanian, G., 2011. Random amplified polymorphic DNA (RAPD) markers and its applications. *Sci Vis*, 11(3), 116–124.

Larkin, P. J., Scowcroft, W. R., 1981. Somaclonal variation—a novel source of variability from cell cultures for plant improvement. *Theoretical and Applied Genetics*, 60(4), 197–214.

Leela Sahijram, L. S., Soneji, J. R., Bollamma, K. T., 2003. Invited review: analyzing somaclonal variation in micropropagated bananas (*Musa* spp.). *In Vitro Cellular & Developmental Biology – Plant*, 39, 551–556.

Leva, A. R., Petruccelli, R., Rinaldi, L. M. R., 2012. Somaclonal variation in tissue culture: a case study with olive, 123–150. In *Recent advances in plant in vitro culture*. InTech.

Li, R., Bruneau, A. H., Qu, R., 2010. Tissue culture-induced morphological somaclonal variation in St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze]. *Plant Breeding*, 129(1), 96–99.

Linacero, R., Rueda, J., Esquivel, E., Bellido, A., Domingo, A., Vázquez, A. M., 2011. Genetic and epigenetic relationship in rye, *Secale cereale* L., somaclonal variation within somatic embryo-derived plants. *In Vitro Cellular & Developmental Biology – Plant*, 47(5), 618–628.

Majumder, S., Igamberdiev, A. U., Debnath, S. C., 2025. Somaclonal variation and clonal fidelity in commercial micropropagation: challenges and perspectives. *Agronomy*, 15(6), 1489.

Manchanda, P., Sharma, D., Kaur, G., Kaur, H., Vanshika, 2025. Exploring the significance of somaclonal variations in horticultural crops. *Molecular Biotechnology*, 67(6), 2185–2203.

Manchanda, P., Sharma, D., Kaur, G., Kaur, H., Vanshika, 2025. Exploring the significance of somaclonal variations in horticultural crops. *Molecular Biotechnology*, 67(6), 2185–2203.

Martínez-Estrada, E., Caamal-Velázquez, J. H., Salinas-Ruíz, J., Bello-Bello, J. J., 2017. Assessment of somaclonal variation during sugarcane micropropagation in temporary immersion bioreactors by intersimple sequence repeat (ISSR) markers. *In Vitro Cellular & Developmental Biology – Plant*, 53(6), 553–560.

Miguel, C., Marum, L., 2011. An epigenetic view of plant cells cultured *in vitro*: somaclonal variation and beyond. *Journal of Experimental Botany*, 62(11), 3713–3725.

Mo, X. Y., Long, T., Liu, Z., Lin, H., Liu, X. Z., Yang, Y. M., Zhang, H. Y., 2009. AFLP analysis of somaclonal variations in *Eucalyptus globulus*. *Biologia Plantarum*, 53(4), 741–744.

Mujib, A., 2005. Colchicine induced morphological variants in pineapple. *Plant Tissue Culture and*

Biotechnology, 15(2), 127–133.
Ngezahayo, F., Dong, Y., Liu, B., 2007. Somaclonal variation at the nucleotide sequence level in rice (*Oryza sativa* L.) as revealed by RAPD and ISSR markers, and by pairwise sequence analysis. *Journal of Applied Genetics*, 48(4), 329–336.
Polanco, C., Ruiz, M. L., 2002. AFLP analysis of somaclonal variation in *Arabidopsis thaliana* regenerated plants. *Plant Science*, 162(5), 817–824.
Prado, M. J., Rodriguez, E., Rey, L., González, M. V., Santos, C., Rey, M., 2010. Detection of somaclonal variants in somatic embryogenesis-regenerated plants of *Vitis vinifera* by flow cytometry and microsatellite markers. *Plant Cell, Tissue and Organ Culture*, 103(1), 49–59.
Puente, R., González, A. I., Ruiz, M. L., Polanco, C., 2008. Somaclonal variation in rye (*Secale cereale* L.) analyzed using polymorphic and sequenced AFLP markers. *In Vitro Cellular & Developmental Biology – Plant*, 44(5), 419–426.
Rahman, M., Rajora, O., 2001. Microsatellite DNA somaclonal variation in micropropagated trembling aspen (*Populus tremuloides*). *Plant Cell Reports*, 20(6), 531–536.
Rajan, R. P., Singh, G., 2021. A review on application of somaclonal variation in important horticulture crops. *Plant Cell Biotechnol. Mol. Biol*, 22, 161–175.
Ranghoo-Sanmukhiya, V. M., 2020. Somaclonal variation and methods used for its detection. In *Propagation and genetic manipulation of plants*, 1–18. Singapore: Springer Singapore.
Saker, M. M., Bekheet, S. A., Taha, H. S., Fahmy, A. S., Moursy, H. A., 2000. Detection of somaclonal variations in tissue culture-derived date palm plants using isoenzyme analysis and RAPD fingerprints. *Biologia Plantarum*, 43(3), 347–351.
Sales, E. K., Butardo, N. G., 2014. Molecular analysis of somaclonal variation in tissue culture derived bananas using MSAP and SSR markers. *Int J Biol Vet Agric Food Eng*, 8(6), 615–622.
Sarasan, V., Cripps, R., Ramsay, M. M., Atherton, C., McMichen, M., Prendergast, G., Rowntree, J. K., 2006. Conservation *in vitro* of threatened plants—progress in the past decade. *In Vitro Cellular & Developmental Biology – Plant*, 42(3), 206–214.
Skirvin, R. M., Janick, J., 1976. Tissue culture-induced variation in scented *Pelargonium* spp. 1. *Journal of the American Society for Horticultural Science*, 101(3), 281–290.
Skirvin, R. M., McPheeters, K. D., Norton, M., 1994. Sources and frequency of somaclonal variation. *HortScience*, 29(11), 1232–1237.
Smith, M. K., Drew, R. A., 1990. Current applications of tissue culture in plant propagation and improvement. *Australian Journal of Plant Physiology*, 17(3), 267–289.
Steward, F. C., Mapes, M. O., Mears, K., 1958. Growth and organized development of cultured cells. II. Organization in cultures grown from freely suspended cells. *American Journal of Botany*, 705–708.
Taha, M. A., Tawfik, A. A., Mahmoud, A. M., Mohamed, M. F., 2024. Assessment of somaclonal variants of *Salvia splendens* at different subcultures using molecular markers. *Assiut Journal of Agricultural Sciences*, 55(3), 78–96.
Taşpınar, M. S., Tosun, M., 2002. İzoenzim elektroforez tekniğinin bitki ıslahında kullanımı. *Research in Agricultural Sciences*, 33(4).
Thorpe, T. A., 2007. History of plant tissue culture. *Molecular Biotechnology*, 37(2), 169–180.
Us-Camas, R., Rivera-Solís, G., Duarte-Aké, F., De-la-Peña, C., 2014. *In vitro* culture: an epigenetic challenge for plants. *Plant Cell, Tissue and Organ Culture*, 118(2), 187–201.
Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, 23(21), 4407–4414.

Bölüm 11

HÜCRE SÜSPANSİYON KÜLTÜRÜ

Başar SEVİNDİK [1]

11.1 GİRİŞ VE TARİHSEL SÜREÇ

Bitkilerin organize olmuş veya organize olmamış hücrelerinin kültüre alınması uzun yıllardır uygulanan bir tekniktir. Bu süreç içerisinde çok farklı metotlar geliştirilmiştir. Ayrıca bu geliştirilen tekniklerin kombine olarak kullanılması bu tekniğin hızla gelişmesine olanak sağlamakla birlikte bitki biyoteknolojisinde sekonder metabolitlerin üretimi gibi farklı alanlarda da yaygın şekilde kullanılmasına sebep olmuştur. Hücrelerin kültüre alınıp organize yapıların oluşturulmasında genetik manipülasyonlar oldukça önemlidir. Bu yönlendirmeler bazı genlerin ifadesinin kaybolması veya bazı genlerin ifadesinin ortaya çıkması ile olabilmektedir. Hücre kültürlerindeki başarı veya başarısızlık kültür başlangıcı ile ilişkilidir. Başlangıçta bitkisel materyallerin seçimi, başlangıç materyallerinin fizyolojik durumu, kullanılan besin ortamı, hazırlık aşamasındaki bazı faktörler başarıyı doğrudan etkileyen faktörlerdir. *In vitro* doku kültürü tekniklerinin farklı formları göz önüne alındığında hücre süspansiyon kültürü doğal bileşiklerin geniş ölçekli üretimi ve üretim sırasındaki kültürlerin homojenitesi açısından oldukça uygun bir tekniktir. Bu geçmişte yapılan çalışmalarda ve ticari üretimlerde uygulamalı olarak da kanıtlanmıştır. Bu bölümde hücre kültürleri, yapılan üretim ve buna bağlı olarak hücre süspansiyon kültürleri ile ilgili pratik bilgiler verilecektir. Bu bölüm kapsamında biyoreaktörlerin gelişim süreci ve hücre süspansiyon kültürüne katkıları ve işleyişi konularına da değinilecektir. Ayrıca bu alandaki gelişmeler ile ilgili literatürlerden bahsedilecek ve yeniliklere değinilecektir.

In vitro teknolojilerin gelişmesi ile bitki hücre kültürü bu konu içerisinde çok geniş bir başlık haline gelmiştir. Bu teknik ilk kullanılmaya başlandığında bitkilerin farklı organları, kökleri, veya organize olmamış hücre toplulukları mikrokültür sistemleri ile, erlenmayer

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miktarı, havalandırma tipleri, çalkalama şekli ve hücre yoğunluğu farklılık teşkil etmektedir. Katı kültüre göre oldukça avantajlı olan bu sistemler ile elde edilen bitkilerin daha kolay gelişim gösterdiği ve katı kültürde meydana gelen zararlanmalardan daha az etkilendiği bilinmektedir. Bunların yanı sıra biyoreaktör sistemlerinin, geniş ölçekli üretimde verim maliyeti, kültür stabilitesi, sistemin optimizasyonunda karşılaşılan problemler gibi önemli dezavantajları da mevcuttur. Biyoreaktörler ile bitki hücrelerinin çoğaltımı ve sekonder metabolit üretimi, 1950'lerde başlasa da günümüze kadar gelen süreçte oldukça hızlı ilerlemiş ve şu an yeni nesil geçici daldırma sistemleri klonal çoğaltımda yaygın bir şekilde kullanılmaya başlanmıştır.

KAYNAKLAR

Akçam Oluk, E., 2006. Bitki Hücre Süspansiyon Kültürleri ve Sekonder Metabolit Üretimi.

Archambault, J., Lavoie L, Williams, R.D.,1995. Nutritional aspects of Daucus carota somatic embryo cultures performed in bioreactors. In: Terzi M, Cella R, Falavigna A, eds. Current Issues in Plant Molecular and Cellular Biology. Dordrecht: Kluwer Acad Publ, 681-87.

Buddendorf-Joosten, J. M. C., Woltering, E. J., 1994. Components of the gaseous environment and their effects on plant growth and development *in vitro*. In: Lumsden PJ, Nicholas JR and Davies WJ, eds. Physiology Growth and Development of Plants in Culture. Dordrecht: Kluwer Acad Publ, 165-90.

Cazzulino, D., Pederson, H., Chin, C. K., 1991. Bioreactors and image analysis for scale-up and plant propagation. In: Vasil IK, ed. Cell Culture and Somatic Cell Genetics of Plants. San Diego: Academic Press, 147-77.Chen, S., Zhao, B., Wang, X., Yuan, X., Wang, Y., 2004. Promotion of the growth of *Crocus sativus* cells and the production of crocin by rare earth elements. Biotechnol Lett, 26, 27–30

Crespi-Perellino, N., Guicciardi, A., Malyszko, G., 1986. Occurrence of indole alkaloids in Ailanthus altissima cell cultures. J Nat Prod, 49, 1010–14

Dar, Z. A., Meena, P. D,, Kumar, A., 2012. Principles of plant breeding. Pointer Publishers, Jaipur, p 250.

De-Eknamkul, W., Ellis, B. E., 2007. Rosmarinic acid production and growth characteristics of *Anchusa officinalis* cell suspension cultures. Planta Med, 50, 346–50

Fujita, Y., Maeda, Y., Suga, C., Morimoto, T. 1983. Production of shikonin derivatives by cell suspension cultures of Lithospermum erythrorhizon. III. Comparison of shikonin derivatives of cultured cells and Ko-shikon. Plant cell reports, 2(4), 192-193.

Gautheret, R. J., 1934. Culture de tissu cambial. C R Acad Sci Paris 198, 2195–2196.

Haberlandt, G.,1902. Culturversuche mit isolierten Pflanzenzellen. Sitzungsber. Kais. Akad. Wiss.—Math. Naturw. Klasse, Wien Bd. CXI, 69–92.

Hall, R. D. (2000). Plant cell culture initiation. Molecular biotechnology, 16(2), 161-173.

Härtel, O.,2003. Gottlieb Haberlandt (1854–1945): a portrait. In: Laimer M, Rücker W (eds) Plant tissue culture. Springer, Vienna.Hippolyte, I., Marin, B., Baccou, J. C., Jonard, R., 1992. Growth and rosmarinic acid production in cell suspension cultures of *Salvia officinalis* L. Plant Cell Rep, 11, 109–12.

Huang, F., Kutchan, T. M., 2000. Distribution of morphinan and benzophenanthridine alkaloid gene transcript accumulation in *Papaver somniferum*. Phytochemistry, 53, 555–64.

Ilan, A., Ziv, M., Halevy, A. A.,1995. Propagation and corm development of Brodiaea in liquid cultures. Scientia Hort, 63,101-12.

Imseng, N., Schillberg, S., Schürch, C., Schmid, D., Schütte, K., Gorr, G., Eibl D., Eibl, R., 2014.

Suspension culture of plant cells under heterotrophic conditions. Industrial scale suspension culture of living cells, 224-258.

Jay, V., Genestier, S., Courduroux, J.C., 1994. Bioreactor studies on the effect of medium pH on carrot (Daucus carota L.) somatic embryogenesis. Plant Cell Tis Org Cult, 36:205-209.

Kang, S., Min, J., Kim, Y., 2009. Effect of biotic elicitors on the accumulation of bilobalide and ginkgolides in *Ginkgo biloba* cell cultures. J Biotechnol, 139, 84–8.

Kaya, S., 2015. Triakontanol'un Serbest Ve Tutuklanmış Capsıcum annuum L. Hücre Süspansiyon Kültüründe Kapsaisin Üretimi Üzerine Etkisi. Niğde Ömer Halisdemir Üniversitesi Fen Bilimleri Enstitüsü Yüksek Lisans Tezi Sf. 62.

Kshirsagar, P. R., Chavan, J. J., Umdale, S. D., Nimbalkar, M. S., Dixit, G. B., Gaikwad, N. B., 2015. Highly efficient *in vitro* regeneration, establishment of callus and cell suspension cultures and RAPD analysis of regenerants of Swertia lawii Burkill. Biotechnology Reports, 6, 79-84.

Kumar, A., Roy, S., 2011. Plant tissue culture and applied plant biotechnology. Avishkar Publishers, Jaipur, 346 pp.

Kumar A, Sopory S (eds) (2010) Applications of plant biotechnology: *in vitro* propagation, plant transformation and secondary metabolite production. I.K. International, New Delhi, 606 pp.

Luttman, R., Florek, P., Preil, W. 1994. Silicone-tubing aerated bioreactors for somatic embryo production. Plant Cell Tiss Org Cult, 39(2), 157-70.

Merillon, J. M.,1999. Large-scale production in bioreactors. In: Ramawat KG, Merillon JM, eds. Biotechnology Secondary Metabolites. Enfield: Science publishers Inc, 331-50.

Miller, C. O., Skoog, F., Von Saltza, M. H., Strong, F., 1955. J Am Chem SOC, 77,1392-93.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue. Physiol Plant. 15.473–497.

Mühlbach, H., 1998. Use of plant cell cultures in biotechnology. Biotech Ann Rev, 4,113-71.

Neumann, K. H., Kumar, A., Imani, J., 2020. Cell Suspension Cultures. In Plant Cell and Tissue Culture–A Tool in Biotechnology (pp. 61-69). Springer, Cham.

Nobecourt, P.C., 1937. Culture en série de tissus végétaux sur le milieu artificiel. C R Acad Sci Paris 205:521–523.

Orihara, Y., Ebizuka, Y., 2010. Production of triterpene acids by cell-suspension cultures of Olea europaea. Chem Pharm Bull (Tokyo), 55, 341–7Pinoargote-Chang, M., Corozo-Quiñónez, L., Saltos-Rezabala, L. A., & Arias Echeverri, J. P. (2025). Annona deceptrix as a potential biofactory for secondary metabolites using plant cell and tissue cultures. *Scientia Agropecuaria*, *16*(3), 439-456.

Pras, N., Woerdenbag, H. J., Batterman, S., 1993. Mucuna pruriens: improvement of the biotechnological production of the anti-Parkinson drug L-dopa by plant cell selection. Pharm World Sci, 15, 263–8.

Praveen, N., Murthy, H. N., Chung, I. M., 2011. Improvement of growth and gymnemic acid production by altering the macro elements concentration and nitrogen source supply in cell suspension cultures of *Gymnema sylvestre* R. Br Ind Crop Prod, 33, 282–6.

Rehwald, L., 1927. Zeitschr Pflanzenkrankenkeiten Pflanzenschutz, 37,65–86.

Robins, R. I., Secondary products from cultured cells and organs: Molecular and cellular approaches. In: Dixon RA, Gonzales RA, eds. Plant Cell Culture. Oxford: IRL Press, 1994:169-97.

Ruffoni, B., Pistelli, L., Bertoli, A., Pistelli, L., 2010. Plant cell cultures: bioreactors for industrial production. In Bio-farms for nutraceuticals (pp. 203-221). Springer, Boston, MA.

Schleiden M. J., 1838. Beitrage zur Phytogenesis. Archiv fuer Anatomie und Physiologie in Verbindung mit mehreren gelehrten herausgegeben von Johannes Mueller, Verlag von veit ET COMP. Berlin, pp. 137– 176.

Schwann, T., 1839. Mikroskopishe Untersuchungen uber die Ubereinstimmung in der Struktur unddem Wachstun der Tiere und Pflanzen, (Smith, H., transl.) London, Sydenham Society.

Scragg, A. H., 1990. Fermentation systems for plant cells. In: Charlwood BV, Rhodes MJC, eds. Secondary Products from Plant Tissue Culture. London: Clarendon Press, 243-63.

Scragg, A. H., 1992 Large-scale plant cell culture: methods, applications and products. Curr Opin Biotech, 3,105-09.

Stafford, A., Morris, P., Fowler, M. W., 1986 Plant cell biotechnology: A perspective. Enz Microbial Technol, 8,578-97.

Stuart, D. A., Strickland, S. G., Walker, K. A.,1987 Bioreactor production of alfalfa somatic embryos. HortScience, 22,800-03.

Sumaryono, W., Proksch, P., Hartmann, T., 1991. Induction of rosmarinic acid accumulation in cell suspension cultures of *Orthosiphon aristatus* after treatment with yeast extract. Phytochemistry, 30, 3267–71.

Sun, X., Linden, J. C., 1999. Shear stress effects on plant cell suspension cultures in a rotating wall vessel bioreactor. J Ind Microbiol biotechnol, 22,44-47.

Suri, S. S., Sharma, R., Ramawat, K. G., 2000. Production of secondary metabolites in bioreactors. In: Khan IA, Khanum A, eds. Role of Biotechnology in Medicinal and Aromatic Plants Vol II. Hyderabad: UKAAZ Publications, 437-51.

Szabo, E., Thelen, A., Petersen, M., 1999. Fungal elicitor preparations and methyl jasmonate enhance rosmarinic acid accumulation in suspension cultures of *Coleus blumei*. Plant Cell Rep, 18, 485–9

Taha, H.S., Abd El-Kawy, A. M., Fathalla, M. A. E., 2012. A new approach for achievement of inulin accumulation in suspension cultures of Jerusalem artichoke (*Helianthus tuberosus*) using biotic elicitors. J Genet Eng Biotechnol, 10, 33–8

Takayama, S., Akita, M.. 1998. Bioreactor techniques for large-scale culture of plant propagules. Adv Hort Sci, 12,93-100.

Titova, M., Popova, E., Nosov, A., 2024. Bioreactor Systems for Plant Cell Cultivation at the Institute of Plant Physiology of the Russian Academy of Sciences: 50 Years of Technology Evolution from Laboratory to Industrial Implications. *Plants*, *13*(3), 430.

Tulecke, W., Nickel, L. G., 1959. Production of large amounts of plant tissue by submerged cultures. Science, 130,863-64.

Waltz, E., 2016 CRISPR-edited crops free to enter market, skip regulation. Nat Biotechnol, 34(6), 582–582. https://doi.org/10.1038/nbt0616-582

White, P. H., 1939. Controlled differentiation in a plant tissue culture. Bull Torrey Bot Club, 66,507–519.

Xu, J., Ge, X., Dolan, M. C., 2011. Towards high-yield production of pharmaceutical proteins with plant cell suspension cultures. *Biotechnology advances*, *29*(3), 278-299.

Xu, X., Zhang, W., Cao, X., Xue, S., 2011. Abietane diterpenoids synthesized by suspension-cultured cells of Cephalotaxus fortunei. Phytochem Lett, 4, 52–5.

Yamamoto, H., Yazaki, K., Inoue, K., 2000. Simultaneous analysis of shikimate-derived secondary metabolites in Lithospermum erythrorhizon cell suspension cultures by high-performance liquid chromatography. J Chromatogr B Biomed Sci Appl, 738, 3–15

Zenk, M. H., 1978. The impact of plant cell culture on industry. In: Thorpe TA, ed. Frontiers of Plant Tissue Culture. Calgary: Int Ass for Plant Tissue Culture,1-13.

Zhao, D., Fu, C., Han, Y., Lu, D., 2005a. Effects of elicitation on jaceosidin and hispidulin production in cell suspension cultures of Saussurea medusa. Process Biochem, 40, 739–45.

Ziv, M., Hadar, A.,1991. Morphogenic pattern of Nephrolepsis exaltata Bostoniensis in agar-gelled or liquid culture. Implication for mass propagation. Israel J Bot, 40,7-16.

Bölüm 12

SEKONDER METABOLİT ÜRETİMİ

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12.1 GİRİŞ

Bitkiler, çevresel stres faktörlerine karşı geliştirdikleri savunma mekanizmaları kapsamında, primer metabolizma dışında yer alan ve doğrudan büyüme veya gelişimle ilişkili olmayan bileşikler üretmektedirler. Bu bileşikler "sekonder metabolitler" olarak adlandırılmaktadır. Sekonder metabolitler özellikle bitkilerin hayatta kalma stratejilerinde, patojenlere karşı savunmada, simbiyotik ilişkilerde ve çevresel adaptasyonlarında kritik rol üstlenmektedir (Selwal ve ark., 2024). Sekonder metabolitler antioksidan, antimikrobiyal, antikanser gibi geniş biyolojik aktivitelere sahiptir ve sahip oldukları biyoaktiviteler nedeniyle farmasötik, kozmetik, gıda ve tarım sektörlerinde yoğun olarak kullanılmaktadır. Bu durum sekonder metabolitlerin önemini ve ticari değerini arttırmaktadır. Ancak bu bileşiklerin doğal koşullarda üretimi sınırlı ve düzensizdir. Bu nedenle, bitki biyoteknolojisinin sunduğu imkanlar ile metabolit üretim miktarlarının artırılması, sürdürülebilir ve kontrollü bir alternatif olarak öne çıkmaktadır (Wu ve ark., 2023). Bitki biyoteknolojisi, genetik mühendislik, hormon uygulamaları, elisitör kullanımı ve kültür ortamı optimizasyonu gibi yöntemlerle metabolit üretimini yönlendirme imkânı sunmaktadır. Mikrobiyal biyoteknoloji, genetik mühendisliği, metabolit mühendisliği ve doku kültürü teknikleri çevresel değişkenlerden bağımsız, yıl boyu üretim sağlayan, genetik stabilitesi yüksek ve ölçeklenebilir sistemler sunmaktadır (Fazili ve ark., 2022).

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avantajlar sunar. E. coli, Saccharomyces cerevisiae gibi model mikroorganizmaların yanı sıra, yeni mikrobiyal şasi türlerinin geliştirilmesiyle daha karmaşık metabolitlerin üretimi mümkün hale gelmektedir. Yapay zekâ destekli genom mühendisliği, bu alanda yeni fırsatlar yaratmaktadır (Han ve Miao, 2024).

- Doku Kültürü ve İn Vitro Teknolojiler: Bitki doku ve hücre kültürü teknikleri, yıl boyu stabil ve ölçeklenebilir sekonder metabolit üretimi için sürdürülebilir bir alternatiftir. Özellikle saçak kök, hücre süspansiyon ve organ kültürleri, biyoteknolojik üretimde öne çıkmaktadır. Bu sistemlerin optimizasyonu ve ticari ölçeğe taşınması, gelecekte daha fazla önem kazanacaktır (Wawrosch ve Zotchev, 2021; Ozyigit ve ark., 2023).
- Elisitör ve Çevresel Faktörlerin Kullanımı: Elisitör (uyarıcı) uygulamaları ve çevresel stres faktörlerinin kontrollü kullanımı, bitkilerde sekonder metabolit üretimini artırmak için etkili stratejilerdir. Bu tekniklerin, biyoreaktör ve *in vitro* sistemlerle entegrasyonu, yüksek verimli ve sürdürülebilir üretim için önerilmektedir (Reshi ve ark., 2023).
- Endofitik Mikroorganizmalar ve Ortak Kültürler: Endofitik bakteri ve mantarların sekonder metabolit üretiminde kullanımı, yeni ve biyoaktif bileşiklerin keşfi için gelecek vadetmektedir. Ortak kültür sistemleriyle yeni metabolitlerin sentezi teşvik edilebilir (Tidke ve ark., 2018; Singh ve Kumar, 2023).

KAYNAKLAR

Abdel-Farid, I., Marghany, M., Rowezek, M., & Sheded, M., 2020. Effect of Salinity Stress on Growth and MetabolomicProfiling of *Cucumis sativus* and *Solanum lycopersicum*. Plants, 9. https://doi.org/10.3390/plants9111626.

Aguilar-Méndez, E. D., Monribot-Villanueva, J. L., Guerrero-Analco, J. A., & De-la-Peña, C., 2024. Chlorophyll deficiency in *Agave angustifolia* Haw.: unveiling the impact on secondary metabolite production. Planta, 260(4), 77.

Ahmad, N., Rab, A., Ahmad, N., 2016. Light-induced biochemical variations in secondary metabolite production and antioxidant activity in callus cultures of Stevia rebaudiana (Bert).. Journal of photochemistry and photobiology. B, Biology, 154, 51-6. https://doi.org/10.1016/j.jphotobiol.2015.11.015

Ahmadpoor, F., Zare, N., Asghari, R., Sheikhzadeh, P., 2022. Sterilization protocols and the effect of plant growth regulators on callus induction and secondary metabolites production in *in vitro* cultures *Melia azedarach* L. AMB Express, 12. https://doi.org/10.1186/s13568-022-01343-8

Ahsan, S. M., Injamum-Ul-Hoque, M., Shaffique, S., Ayoobi, A., Rahman, M. A., Rahman, M. M., & Choi, H. W., 2024. Illuminating *Cannabis sativa* L.: The power of light in enhancing C. sativa growth and secondary metabolite production. Plants, 13(19), 2774.

Alamgir, A. N. M., 2018. Secondary Metabolites: Secondary Metabolic Products Consisting of C and H; C, H, and O; N, S, and P Elements; and O/N Heterocycles. Therapeutic Use of Medicinal Plants and their Extracts: Volume 2 (C. 74, ss. 165-309). Springer International Publishing. https://doi.org/10.1007/978-3-319-92387-1_3

Alcalde, M., Perez-Matas, E., Escrich, A., Cusido, R., Palazón, J., Bonfill, M., 2022. Biotic Elicitors in Adventitious and Hairy Root Cultures: A Review from 2010 to 2022. Molecules, 27. https://doi.org/10.3390/molecules27165253.

Ali, A., Mashwani, Z., Raja, N., Mohammad, S., Luna-Arias, J., Ahmad, A., Kaushik, P., 2023. Phytomediated selenium nanoparticles and light regimes elicited *in vitro* callus cultures for biomass accumulation and secondary metabolite production in *Caralluma tuberculata*. Frontiers in Plant Science, 14. https://doi.org/10.3389/fpls.2023.1253193

Ali, A., Mohammad, S., Khan, M., Raja, N., Arif, M., Kamil, A., Mashwani, Z., 2019. Silver nanoparticles elicited *in vitro* callus cultures for accumulation of biomass and secondary metabolites in *Caralluma tuberculata*. Artificial Cells, Nanomedicine, and Biotechnology, 47, 715 - 724. https://doi.org/10.1080/21691401.2019.1577884

Anjitha, K., Sameena, P., Puthur, J., 2021. Functional aspects of plant secondary metabolites in metal stress tolerance and their importance in pharmacology. Plant Stress. https://doi.org/10.1016/j.stress.2021.100038.

Arora, L., Narula, A., 2017. Gene Editing and Crop Improvement Using CRISPR-Cas9 System. Frontiers in Plant Science, 8. https://doi.org/10.3389/fpls.2017.01932.

Badyal, S., Singh, H., Yadav, A. K., Sharma, S., Bhushan, I., 2020. Plant secondary metabolites and their uses. Plant Archives, 20(2), 3336-40.

Bakır, Ö., 2020. Sekonder metabolitler ve rolleri. Uluslararası Anadolu Ziraat Mühendisliği Bilimleri Dergisi, 2(4), 39-45. https://dergipark.org.tr/en/pub/uazimder/issue/57919/760126

Banaev, E. V., Erst, A. A., Khramova, E. P., Tomoshevich, M. A., Shaldaeva, T. M., 2024. *In vitro* culture of *Atraphaxis frutescens* (L.) K. Koch: effects of D-mannitol and pH on a propagation coefficient, secondary-metabolite levels, and antiradical activity. Plant Cell, Tissue and Organ Culture (PCTOC), 156(2), 46.

Baransel, G. Ş., Yücel, O., Yıldırım, E., Kalyon, G., Emik, S., Erol, A., Kara, N. T., 2025. Green synthesized silver nanoparticles enhance drought tolerance in cotton plants cultured *in vitro*. Physiology and Molecular Biology of Plants, 1-20.

Baydu, F. Y., Bektaş, E., 2025. Effects of Zinc Oxide Nanoparticles on Growth and Secondary Metabolite Accumulation in In Vitro Culture of Ocimum basilicum L. *Journal of Apitherapy and Nature*, *8*(1), 37-60.

Bhambhani, S., Kondhare, K. R., Giri, A. P., 2021. Diversity in Chemical Structures and Biological Properties of Plant Alkaloids. Molecules, 26(11), 3374. https://doi.org/10.3390/molecules26113374

Bhaskar, R., Xavier, L., Udayakumaran, G., Kumar, D., Venkatesh, R., Nagella, P., 2021. Biotic elicitors: a boon for the *in-vitro* production of plant secondary metabolites. Plant Cell, Tissue and Organ Culture (PCTOC), 149, 7 - 24. https://doi.org/10.1007/s11240-021-02131-1.

Bhavaniramya, S., Vishnupriya, S., Al-Aboody, M. S., Vijayakumar, R., Baskaran, D., 2019. Role of essential oils in food safety: Antimicrobial and antioxidant applications. Grain & Oil Science and Technology, 2(2), 49-55. https://doi.org/10.1016/j.gaost.2019.03.001

Bistgani, Z., Hashemi, M., Dacosta, M., Craker, L., Maggi, F., Morshedloo, M., 2019. Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of *Thymus vulgaris* L. and Thymus daenensis Celak. Industrial Crops and Products. https://doi.org/10.1016/J.INDCROP.2019.04.055.

Borah, A., Singh, S., Chattopadhyay, R., Kaur, J., Bari, V., 2024. Integration of CRISPR/Cas9 with multi-omics technologies to engineer secondary metabolite productions in medicinal plant: Challenges and Prospects. Functional & integrative genomics, 24 6, 207 . https://doi.org/10.1007/s10142-024-01486-w.

Bulut, M., 2025. Chemodiversity of sulfur-containing metabolites emphasizing the ecophysiology of Allium plants and the developmental innovations in bulb formation. *Current Opinion in Plant Biology*, *85*, 102724.

Cabañas-García, E., Areche, C., Gómez-Aguirre, Y., Bórquez, J., Muñoz, R., Cruz-Sosa, F., Balch, E., 2021. Biomass production and secondary metabolite identification in callus cultures of *Coryphantha macromeris* (Engelm.) Britton & Rose (Cactaceae), a traditional medicinal plant. South

African Journal of Botany, 137, 1-9. https://doi.org/10.1016/j.sajb.2020.10.002

Cambaz, E., Çördük, N., 2023. Secondary Metabolite Production in Callus Culture of *Verbascum scamandri* Murb.. Acta Societatis Botanicorum Poloniae. https://doi.org/10.5586/asbp/165894

Caser, M., Chitarra, W., D'Angiolillo, F., Perrone, I., Demasi, S., Lovisolo, C., ... Scariot, V., 2019. Drought stress adaptation modulates plant secondary metabolite production in *Salvia dolomitica* Codd. Industrial Crops and Products, 129, 85-96.

Chakraborty, A., Mukherjee, S., Biswas, D., Santra, I., Halder, T., Alam, M. M., ... Ghosh, B., 2024. Elite chemotype selection, antipathogenic activities, secondary metabolite fingerprinting of *in vitro* regenerated *Tinospora cordifolia* (Willd.) Hook. f. & Thomson–a plant with multipurpose therapeutic significance. Plant Cell, Tissue and Organ Culture (PCTOC), 157(2), 48.

Chamkhi, I., Benali, T., Aanniz, T., Menyiy, N., Guaouguaou, F., Omari, N., El-Shazly, M., Zengin, G., Bouyahya, A., 2021. Plant-microbial interaction: The mechanism and the application of microbial elicitor induced secondary metabolites biosynthesis in medicinal plants. Plant physiology and biochemistry: PPB, 167, 269-295. https://doi.org/10.1016/j.plaphy.2021.08.001.

Chattopadhyay, S., Farkya, S., Srivastava, A., Bisaria, V., 2002. Bioprocess considerations for production of secondary metabolites by plant cell suspension cultures. Biotechnology and Bioprocess Engineering, 7, 138-149. https://doi.org/10.1007/BF02932911.

Chen, D., Mubeen, B., Hasnain, A., Rizwan, M., Adrees, M., Naqvi, S., Iqbal, S., Kamran, M., El-Sabrout, A., Elansary, H., Mahmoud, E., Alaklabi, A., Sathish, M., Din, G., 2022. Role of Promising Secondary Metabolites to Confer Resistance Against Environmental Stresses in Crop Plants: Current Scenario and Future Perspectives. Frontiers in Plant Science, 13. https://doi.org/10.3389/fpls.2022.881032.

Chrysargyris, A., Papakyriakou, E., Petropoulos, S. A., Tzortzakis, N., 2019. The combined and single effect of salinity and copper stress on growth and quality of Mentha spicata plants. Journal of hazardous materials, 368, 584-593.

Cuadra, P., Harborne, J. B., Waterman, P. G, 1997. Increases in surface flavonols and photosynthetic pigments in Gnaphalium luteo-album in response to UV-B radiation. Phytochemistry, 45(7), 1377-1383.

Çalışkan, T., Hatipoğlu, R., Kırıcı, S., 2019. Sekonder bitki metabolitlerinin *in vitro* koşullarda üretimi. Turkish Journal of Agriculture-Food Science and Technology, 7(7), 971-980. https://agrifoodscience.com/index.php/TURJAF/article/view/2447

Darigh, F., Iranbakhsh, A., Ardebili, Z. O., Ebadi, M., Hassanpour, H., 2022. Simulated microgravity contributed to modification of callogenesis performance and secondary metabolite production in Cannabis Indica. *Plant Physiology and Biochemistry*, *186*, 157-168.

Del-Saz, N., Iglesias-Sanchez, A., Alonso-Forn, D., López-Gómez, M., Palma, F., Clemente[L] Moreno, M., Fernie, A., Ribas-Carbó, M., Florez-Sarasa, I., 2022. The Lack of Alternative Oxidase 1a Restricts in vivo Respiratory Activity and Stress-Related Metabolism for Leaf Osmoprotection and Redox Balancing Under Sudden Acute Water and Salt Stress in *Arabidopsis thaliana*. Frontiers in Plant Science, 13. https://doi.org/10.3389/fpls.2022.833113.

Derbassi, N. B., Pedrosa, M. C., Heleno, S., Carocho, M., Ferreira, I. C., Barros, L., 2022. Plant volatiles: Using Scented molecules as food additives. Trends in Food Science & Technology, 122, 97-103.

Desta, B., Amare, G., 2021. Paclobutrazol as a plant growth regulator. Chemical and Biological Technologies in Agriculture, 8, 1-15. https://doi.org/10.1186/s40538-020-00199-z.

Devi, A., Devi, K., Devi, P., Devi, M., Das, S., 2023. Metabolic engineering of plant secondary metabolites: prospects and its technological challenges. Frontiers in Plant Science, 14. https://doi.org/10.3389/fpls.2023.1171154.

Devrnja, N., Milutinović, M., Savić, J., 2022. When scent becomes a weapon—Plant essential oils as potent bioinsecticides. Sustainability, 14(11), 6847. https://www.mdpi.com/2071-1050/14/11/6847

Dubey, S., Shri, M., Gupta, A., Rani, V., Chakrabarty, D., 2018. Toxicity and detoxification of heavy metals during plant growth and metabolism. Environmental Chemistry Letters, 16, 1169-1192. https://doi.org/10.1007/s10311-018-0741-8.

Ejsmont, W., Kiss, A. K., Grzegorczyk-Karolak, I., 2025. Optimization of *In Vitro* Shoot Culture Parameters for Enhanced Biomass and Rosmarinic Acid Production in Salvia atropatana. Molecules, 30(12), 2654.

El Sherif, F., AlDayel, M., Ismail, M. B., Alrajeh, H. S., Younis, N. S., Khattab, S., 2023. Bio-stimulant for improving *Simmondsia chinensis* secondary metabolite production, as well as antimicrobial activity and wound healing abilities. Plants, 12(18), 3311.

Elshafie, H. S., Camele, I., Mohamed, A. A., 2023. A comprehensive review on the biological, agricultural and pharmaceutical properties of secondary metabolites based-plant origin. International journal of molecular sciences, 24(4), 3266. https://www.mdpi.com/1422-0067/24/4/3266

Fàbregas, N., Fernie, A., 2019. The metabolic response to drought. Journal of Experimental Botany, 70, 1077–1085. https://doi.org/10.1093/jxb/ery437.

Faehnrich, B., Franz, C., Nemaz, P., Kaul, H.-P., 2021. Medicinal plants and their secondary metabolites – State of the art and trends in breeding, analytics and use in feed supplementation– with special focus on German chamomile. Journal of Applied Botany and Food Quality, 61-74 Pages. https://doi.org/10.5073/JABFQ.2021.094.008

Falsini, S., Ballantini, S., Pittella, A., Fico, G., Giuliani, C., Palchetti, E., ... Innocenti, M., 2025. Secondary Metabolites Composition and Their Histochemical Localization in the Fruit of *Piper malgassicum* Papini, Palchetti, Gori and Rota Nodari (Piperaceae). Chemistry & Biodiversity, e03289.

Fazili, M., Bashir, I., Ahmad, M., Yaqoob, U., Geelani, S., 2022. *In vitro* strategies for the enhancement of secondary metabolite production in plants: a review. Bulletin of the National Research Centre, 46. https://doi.org/10.1186/s42269-022-00717-z.

Ganie, I. B., Ahmad, Z., Shahzad, A., Zaushintsena, A., Neverova, O., Ivanova, S., ... Tahseen, S., 2022. Biotechnological intervention and secondary metabolite production in *Centella asiatica* L. *Plants*, *11*(21), 2928.

Ge, W., Xin, J., Tian, R., 2023. Phenylpropanoid pathway in plants and its role in response to heavy metal stress: a review. Sheng wu Gong Cheng xue bao= Chinese Journal of Biotechnology, 39(2), 425-445. https://doi.org/10.13345/j.cjb.220338.

Ghasemi, S., Kumleh, H., Kordrostami, M., 2018. Changes in the expression of some genes involved in the biosynthesis of secondary metabolites in *Cuminum cyminum* L. under UV stress. Protoplasma, 256, 279 - 290. https://doi.org/10.1007/s00709-018-1297-y.

Ghosh, S., Adhikari, S., Adhikari, A., Hossain, Z., 2021. Contribution of plant miRNAome studies towards understanding heavy metal stress responses: Current Status and Future Perspectives. Environmental and Experimental Botany. https://doi.org/10.1016/j.envexpbot.2021.104705.

Golkar, P., Taghizadeh, M., 2018. *In vitro* evaluation of phenolic and osmolite compounds, ionic content, and antioxidant activity in safflower (*Carthamus tinctorius* L.) under salinity stress. Plant Cell, Tissue and Organ Culture (PCTOC), 134, 357-368. https://doi.org/10.1007/s11240-018-1427-4.

Goncharuk, E., Zagoskina, N., 2023. Heavy Metals, Their Phytotoxicity, and the Role of Phenolic Antioxidants in Plant Stress Responses with Focus on Cadmium: Review. Molecules, 28. https://doi.org/10.3390/molecules28093921.

Hachlafi, N. E., Aanniz, T., Menyiy, N. E., Baaboua, A. E., Omari, N. E., Balahbib, A., Shariati, M. A., Zengin, G., Fikri-Benbrahim, K., Bouyahya, A., 2023. *In Vitro* and *In Vivo* Biological Investigations of Camphene and Its Mechanism Insights: A Review. Food Reviews International, 39(4), 1799-1826. https://doi.org/10.1080/87559129.2021.1936007

Halder, M., Sarkar, S., Jha, S., 2019. Elicitation: A biotechnological tool for enhanced production of secondary metabolites in hairy root cultures. Engineering in Life Sciences, 19, 880 - 895.

https://doi.org/10.1002/elsc.201900058.
Han, T., Miao, G., 2024. Strategies, Achievements, and Potential Challenges of Plant and Microbial Chassis in the Biosynthesis of Plant Secondary Metabolites. Molecules, 29. https://doi.org/10.3390/molecules29092106.
Hasanuzzaman, M., Zhou, M., Shabala, S., 2023. How Does Stomatal Density and Residual Transpiration Contribute to Osmotic Stress Tolerance?. Plants, 12. https://doi.org/10.3390/plants12030494.
He, J., Yao, L., Pecoraro, L., Liu, C., Wang, J., Huang, L., Gao, W., 2022. Cold stress regulates accumulation of flavonoids and terpenoids in plants by phytohormone, transcription process, functional enzyme, and epigenetics. Critical Reviews in Biotechnology, 43, 680 - 697. https://doi.org/10.1080/07388551.2022.2053056.
Hill, C. R., Shafaei, A., Balmer, L., Lewis, J. R., Hodgson, J. M., Millar, A. H., Blekkenhorst, L. C., 2023. Sulfur compounds: From plants to humans and their role in chronic disease prevention. Critical Reviews in Food Science and Nutrition, 63(27), 8616-8638. https://doi.org/10.1080/10408398.2022.2057915
Humbal, A., Pathak, B., 2023. Harnessing nanoparticle-mediated elicitation in plant tissue culture: a promising approach for secondary metabolite production. Plant Cell, Tissue and Organ Culture (PCTOC), 155, 385 - 402. https://doi.org/10.1007/s11240-023-02612-5.
Hussein, R. A., El-Anssary, A. A., 2019. Plants secondary metabolites: The key drivers of the pharmacological actions of medicinal plants. Herbal medicine, 1(3), 11-30.
Ibrahim, M. H., Chee Kong, Y., Mohd Zain, N. A., 2017. Effect of cadmium and copper exposure on growth, secondary metabolites and antioxidant activity in the medicinal plant Sambung Nyawa (*Gynura procumbens* (Lour.) Merr). Molecules, 22(10), 1623.
Inam, M., Attique, I., Zahra, M., Khan, A., Hahim, M., Hano, C., Anjum, S., 2023. Metal oxide nanoparticles and plant secondary metabolism: unraveling the game-changer nano-elicitors. Plant Cell, Tissue and Organ Culture (PCTOC), 155, 327 - 344. https://doi.org/10.1007/s11240-023-02587-3.
In-On, A., Thananusak, R., Ruengjitchatchawalya, M., Vongsangnak, W., Laomettachit, T., 2022. Construction of light-responsive gene regulatory network for growth, development and secondary metabolite production in Cordyceps militaris. *Biology*, *11*(1), 71.
Iqbal, S., Begum, F., Rabaan, A. A., Aljeldah, M., Al Shammari, B. R., Alawfi, A., Alshengeti, A., Sulaiman, T., Khan, A., 2023. Classification and multifaceted potential of secondary metabolites produced by Bacillus subtilis group: A comprehensive review. Molecules, 28(3), 927. https://www.mdpi.com/1420-3049/28/3/927
Isah, T., Umar, S., Mujib, A., Sharma, M. P., Rajasekharan, P. E., Zafar, N., Frukh, A., 2018. Secondary metabolism of pharmaceuticals in the plant *in vitro* cultures: strategies, approaches, and limitations to achieving higher yield. Plant Cell, Tissue and Organ Culture (PCTOC), 132(2), 239-265.
Jain, D., Bisht, S., Parvez, A., Singh, K., Bhaskar, P., Koubouris, G., 2024. Effective Biotic Elicitors for Augmentation of Secondary Metabolite Production in Medicinal Plants. Agriculture. https://doi.org/10.3390/agriculture14060796.
Jaiswal, D., Pandey-Rai, S., Agrawal, S., 2021. Untangling the UV-B radiation-induced transcriptional network regulating plant morphogenesis and secondary metabolite production. Environmental and Experimental Botany. https://doi.org/10.1016/j.envexpbot.2021.104655.
Jakovljević, D., Stanković, M., Warchoł, M., Skrzypek, E., 2022. Basil (*Ocimum* L.) cell and organ culture for the secondary metabolites production: a review. Plant Cell, Tissue and Organ Culture (PCTOC), 149, 61 - 79. https://doi.org/10.1007/s11240-022-02286-5.
Jamwal, K., Bhattacharya, S., Puri, S., 2018. Plant growth regulator mediated consequences of secondary metabolites in medicinal plants. Journal of applied research on medicinal and aromatic plants, 9, 26-38.

Javed, R., Gürel, E., 2019. Salt stress by NaCl alters the physiology and biochemistry of tissue culture-grownStevia rebaudiana Bertoni. Turkish Journal of Agriculture and Forestry, 43(1), 11-20.

Kamarul Zaman, M. A., Azzeme, A. M., Ramle, I. K., Normanshah, N., Ramli, S. N., Shaharuddin, N. A., ... Abdullah, S. N. A., 2020. Induction, multiplication, and evaluation of antioxidant activity of Polyalthia bullata callus, a woody medicinal plant. Plants, 9(12), 1772.

Khalafalla, M., 2025. Plant Cell Suspension Culture for Plant Secondary Metabolite Production: Current Status, Constraints, and Future Solutions. Polish Journal of Environmental Studies. https://doi.org/10.15244/pjoes/195703.

Khalifa, A. M., Abd-ElShafy, E., Abu-Khudir, R., Gaafar, R. M., 2022. Influence of gamma radiation and phenylalanine on secondary metabolites in callus cultures of milk thistle (*Silybum marianum* L.). Journal of Genetic Engineering and Biotechnology, 20(1), 166.

Khatoon, S., Raj, N., Fakhri, K. U., Kumar, S., Manzoor, N., 2025. *In vitro* and in silico antifungal susceptibilities of thymol against Aspergillus flavus. The Microbe, 8, 100490.

Khayri, J., Banadka, A., Rashmi, R., Nagella, P., Alessa, F., Almaghasla, M., 2023. Cadmium toxicity in medicinal plants: An overview of the tolerance strategies, biotechnological and omics approaches to alleviate metal stress. Frontiers in Plant Science, 13. https://doi.org/10.3389/fpls.2022.1047410.

Kidd, P. S., Llugany, M., Poschenrieder, C. H., Gunse, B., Barcelo, J., 2001. The role of root exudates in aluminium resistance and silicon‐induced amelioration of aluminium toxicity in three varieties of maize (*Zea mays* L.). Journal of experimental botany, 52(359), 1339-1352.

Krif, G., El Aissami, A., Mokrini, F., 2025. First Report on the Parasitic Potential of Paraconiothyrium cyclothyrioides Against Meloidogyne incognita. Journal of Phytopathology, 173(4), e70141.

Kruszka, D., Selvakesavan, R., Kachlicki, P., Franklin, G., 2022. Untargeted metabolomics analysis reveals the elicitation of important secondary metabolites upon treatment with various metal and metal oxide nanoparticles in *Hypericum perforatum* L. cell suspension cultures. Industrial Crops and Products. https://doi.org/10.1016/j.indcrop.2022.114561.

Kumar, S., Saini, R., Suthar, P., Kumar, V., Sharma, R., 2022. Plant Secondary Metabolites: Their Food and Therapeutic Importance. Içinde A. K. Sharma & A. Sharma (Ed.), Plant Secondary Metabolites (ss. 371-413). Springer Nature Singapore. https://doi.org/10.1007/978-981-16-4779-6_12

Kumokita, R., Bamba, T., Inokuma, K., Yoshida, T., Ito, Y., Kondo, A., Hasunuma, T., 2022. Construction of an L-tyrosine chassis in Pichia pastoris enhances aromatic secondary metabolite production from glycerol. *ACS Synthetic Biology*, *11*(6), 2098-2107.

Künstler, A., Gullner, G., Ádám, A. L., Kolozsváriné Nagy, J., Király, L., 2020. The versatile roles of sulfur-containing biomolecules in plant defense—A road to disease resistance. *Plants*, *9*(12), 1705.

Lajayer, A., Ghorbanpour, M., Nikabadi, S., 2017. Heavy metals in contaminated environment: Destiny of secondary metabolite biosynthesis, oxidative status and phytoextraction in medicinal plants. Ecotoxicology and environmental safety, 145, 377-390. https://doi.org/10.1016/j.ecoenv.2017.07.035.

Li, M., Li, X., Zhang, X., Ai, J., Shi, G., Wang, Z., Liang, L., Liu, J., Sun, D., 2025. Optimization of somatic embryogenesis system and accumulation of secondary metabolites in callus of Schisandra chinensis (Turcz.) Baill. Industrial Crops and Products. https://doi.org/10.1016/j.indcrop.2025.120572.

Lugan, R., Niogret, M., Leport, L., Guégan, J., Larher, F., Savouré, A., Kopka, J., Bouchereau, A., 2010. Metabolome and water homeostasis analysis of Thellungiella salsuginea suggests that dehydration tolerance is a key response to osmotic stress in this halophyte. The Plant journal: for cell and molecular biology, 64 2, 215-29. https://doi.org/10.1111/j.1365-313X.2010.04323.x.

Lupo, M., Alfieri, G., Filippi, S., Modesti, M., Brunori, E., Pacchiarelli, A., ... Silvestri, C., 2025. Red-leaf hazelnut: Biotechnological approaches for secondary metabolite production and potential biological activities. Plant Physiology and Biochemistry, 229, 110329.

Ma, B., Li, Y., Wang, T., Li, D., Jia, S., 2025. Advances in CRISPR/Cas9-Based Gene Editing in Filamentous Fungi. Journal of Fungi, 11(5), 350.

Mai, N. T. N., Phong, T. H., Cuong, D. M., Luan, V. Q., Tung, H. T., Phuong, H. T. N., ... Nhut, D. T., 2025. The changes of ethylene gas accumulation, antioxidant system activity, and secondary metabolite synthesis during *in vitro* adventitious root formation of Phyllanthus amarus. Plant Cell, Tissue and Organ Culture (PCTOC), 160(1), 11.

Maleki, M., Ghorbanpour, M., Kariman, K., 2017. Physiological and antioxidative responses of medicinal plants exposed to heavy metals stress. Plant Gene, 11, 247-254. https://doi.org/10.1016/J.PLGENE.2017.04.006.

Mamdouh, D., Smetanska, I., 2022. Optimization of callus and cell suspension cultures of Lycium schweinfurthii for improved production of phenolics, flavonoids, and antioxidant activity. Horticulturae, 8(5), 394.

Maresca, V., Teta, R., Finamore, C., Cianciullo, P., Sorbo, S., D'Auria, M., Basile, A., 2023. Heavy metal stress induces adaptative responses in the liverwort *Conocephalum conicum* L. (Dum.): an integrated biologic and metabolomic study. Environmental and Experimental Botany. https://doi.org/10.1016/j.envexpbot.2023.105292.

Markowski, M., Czarnomska, Z., Tomiczak, K., Mikuła, A., Granica, S., Podwyszyńska, M., Szypuła, W., 2024. The influence of cryopreservation via encapsulation-dehydration on growth kinetics, embryogenic potential and secondary metabolite production of cell suspension cultures of Gentiana capitata Buch.-Ham. ex D.Don and *Gentiana decumbens* L. Industrial Crops and Products. https://doi.org/10.1016/j.indcrop.2024.118349.

Martínez-Chávez, L., Hernández-Ramírez, M., Feregrino-Pérez, A., Escalante, K., 2024. Cutting-Edge Strategies to Enhance Bioactive Compound Production in Plants: Potential Value of Integration of Elicitation, Metabolic Engineering, and Green Nanotechnology. Agronomy. https://doi.org/10.3390/agronomy14122822.

Mazhar, M. W., Ishtiaq, M., Maqbool, M., Jafri, F. I., Siddiqui, M. H., Alamri, S., Akhtar, M. S., 2024. Synergistic effects of selenium nanoparticles and LED light on enhancement of secondary metabolites in sandalwood (*Santalum album*) plants through in-vitro callus culturing technique. PeerJ, 12, e18106.

Melato, F. A., Regnier, T., McCrindle, R. I., Mokgalaka, N. S., 2012. Impact of metals on secondary metabolites production and plant morphology in vetiver grass (Chrysopogon zizanioides). South African Journal of Chemistry, 65, 178-183.

Micheal, L., 2025. Optimization of *In Vitro* Culture Conditions for Bioactive Compound Biosynthesis in Lepidium sativum Using UV-C and Melatonin Elicitors.

Ming, Q., Su, C., Zheng, C., Jia, M., Zhang, Q., Zhang, H., Rahman, K., Han, T., Qin, L., 2013. Elicitors from the endophytic fungus Trichoderma atroviride promote Salvia miltiorrhiza hairy root growth and tanshinone biosynthesis. Journal of experimental botany, 64 18, 5687-94. https://doi.org/10.1093/jxb/ert342.

Mipeshwaree Devi, A., Khedashwori Devi, K., Premi Devi, P., Lakshmipriyari Devi, M., Das, S., 2023. Metabolic engineering of plant secondary metabolites: Prospects and its technological challenges. Frontiers in Plant Science, 14, 1171154.

Moeini, A., Germann, N., Malinconico, M., Santagata, G., 2021. Formulation of secondary compounds as additives of biopolymer-based food packaging: A review. Trends in Food Science & Technology, 114, 342-354. https://doi.org/10.1016/j.tifs.2021.05.040

Molina, L., Segura, A., 2021. Biochemical and Metabolic Plant Responses toward Polycyclic Aromatic Hydrocarbons and Heavy Metals Present in Atmospheric Pollution. Plants, 10. https://doi.org/10.3390/plants10112305.

Motolinía-Alcántara, E., Castillo-Araiza, C., Rodríguez-Monroy, M., Román-Guerrero, A., Cruz-Sosa, F., 2021. Engineering Considerations to Produce Bioactive Compounds from Plant Cell Suspension Culture in Bioreactors. Plants, 10. https://doi.org/10.3390/plants10122762.

Murthy, H. N., Dandin, V. S., Zhong, J. J., Paek, K. Y., 2014. Strategies for enhanced production of plant secondary metabolites from cell and organ cultures. In Production of biomass and bioactive compounds using bioreactor technology (pp. 471-508). Dordrecht: Springer Netherlands.
Murthy, H., Joseph, K., Hahn, J., Lee, H., Paek, K., Park, S., 2023. Suspension culture of somatic embryos for the production of high-value secondary metabolites. Physiology and Molecular Biology of Plants, 29, 1153-1177. https://doi.org/10.1007/s12298-023-01365-x.
Nahar, N., Zilani, M. N. H., Biswas, P., Billah, M. M., Bibi, S., Albekairi, N. A., ... Hasan, M. N., 2024. Profiling of secondary metabolite and evaluation of anti-diabetic potency of *Crotalaria quinquefolia* (L): *In-vitro*, *in-vivo*, and in-silico approaches. Saudi Pharmaceutical Journal, 32(1), 101887.
Naik, P. M., Al–Khayri, J. M., 2016. Abiotic and biotic elicitors–role in secondary metabolites production through *in vitro* culture of medicinal plants. IntechOpen.
Najafi, F., Mohammadi, S., Asgharian, P., Kosari-Nasab, M., 2025. The impact of activated charcoal and graphite on growth parameters and production of secondary metabolites of Plantago maritima through *in vitro* culture. BMC chemistry, 19(1), 222.
Naji, E. F., Abdulfatah, H. F., Hashim, K. S., 2024. Plant Secondary Metabolites, Their Classification and Biological Roles: A Review. Journal of University of Anbar for Pure Science, 18(1).
Narayani, M., Srivastava, S., 2017. Elicitation: a stimulation of stress in *in vitro* plant cell/tissue cultures for enhancement of secondary metabolite production. Phytochemistry reviews, 16(6), 1227-1252.
Omar, S., Ashokhan, S., Majid, N., Karsani, S., Lau, B., Yaacob, J., 2024. Enhanced azadirachtin production in neem (Azadirachta indica) callus through NaCl elicitation: Insights into differential protein regulation via shotgun proteomics. Pesticide biochemistry and physiology, 199, 105778. https://doi.org/10.1016/j.pestbp.2024.105778.
Ozyigit, I. I., Dogan, I., Hocaoglu-Ozyigit, A., Yalcin, B., Erdogan, A., Yalcin, I. E., Cabi, E., Kaya, Y., 2023. Production of secondary metabolites using tissue culture-based biotechnological applications. Frontiers in Plant Science, 14, 1132555.
Özay, C., Pehlivan, E., 2024. Bitki Sekonder Metabolitlerinin Biyosentezini ve Akümülasyonunu Etkileyen Faktörler. Journal of Faculty of Pharmacy of Ankara University, 48(3), 44-44. https://dergipark.org.tr/en/pub/jfpanu/issue/85047/1488042
Park, S., Paek, K., 2014. Bioreactor Culture of Shoots and Somatic Embryos of Medicinal Plants for Production of Bioactive Compounds. 337-368. https://doi.org/10.1007/978-94-017-9223-3_14.
Pellegrini, M., Ricci, A., Serio, A., Chaves-López, C., Mazzarrino, G., D'Amato, S., Lo Sterzo, C., Paparella, A., 2018. Characterization of Essential Oils Obtained from Abruzzo Autochthonous Plants: Antioxidant and Antimicrobial Activities Assessment for Food Application. Foods, 7(2), 19. https://doi.org/10.3390/foods7020019
Ptak, A., Szewczyk, A., Simlat, M., Pawłowska, B., Warchoł, M., 2024. LED light improves shoot multiplication, steviol glycosides and phenolic compounds biosynthesis in Stevia rebaudiana Bertoni *in vitro* culture. Scientific Reports, 14(1), 30860.
Rabeh, K., Hnini, M., Oubohssaine, M., 2025. A comprehensive review of transcription factor-mediated regulation of secondary metabolites in plants under environmental stress. Stress Biology, 5(1), 15.
Rahimi, M., Mortazavi, M., Mianabadi, A., Debnath, S., 2023. Evaluation of basil (*Ocimum basilicum*) accessions under different drought conditions based on yield and physio-biochemical traits. BMC Plant Biology, 23(1), 523.
Rahmawati, A., Esyanti, R. R., 2014. Analysis of secondary metabolite production in somatic embryo of Pasak Bumi (*Eurycoma longifolia* Jack.). Procedia Chemistry, 13, 112-118.
Rahmawati, A., Faizal, A., Iriawati, Othman, R., Nugrahapraja, H., Esyanti, R. R., 2025. Growth and steviol glycoside analysis on *Stevia rebaudiana* shoot culture elicited by methyl jasmonate: insight from transcriptomics. Plant Cell, Tissue and Organ Culture (PCTOC), 162(2), 48.
Rai, R., Meena, R. P., Smita, S. S., Shukla, A., Rai, S. K., Pandey-Rai, S., 2011. UV-B and UV-C

pre-treatments induce physiological changes and artemisinin biosynthesis in *Artemisia annua* L.–An antimalarial plant. Journal of photochemistry and photobiology B: Biology, 105(3), 216-225.

Ramakrishna, W., Kumari, A., Rahman, N., Mandave, P., 2021. Anticancer Activities of Plant Secondary Metabolites: Rice Callus Suspension Culture as a New Paradigm. Rice Science. https://doi.org/10.1016/j.rsci.2020.11.004

Ramani, S., Chelliah, J., 2007. UV-B-induced signaling events leading to enhanced-production of catharanthine in Catharanthus roseus cell suspension cultures. BMC plant biology, 7(1), 61.

Rao, M., Zheng, B., 2025. The Role of Polyphenols in Abiotic Stress Tolerance and Their Antioxidant Properties to Scavenge Reactive Oxygen Species and Free Radicals. Antioxidants, 14. https://doi.org/10.3390/antiox14010074.

Rao, S., Usha, K., Arjun., 2015. Production of secondary metabolites from callus cultures of *Centella asiatica* (L.) Urban. *Annals of Phytomedicine-An International Journal*, *4*(1), 74-78.

Regvar, M., Bukovnik, U., Likar, M., Kreft, I., 2012. UV-B radiation affects flavonoids and fungal colonisation in Fagopyrum esculentum and F. tataricum. Central European Journal of Biology, 7(2), 275-283.

Reshi, Z., Ahmad, W., Lukatkin, A., Javed, S., 2023. From Nature to Lab: A Review of Secondary Metabolite Biosynthetic Pathways, Environmental Influences, and *In Vitro* Approaches. Metabolites, 13. https://doi.org/10.3390/metabo13080895.

Ritonga, F., Chen, S., 2020. Physiological and Molecular Mechanism Involved in Cold Stress Tolerance in Plants. Plants, 9. https://doi.org/10.3390/plants9050560.

Rui, Z., Xiu-Juan, W., Wei, G., 2020. Regulation mechanism of plant hormones on secondary metabolites. *Zhongguo Zhong yao za zhi= Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica*, *45*(17), 4205-4210. https://doi.org/10.19540/j.cnki.cjcmm.20190129.007.

Saeidnejad, A., 2021. Plant Growth Regulators and Secondary Metabolites, Downregulation and Upregulation. 388-399. https://doi.org/10.1201/9781003093640-25.

Sanyal, A. J., Anstee, Q. M., Trauner, M., Lawitz, E. J., Abdelmalek, M. F., Ding, D., ... Myers, R. P., 2022. Cirrhosis regression is associated with improved clinical outcomes in patients with nonalcoholic steatohepatitis. *Hepatology*, *75*(5), 1235-1246.

Saragoça, A., Silva, A. C., Varanda, C. M., Materatski, P., Ortega, A., Cordeiro, A. I., Telo da Gama, J., 2025. Current Context of *Cannabis sativa* Cultivation and Parameters Influencing Its Development. Agriculture, 15(15), 1635.

Selwal, N., Goutam, U., Akhtar, N., Sood, M., Kukreja, S., 2024. Elicitation: "A Trump Card" for Enhancing Secondary Metabolites in Plants. Journal of Plant Growth Regulation. https://doi.org/10.1007/s00344-024-11294-y.

Semenova, N., Ivanitskikh, A., Uyutova, N., Smirnov, A., Proshkin, Y., Burynin, D., Kachan, S., Sokolov, A., Dorokhov, A., Chilingaryan, N., 2024. Effect of UV Stress on the Antioxidant Capacity, Photosynthetic Activity, Flavonoid and Steviol Glycoside Accumulation of *Stevia rebaudiana* Bertoni. Horticulturae. https://doi.org/10.3390/horticulturae10030210.

Setyawati, A., Samanhudi, S., Prameswari, W., Syukri, D., Ramadhani, D. F., Talitha, O., 2023. *In Vitro* Propagation and Secondary Metabolite Production of Medicinal Plant of Euchresta horsfieldii (Lesch) Benn. Plant Breeding and Biotechnology, 11(1), 34-48.

Shasmita, Behera, S., Mishra, P., Samal, M., Mohapatra, D., Monalisa, K., Naik, S. K., 2023. Recent advances in tissue culture and secondary metabolite production in *Hypericum perforatum* L. Plant Cell, Tissue and Organ Culture (PCTOC), 154(1), 13-28.

Singh, P., Singh, A., Choudhary, K., 2023. Revisiting the role of phenylpropanoids in plant defense against UV-B stress. Plant Stress. https://doi.org/10.1016/j.stress.2023.100143.

Smetanska, I., 2008. Production of secondary metabolites using plant cell cultures. Advances in biochemical engineering/biotechnology, 111, 187-228. https://doi.org/10.1007/10_2008_103.

Song, X., Zhu, Y., Bao, Y., 2025. Identification and characteristics of differentially expressed genes under UV-B stress in *Gossypium hirsutum*. Frontiers in Plant Science, 15. https://doi.

org/10.3389/fpls.2024.1529912.
Souffront, D. K. S., Salazar-Amoretti, D., Jayachandran, K., 2022. Influence of vermicompost tea on secondary metabolite production in tomato crop. *Scientia Horticulturae*, *301*, 111135.
Sreekissoon, A., Chen, W., Viljoen, A. M., Finnie, J. F., Van Staden, J., 2025. The Effects of Light, Salt, and Osmotic Stress on Mesembryanthemum tortuosum Growth and Mesembrine[L] Type Alkaloid Production *In Vitro*. Physiologia Plantarum, 177(4), e70385.
Sreenikethanam, A., Raj, S., J., R., Gugulothu, P., Bajhaiya, A., 2022. Genetic Engineering of Microalgae for Secondary Metabolite Production: Recent Developments, Challenges, and Future Prospects. Frontiers in Bioengineering and Biotechnology, 10. https://doi.org/10.3389/fbioe.2022.836056.
Sudheer, W. N., Thiruvengadam, M., Nagella, P., 2023. A comprehensive review on tissue culture studies and secondary metabolite production in *Bacopa monnieri* L. Pennell: A nootropic plant. Critical reviews in biotechnology, 43(6), 956-970.
Swallah, M. S., Sun, H., Affoh, R., Fu, H., Yu, H., 2020. Antioxidant Potential Overviews of Secondary Metabolites (Polyphenols) in Fruits. International Journal of Food Science, 2020(1), 9081686. https://doi.org/10.1155/2020/9081686
Szabó, B., Tyihák, E., Szabó, G., Botz, L., 2003. Mycotoxin and drought stress induced change of alkaloid content of Papaver somniferum plantlets. Acta Botanica Hungarica, 45(3-4), 409-417.
Takshak, S., Agrawal, S., 2019. Defense potential of secondary metabolites in medicinal plants under UV-B stress. Journal of photochemistry and photobiology. B, Biology, 193, 51-88. https://doi.org/10.1016/j.jphotobiol.2019.02.002.
Tarigholizadeh, S., Motafakkerazad, R., Kosari-Nasab, M., Movafeghi, A., Mohammadi, S., Sabzi, M., Talebpour, A., 2021. Influence of plant growth regulators and salicylic acid on the production of some secondary metabolites in callus and cell suspension culture of *Satureja sahendica* Bornm. Acta agriculturae Slovenica. https://doi.org/10.14720/aas.2021.117.4.773.
Teoh, E. S., 2016. Secondary Metabolites of Plants. Medicinal Orchids of Asia (ss. 59-73). Springer International Publishing. https://doi.org/10.1007/978-3-319-24274-3_5
Terletskaya, N., Shadenova, E., Litvinenko, Y., Ashimuly, K., Erbay, M., Mamirova, A., Nazarova, I., Meduntseva, N., Kudrina, N., Korbozova, N., Djangalina, E., 2024. Influence of Cold Stress on Physiological and Phytochemical Characteristics and Secondary Metabolite Accumulation in Microclones of *Juglans regia* L.. International Journal of Molecular Sciences, 25. https://doi.org/10.3390/ijms25094991.
Thakur, A., Morya, S., Kumar, D., Ahmed, J., Mac Regenstein, J., Fuoco, D., 2025. Potential of Quinoa as a Source of Secondary Metabolites for Human Health. In Plant Secondary Metabolites (pp. 274-293). CRC Press.
Thoma, F., Somborn-Schulz, A., Schlehuber, D., Keuter, V., Deerberg, G., 2020. Effects of Light on Secondary Metabolites in Selected Leafy Greens: A Review. Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.00497.
Tidke, S., Kiran, S., Giridhar, P., Gokare, R., 2019. Current Understanding and Future Perspectives of Endophytic Microbes vis-a-vis Production of Secondary Metabolites. Reference Series in Phytochemistry. https://doi.org/10.1007/978-3-319-76900-4_12-1.
Tomiczak, K., Mikuła, A., Niedziela, A., Wójcik-Lewandowska, A., Domżalska, L., Rybczyński, J., 2019. Somatic Embryogenesis in the Family Gentianaceae and Its Biotechnological Application. Frontiers in Plant Science, 10. https://doi.org/10.3389/fpls.2019.00762.
Tripathi, D., Meena, R., Pandey-Rai, S., 2021. Short term UV-B radiation mediated modulation of physiological traits and withanolides production in *Withania coagulans* (L.) Dunal under in-vitro condition. Physiology and Molecular Biology of Plants, 27, 1823 - 1835. https://doi.org/10.1007/s12298-021-01046-7.
Twaij, B. M., Hasan, M. N., 2022. Bioactive secondary metabolites from plant sources: Types, synthesis, and their therapeutic uses. International Journal of Plant Biology, 13(1), 4-14. https://www.mdpi.com/2037-0164/13/1/3

Ullrich, C. I., Aloni, R., Saeed, M. E., Ullrich, W., Efferth, T., 2019. Comparison between tumors in plants and human beings: Mechanisms of tumor development and therapy with secondary plant metabolites. Phytomedicine, 64, 153081.

Ülger, T. G., Ayhan, N. Y., 2020. Bitki sekonder metabolitlerinin sağlık üzerine fonksiyonel etkileri. Acıbadem Üniversitesi Sağlık Bilimleri Dergisi, 3, 384-390. https://dergipark.org.tr/en/download/article-file/1701934

Vázquez-Flota, F., Monforte-González, M., Miranda-Ham, M., 2016. Application of Somatic Embryogenesis to Secondary Metabolite-Producing Plants. 455-469. https://doi.org/10.1007/978-3-319-33705-0_25.

Verma, V., Ravindran, P., Kumar, P. P., 2016. Plant hormone-mediated regulation of stress responses. BMC Plant Biology, 16(1), 86. https://doi.org/10.1186/s12870-016-0771-y

Vuran, N. E., Türker, M., 2021. Bitki doku kültürlerinde sekonder metabolit miktarını arttırmaya yönelik uygulamalar. International Journal of Advances in Engineering and Pure Sciences, 33(3), 487-498. https://dergipark.org.tr/en/pub/jeps/issue/64843/900129

Wang, G., Sun, X., Li, Y., Wang, Y., Jin, C., 2025. The role of UV-B radiation in modulating secondary metabolite biosynthesis and regulatory mechanisms in medicinal plants. BioResources. https://doi.org/10.15376/biores.20.2.wang.

Wawrosch, C., Zotchev, S., 2021. Production of bioactive plant secondary metabolites through *in vitro* technologies—status and outlook. Applied Microbiology and Biotechnology, 105, 6649 - 6668. https://doi.org/10.1007/s00253-021-11539-w.

Wawrosch, C., Zotchev, S. B., 2021. Production of bioactive plant secondary metabolites through *in vitro* technologies—Status and outlook. Applied Microbiology and Biotechnology, 105(18), 6649-6668. https://doi.org/10.1007/s00253-021-11539-w

Wu, P., Wang, G., Cao, Z., Liu, Y., Xia, N., Wang, Q., ... Chen, Z., 2023. Effects of different light qualities and plant growth regulators on the growth and secondary metabolite content of Lonicera macranthoides seedlings. *In Vitro* Cellular & Developmental Biology-Plant, 59(5), 536-546.

Wu, T., Kerbler, S., Fernie, A., Zhang, Y., 2021. Plant cell cultures as heterologous bio-factories for secondary metabolite production. Plant Communications, 2. https://doi.org/10.1016/j.xplc.2021.100235.

Xiao, C., Xu, C., Zhang, J., Jiang, W., Zhang, X., Yang, C., ... Zhou, T., 2022. Soil Microbial Communities Affect the Growth and Secondary Metabolite Accumulation in *Bletilla striata* (Thunb.) Rchb. f. Frontiers in Microbiology, 13, 916418.

Yue, W., Ming, Q., Lin, B., Rahman, K., Zheng, C., Han, T., Qin, L., 2016. Medicinal plant cell suspension cultures: pharmaceutical applications and high-yielding strategies for the desired secondary metabolites. Critical Reviews in Biotechnology, 36, 215 - 232. https://doi.org/10.3109/07388551.2014.923986.

Zandavar, H., Babazad, M. A., 2023. Secondary Metabolites: Alkaloids. Herbs and Spices: New Advances, 79.

Zhang, Y., Qi, G., Yao, L., Huang, L., Wang, J., Gao, W., 2022. Effects of Metal Nanoparticles and Other Preparative Materials in the Environment on Plants: From the Perspective of Improving Secondary Metabolites. Journal of agricultural and food chemistry. https://doi.org/10.1021/acs.jafc.1c05152.

Zhao, K., Lan, Y., Shi, Y., Duan, C., Yu, K., 2024. Metabolite and transcriptome analyses reveal the effects of salinity stress on the biosynthesis of proanthocyanidins and anthocyanins in grape suspension cells. Frontiers in Plant Science, 15. https://doi.org/10.3389/fpls.2024.1351008.

Zhong, C., Cao, X., Hu, J., Zhu, L., Zhang, J., Huang, J., Jin, Q., 2017. Nitrogen Metabolism in Adaptation of Photosynthesis to Water Stress in Rice Grown under Different Nitrogen Levels. Frontiers in Plant Science, 8. https://doi.org/10.3389/fpls.2017.01079.

Bölüm 13

BİTKİ GENETİK KAYNAKLARININ KORUNMASI

Başar SEVİNDİK [1]
Yeşim YALÇIN MENDİ [2]

13.1 BİTKİ GENETİK KAYNAKLARININ ÖNEMİ

Genetik kaynaklar; bitki, hayvan ve mikroorganizmalardan oluşan ve hem günümüzde hem de gelecekte ekonomik, ekolojik ve bilimsel açıdan değer taşıyan biyolojik materyaller olarak tanımlanır. Bu kaynakların kullanımı, bilimsel yöntemlerle bilgi toplanması, yararlı özelliklerin araştırılması, ticari ürünlerin geliştirilmesi ve sürdürülebilir biçimde korunması süreçlerini kapsar. Modern biyoteknolojinin hızlı gelişimi, yalnızca biyolojik çeşitlilik hakkındaki bilgilerimizi derinleştirmekle kalmamış, aynı zamanda gıda güvenliği, ilaç geliştirme, iklim değişikliğine uyum ve yeni ürünlerin tasarımı gibi çok sayıda alanın ortaya çıkmasına da katkı sağlamıştır. Bununla birlikte biyolojik çeşitlilik kaybı, günümüzde karşı karşıya kalınan en kritik küresel sorunlardan biridir (Hawkes ve ark., 2012). Bitkiler, ekosistemlerde birincil üretici olarak enerji akışını sağlamakta, biyokimyasal döngüleri düzenlemekte ve ekolojik dengeyi korumakta temel bir rol üstlenmektedir. Tarih boyunca insan faaliyetleri—tarım, endüstri, tıbbi kullanım ve kültürel pratikler—bitki biyoçeşitliliği üzerinde baskı yaratmıştır. Dünya Sağlık Örgütü'ne göre, günümüzde dünya nüfusunun yaklaşık %80'i birincil tedavi aracı olarak doğrudan bitkilerden veya bitkisel kökenli ürünlerden faydalanmaktadır (Gaio-Oliveira ve ark., 2017). İklim değişikliği, habitat tahribatı, hızlı nüfus artışı ve yanlış kullanım uygulamaları ise genetik erozyonu hızlandıran başlıca faktörlerdir. Doğadaki ve tarımsal sistemlerdeki bitki biyoçeşitliliğindeki azalma, bitkiler üzerine çalışan araştırmacıları bitki türlerinin korunması için alternatif arayışlara itmiştir (Reed, 2008). Bitki genetik kaynaklarının ko-

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tirilmelidir. Doku kültürü teknikleri kullanılarak farklı türler için klonal çoğaltım protokleri geliştirilmiş olsa da günümüzde birçok bitki için uzun süreli koruma amacıyla yapılan çalışmalar yetersiz kalmaktadır. Ticari olarak kullanılan türlerde gerçekleştirilmiş olan türlerde belirli oranlarda başarı sağlanmış olsa da özellikle yabani tiplerin ve endemik türlerin korunması için daha fazla çalışma yapmak gerekmektedir. Bu çalışmalar yapılırken bazı türler için yüksek oranda güvenilir ve kaliteli saklama protokolleri geliştirilmesi ve meydana gelebilecek olan ve genetik kaynakların saklanmasında istenmeyen bir durum olan somaklonal varyasyonu saptayabilecek moleküler markırların da geliştirilmesi önem arz etmektedir.

KAYNAKLAR

Ashmore, S.E., 1997. Current *in vitro* conservation techniques. In: F. Engelmann (Ed.), Status Report on the Development and Application of in vitro Techniques for the Conservation and Use of Plant Genetic Resources, pp.5–18. IPGRI. Rome, Italy.

Bachiri, Y., Song, G. Q., Plessis, P., Shoar-Ghaffari, A., Rekab, T., Morisset., C., 2001. Routine cryopreservation of kiwifruit (*Actinidia* spp) germplasm by encapsulation- dehydration: importance of plant growth regulators. CryoLetters 22, 61–74.

Banerjee, N., De Langhe, E., 1985. A tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of *Musa* (banana and plantain). Plant Cell Rep 4,351–354

Benson, E. E., 1990. Free radical damage in stored plant germplasm. International Board for Plant Genetic Resources, Rome.

Benson, E. E., 2004. Cryoconserving algal and plant diversity: Historical perspec- tives and future challenges. In: Fuller B, Lane N, Benson EE (eds) Life in the Frozen State. CRC Press, London, pp. 299-328.

Bertrand-Desbrunais, A., Noirot, M., & Charrier, A., 1992. Slow growth in vitro conservation of coffee (Coffea spp.). Plant Cell, Tissue and Organ Culture (PCTOC), 31(2), 105-110.

Chin, H. F., 1988. Cryopreservation of Veitchia and Howea palm embryos: non-development of the haustorium. Cryo-Letters, 9, 372-379.

Cho, E. G., Hor, Y. L., Kim, H. H., Ramanatha Rao, V., Engelmann, F., 2002. Cryopreservation of *Citrus madurensis* zygotic embryonic axes by vitrification: Importance of pregrowth and preculture conditions. CryoLetters, 23, 325–332

Clavero-Ramirez, I., Galvez-Farfan, J., Lopez-Aranda, J. M., Gonzalez-Benito, M. E., 2005. Apex cryopreservation of several strawberry genotypes by two encapsulation- dehydration methods. CryoLetters, 26, 17–24.

Day, J. G., McClellan, M. R., 1995. Cryopreservation and freeze-drying protocols. Methods in Molecular Biology Volume 38. Humana Press, Totowa, New Jersey.

de Oliveira Prudente, D., de Souza, L. B., & Paiva, R., 2019. Synthetic Seeds: Prospects and Advances in Cryopreservation. In Synthetic Seeds pp. 417-437. Springer, Cham.

Decruse, S. W., Seeni, S., Pushpangadan, P., 1999. Cryopreservation of alginate coated shoot tips of *in vitro* grown Holostemma annulare (Roxb.) K. Schum, an endangered medicinal plant: Influence of preculture and DMSO treatment on survival and regeneration. CryoLetters, 20, 243–250

Dereuddre, J., Scottez, C., Arnaud, Y., Duron, M., 1990. Effets d'un endurcissement au froid des vitroplants de poirier (Pyrus communis L. cv. Beurré Hardy) sur la résistance des apex axillaires à une congélation dans l'azote liquide. C R Acad Sci Paris, 310(3), 317–323.

Edwards, S., Kelbessa, E., 1999. Indicators to determine the level of threat to tree species. In: Proceedings of Forest Genetic Resources Conservation: Principles, Strategies and Actions, (Eds S. Edwards, A. Demise, T. Bekele and H. Gunther). Institute of Biodiversity Conservation and Research with GTZ, Addis Ababa.

Ellis, R. H., Hong, T. D., & Roberts, E. H., 1990. An intermediate category of seed storage behaviour? I. Coffee. Journal of Experimental Botany, 41(9), 1167-1174.

Engelmann, F., 1997. *In vitro* conservation methods. In: Ford-Lloyd BV, Newburry JH, Callow JA (eds) Biotechnology and Plant Genetic Resources: Conservation and Use. CAB International, Wellingford, pp 119–162.

Engelmann, F. 2000. Importance of cryopreservation for the conservation of plant genetic resources. In: Engelmann F, Takagi H (eds) Cryopreservation of Tropical Plant Germplasm—current Research Progress and Applications. JIRCAS, Tsukuba/IPGRI, Rome, pp 8–20.

Engelmann, F., Takagi, H., 2000. Cryopreservation of Tropical Plant Germplasm: Current Research Progress and Applications. JIRCAS, Tsukuba and IPGRI, Rome

Engelmann, F., & Engels, J. M. M., 2002. Technologies and strategies for *ex situ* conservation. Managing plant genetic diversity, 89-103.

Engels, J. M., & Wood, D., 1999. Conservation of agrobiodiversity. Agrobiodiversity: characterization, utilization and management., 355-385.

Escobar, R. H., Mafla, G., Roca, W. M., 1997. A methodology for recovering cassava plants from shoot tips maintained in liquid nitrogen. Plant Cell Rep, 16, 474– 478.

Fabre, J., & Dereuddre, J., 1990. Encapsulation-dehydration: a new approach to cryopreservation of *Solanum* shoot-tips. Cryo Letters.

Ford-Lloyd, B. V., Engels, J. M. M., Jackson, M., 2014. Genetic resources conservation challenges under the threat of climate change. In: Jackson M, Ford-Lloyd B and Parry M (eds) Plant Genetic Resources and Climate Change. Wallingford, UK: CAB International, pp. 16–37.

Gaio-Oliveira, G., Delicado, A., Martins-Loução, M. A., 2017. Botanic gardens as communicators of plant diversity and conservation. The Botanical Review, 83(3), 282-302.

Geda, M., Balakrishnan, M., 2013. *Ex Situ* Conservation of Biodiversity with Particular Emphasis to Ethiopia. ISRN Biodiversity, 1-11. DOI: 10.1155/2013/985037.

Ghosh, B., Haque, S. M., 2019. Synthetic seeds: An alternative approach for clonal propagation to avoiding the heterozygosity problem of natural botanical seeds. In Synthetic Seeds, pp. 77-112. Springer, Cham.

Gonzalez-Arnao, M.T., Engelmann, F., 2006. Cryopreservation of plant germplasm using the encapsulation- dehydration technique: Review and case study on sugarcane. CryoLetters, 27, 155–168.

Gonzalez-Arnao, M. T., Engelmann, F., Huet, C., Urra, C., 1993. Cryopreservation of encapsulated apices of sugarcane: effect of freezing procedure and histology. CryoLetters 14, 303–308.

Grospietch, M., Stodulkova, E., Zamecnik, J., 1999. Effect of osmotic stress on the dehydration tolerance and cryopreservation of Solanum tuberosum shoot tips. CryoLetters, 20,339–346.

Hao, Y. J., Liu, Q. L., Deng, X. X., 2002. Effect of cryopreservation on apple genetic resources at morphological, chromosomal and molecular levels. Cryobiology, 43, 46–53.

Hawkes, J. G., Maxted, N., Ford-Lloyd, B. V. (2012). The *ex situ* conservation of plant genetic resources. Springer Science & Business Media.

Hirata, K., Phunchindawan, M., Tukamoto, J., Goda, S., Miyamoto, K., 1996. Cryopreservation of microalgae using encapsulation-dehydration. CryoLetters, 17, 321–328.

Hong, T. D., Ellis, R. H., 1996. A protocol to determine seed storage behaviour (No. 1). Bioversity International.

Jeffries, M. J., 1997. Biodiversity and conservation, London: Routledge.

Kartha, K. K., 1985. Cryopreservation of Plant Cells and Organs. CRC Press Inc., Boca Raton, Florida.

Kocak, M., Izgu, T., Sevindik, B., Tutuncu, M., Curuk, P., Simsek, O., Aka Kaçar, Y., da Silva, J. A.

T., Mendi, Y. Y., 2014. Somatic embryogenesis of Turkish *Cyclamen persicum* Mill. Sci Hortic 172:26–33. https://doi.org/10.1016/j.scienta.2014.03.044

Kurt, O., Çelik, N., Göre, M., Kurt, H., 2019. Threats to Biodiversity Bio-Trafficking in Turkey. Turkish Journal of Agriculture-Food Science and Technology, 7(2), 46-51.

Langis, R., Steponkus, P., 1991. Vitrification of isolated rye protoplasts: protection against dehydration injury by ethylene glycol. CryoLetters, 12,107-112.

Lata, H., Moraes, R. M., Bertoni, B., & Pereira, A. M., 2010. *In vitro* germplasm conservation of Podophyllum peltatum L. under slow growth conditions. *In Vitro* Cellular & Developmental Biology-Plant, 46(1), 22-27.

Leveque, C., Mounolou, J. C., 2013. Biyoçeşitlilik, biyolojik devinimler ve koruma (Çeviri Editörleri: Hasan H. Başıbüyük, Ahmet Yılmaz, Sabri Kılınç). Ankara: Palme Yayıncılık, p259.

Mari, S., Engelmann, F., Chabrillange, N., Huet, C., Michaux-Ferrière, N., 1995. Histocytological study of coffee (Coffea racemosa and C. sessiliflora) apices of *in vitro* plantlets during their cryopreservation using the encapsulation-dehydration technique. CryoLetters, 16, 289–298.

Matsumoto, T., Sakai, A., 1995. An approach to enhance dehydration tolerance of alginate-coated dried meristems cooled to –196°C. CryoLetters, 16, 299–306.

Matsumoto, T., Sakai, A., Takahashi, C., Yamada, K., 1995. Cryopreservation of *in vitro*-grown apical meristems of wasabi (Wasabia japonica) by encapsulation- vitrification method. CryoLetters, 16, 189–196.

Maxted, N., Hawkes, J. G., Guarino, L., & Sawkins, M., 1997. Towards the selection of taxa for plant genetic conservation. Genetic Resources and Crop Evolution, 44(4), 337-348.

Mazur, P., 2004. Principles of cryobiology, In: Fuller B, Lane N, Benson EE (eds) Life in the Frozen State. CRC Press, London, pp. 3-66.

Miller, K., Allegretti, M. A., Johnson, N., Jonsson, B., 1995. Measures for conservation of Diodiversity and sustainable use of its components. In: Heywood V. H. (ed.), Global biodiversity assessment. UNEP, Cambridge University Press, Cambridge, pp. 915-1061.

Moore, G., Hawtin, G., 2014. International mechanisms for conservation and use of genetic resources In Plant Genetic Resources and Climate Change, M. Jackson, B. Ford-Lloyd, and M. Parry, eds. (Wallingford, UK: CAB International), http://dx.doi.org/10.1079/9781780641973.0098.

Nadarajan, J., Mansor, M., Krishnapillay, B., Staines, H., Benson, E., Harding, K., 2008. Development of cryopreservation strategies for a recalcitrant seed species using differential scanning calorimetry. Cryopreservation of crop species in Europe, p. 34.

Panis, B., Piette, B., Andre, E., Van den Houwe, I., & Swennen, R., 2009. Droplet vitrification: the first generic cryopreservation protocol for organized plant tissues?. In I International Symposium on Cryopreservation in Horticultural Species 908, pp. 157-162.

Paulet, F., Engelmann, F., Glaszmann, J. C., 1993. Cryopreservation of apices of in vitro plantlets of sugarcane (Saccharum sp.) using encapsulation/dehydration. Plant Cell Rep, 12, 525–529.

Pritchard, D. J., Harrop S. R., 2010. A re-evaluation of the role of *ex situ* conservation in the face of climate change. BG Journal, 7(1):3-6.

Redenbaugh, K., Paasch, B. D., Nichol, J. W., Kossler, M. E., Viss, P. R., Walker, K. A., 1986. Somatic seeds: Encapsulation of asexual plant embryos. Biotechnology, 4, 797–801.

Reed, B. M., 1991. Application of gas-permeable bags for in vitro cold storage of strawberry germplasm. Plant Cell Rep, 10,431–434

Reed, B. M. (2008). Cryopreservation—practical considerations. In Plant cryopreservation: a practical guide (pp. 3-13). Springer, New York, NY.

Sakai, A., 1966. Survival of a plant tissue at super-low temperature IV cell survival with rapid cooling and rewarming. Plant Physiol, 41,1050-1054.

Sakai, A., Kobayashi, S., Oiyama, I., 1990. Cryopreservation of nucellar cells of navel orange (Citrus sinensis Osb. var. brasiliensis Tanaka) by vitrification. Plant Cell Rep, 9, 30-33.

Sakai, A., 1995. Cryopreservation of germplasm of woody plants. In: Bajaj YPS (ed) Biotechnology in Agriculture and Forestry, Cryopreservation of Plant Germplasm I , Springer-Verlag, Berlin, Heidelberg, 32, pp.53-69

Sakai, A., 1997. Potentially valuable cryogenic procedures for cryopreservation of cultured plant meristems. In: Razdan MK, Cocking EC (eds) Conservation of Plant Genetic Resources *In Vitro* Science Publishers, USA, pp .53-66

Sakai, A., Matsumoto T, Hirai D, Niino TI (2000) Newly developed encapsulationdehydration protocol for plant cryopreservation. CryoLetters 21: 53–62.

Schnabel-Preikstas, B., Earle, E. D., Steponkus, P. L., 1992. Cryopreservation of chrysanthemum shoot tips by vitrification. Cryobiology, 29, 739.

Staritsky, G., Zandvoort, E. A., 1985. In vitro propagation and genetic conservation of tropical woody crops. Acta botanica neerlandica, 34, 238.

Uemura, M., Sakai, A., 1980. Survival of carnation (*Dianthus caryophyllus* L.) shoot apices frozen to the temperature of liquid nitrogen. Plant Cell Physiol, 21,85-94.

Walters, C., & Engels, J. (1998). The effects of storing seeds under extremely dry conditions. Seed Science Research, 8(1), 3-8.

Withers, L. A., 1975. Freeze-preservation of cultures cells and tissues. In: Thorpe, TA (ed) Frontiers of Plant Tissue Culture. International Association of Plant Tissue Culture, University of Calgary, Calgary, Alberta, Canada, pp. 297-309.

Withers, L. A., King, P. J., 1980. A simple freezing unit and routine cryopreservation methods for plant cell cultures. CryoLetters, 1, 213-220.

Withers, L. A., 1991. In-vitro conservation. Biological Journal of the Linnean Society, 43(1), 31-42.

Withers, L. A., 1995. Tissue and Organs Culture. Boca Raton: CRC Press, pp. 243–267.

Withers, L. A., Engelmann, F., 1997. *In vitro* conservation of plant genetic resources. Biotechnology in Agriculture. Marcel Dekker Inc. New York, 57-88.

Wolf , H., 1999. Methods and strategies for the conservation of forest genetic resources. In: Edwards S., Demissie A., Bekele T. and Haase G. (eds.), Proceedings of the national workshop on forest genetic resources conservation: Principles, strategies and actions. IBCR and GTZ, Addis Ababa, pp. 83-100.

Wu, Y., Zhao, Y., Engelmann, F., Zhou, M., 2001. Cryopreservation of apple dormant buds and shoot tips. CryoLetters, 22,277–284.

Wyse Jackson, P. S., Sutherland, L. A., 2000. international agenda for botanic gardens in conservation, UK: Botanic Gardens Conservation International (BGCI).

Villalobos, V. M., Engelmann, F., 1995. *Ex situ* conservation of plant germplasm using biotechnology. World journal of microbiology and Biotechnology, 11(4), 375-382.

Yamada, T., Sakai, A., Matsumura, T., Higuchi, S., 1991. Cryopreservation of apical meristems of white clover (Trifolium repens L.) by vitrification. Plant Sci, 78, 81-87.

Yamamoto, S. I., Fukui, K., Niino, T., 2011. A new cryopreservation method for vegetatively propagated plant genetic resources using aluminum cryo-plates. Research Highlights for, 10-11.

Zegeye, H., 2017. Major drivers and consequences of deforestation in Ethiopia: implications for forest conservation. Asian Journal of Science and Technology, 8(08), 5166-5175.

Zhai, Z. Y., Wu, Y. J., Engelmann, F., Chen, R. Z., Zhao, Y. H., 2003. Genetic stability assessments of plantlets regenerated from cryopreserved in vitro cultured grape and kiwi shoot tips using RAPD. CryoLetters, 24, 315–322.

Bölüm 14

PROTOPLAST KÜLTÜRÜ VE FÜZYONU

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14.1 TANIM VE TARİHÇE

Bitki hücresinin hücre duvarı, fiziksel veya kimyasal yöntemlerle uzaklaştırıldığında geriye kalan kısmı protoplast olarak adlandırılır (Naing ve ark., 2021). Yüksek bitkilerden protoplast izolasyonuna yönelik ilk çalışmalar 1892 yılında Klercker tarafından *Stratiotes aloides* L. üzerinde gerçekleştirilmiştir. Klercker'in uyguladığı yöntem esasen mekanik nitelikte olup hücreler uygun bir plazmolit içerisinde tutularak ince bir bıçak yardımıyla kesilmesini içermektedir. Ancak bu yaklaşımın en önemli sınırlaması protoplast izolasyonunun oldukça düşük verimde gerçekleşmesidir (Bhojwani ve ark., 1997). Cocking (1960), yaptığı çalışmada yüksek bitki hücrelerinden çok sayıda protoplastın enzimatik yöntemlerle izole edilebileceğini ortaya koymuştur. Cocking, hücre duvarlarının parçalanması amacıyla *Myrothecium verrucaria* mantar kültürlerinden elde edilen konsantre selülaz enzim çözeltisi kullanılmıştır. Ancak alandaki asıl gelişme selülaz, makerozim ve pektinaz

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nin yaygın kullanımını sınırlamasına rağmen, omik teknolojiler, mikroakışkan sistemler, yapay zekâ optimizasyonu ve CRISPR/Cas9 gibi ileri genom düzenleme yaklaşımlarıyla bu kısıtlar aşılmaktadır. Bu gelişmeler, protoplast temelli yöntemlerin laboratuvarın yanı sıra tarımsal üretim ve ıslah programlarında da etkin kullanılabileceğini göstermektedir. Günümüzde protoplast sistemleri, üç yönlü bir potansiyeli bir arada sunmaktadır: (i) tek hücre seviyesinde "laboratuvar" işlevi görerek moleküler biyoloji çalışmalarını hızlandırmak, (ii) melezleme engellerini aşarak geleneksel yöntemlerle elde edilemeyen yeni genetik kombinasyonların ortaya çıkmasını sağlamak ve (iii) sentetik biyolojide yüksek değerli metabolitlerin üretimi için hücre fabrikalarına dönüşmek. Bu çok yönlü potansiyel, protoplast teknolojisini yalnızca bir yöntem olmaktan çıkarıp, bitki biyoteknolojisinin temel paradigmalarından biri haline getirmektedir. Sonuç olarak, 1960'lı yıllardaki keşiflerinden günümüzdeki ileri biyoteknolojik uygulamalara kadar protoplastlar, basitlik ve karmaşıklığı bir arada barındıran yapıları ile bitki biyolojisinde dönüştürücü bir rol üstlenmiştir. Önümüzdeki dönemde, disiplinler arası yaklaşımlarla daha da güçlenecek olan bu sistemin, moleküler ıslahın ivmelenmesine, sürdürülebilir tarım uygulamalarının gelişmesine ve bitki biyolojisinin temel sorularına yanıt bulunmasına önemli katkılar sunacağı öngörülmektedir.

KAYNAKLAR

Adedeji, O. S., Naing, A. H., Kim, C. K., 2020. Protoplast Isolation and Shoot Regeneration from Protoplast-derived Calli of Chrysanthemum cv. White ND. Plant Cell, Tissue and Organ Culture, 141(3), 571–581. https://doi.org/10.1007/s11240-020-01816-3

Al-Nema, Q. S., & Al-Mallah, M. K., 2020. Electrofusion of Mesophyll Protoplasts from Two Varieties of Sugar Beet (*Beta vulgaris* L.). Journal of Life and Bio-Sciences Research, 1(1), 22-25. https://doi.org/10.38094/jlbsr117

Andersson, M., Turesson, H., Olsson, N., Fält, A. S., Ohlsson, P., Gonzalez, M. N., Samuelsson, M., Hofvander, P., 2018. Genome Editing in Potato via CRISPR-Cas9 Ribonucleoprotein Delivery. Physiologia Plantarum, 164(4), 378–384. https://doi.org/10.1111/ppl.12731

Avila-Peltroche, J., Won, B. Y., Cho, T. O., 2021. Protoplast Isolation From Dictyopteris Pacifica And Scytosiphon Lomentaria, Using A Simple Commercial Enzyme Preparation. Journal of Genetic Engineering and Biotechnology, 19(1). https://doi.org/10.1186/s43141-021-00226-y

Aviv, D., & Galun, E., 1980. Restoration of Fertility in Cytoplasmic Male Sterile (CMS) Nicotiana Sylvestris by Fusion with X-irradiated N. tabacum Protoplasts. Theoretical and Applied Genetics, 58(3–4), 121–127. https://doi.org/10.1007/bf00263102/metrıcs

Babaoğlu, M., & Özcan, S., 2002. Protoplast Kültürü ve Somatik Melezleme. In: Babaoğlu M, Gürel E, Özcan S (eds) Bitki Biyoteknolojisi I Doku Kültürü ve Uygulamaları. Konya, Türkiye, p 89-136.

Barrera, M., Olmedo, B., Narváez, M., Moenne-Locoz, F., Rubio, A., Pérez, C., Cordero-Lara, K., Prieto, H., 2025. Protoplast-based Regeneration Enables CRISPR/Cas9 Application in Two Temperate Japonica Rice Cultivars. Plants, 14(13), 2059. https://doi.org/10.3390/plants14132059

Barsby, T. L., Yarrow, S. A., Shepard, J. F., 1986. A Rapid And Efficient Alternative Procedure for The Regeneration of Plants From Hypocotyl Protoplasts of *Brassica Napus*. Plant Cell Reports, 5(2), 101–103. https://doi.org/10.1007/BF00269244

Bente, H., Foerster, A. M., Lettner, N., Scheid, O. M., 2021. Polyploidy-Associated Paramutation in Arabidopsis is Determined by Small Rnas, Temperature, and Allele Structure. PLoS Genetics, 17(3). https://doi.org/10.1371/journal.pgen.1009444

Bhojwani, S. S., Evans, P. K., Cocking, E. C., 1977. Protoplast Technology in Relation to Crop Plants: Progress and Problems. Euphytica, 26(2), 343–360. https://doi.org/10.1007/BF00026995

Brandt, K. M., Gunn, H., Moretti, N., Zemetra, R. S., 2020. A streamlined Protocol for Wheat (*Triticum aestivum*) Protoplast Isolation and Transformation with CRISPR-Cas Ribonucleoprotein Complexes. Frontiers in Plant Science, 11, 769. https://doi.org/10.3389/fpls.2020.00769

Bright, S. W. J., Northcote, D. H., 1974. Protoplast Regeneration from Normal and Bromodeoxyuridine-resistant Sycamore Callus. Journal of Cell Science, 16(2), 445–463. https://doi.org/10.1242/JCS.16.2.445

Burris, K. P., Dlugosz, E. M., Collins, A. G., Stewart, C. N., Lenaghan, S. C., 2016. Development of a Rapid, Low-Cost Protoplast Transfection System for Switchgrass (*Panicum virgatum* L.). Plant Cell Reports, 35(3), 693–704. https://doi.org/10.1007/s00299-015-1913-7

Burton, R. A., Gidley, M. J., Fincher, G. B., 2010. Heterogeneity in The Chemistry, Structure and Function of Plant Cell Walls. Nature Chemical Biology, 6(10), 724–732. https://doi.org/10.1038/nchembio.439

Carlson, P. S., Smith, H. H., Dearing, R. D., 1972. Parasexual Interspecific Plant Hybridization. Proceedings of the National Academy of Sciences, 69(8), 2292–2294. https://doi.org/10.1073/PNAS.69.8.2292

Chand, P. K., Davey, M. R., Power, J. B., Cocking, E. C., 1988. An Improved Procedure for Protoplast Fusion Using Polyethylene Glycol. Journal of Plant Physiology, 133(4), 480–485. https://doi.org/10.1016/S0176-1617(88)80041-5

Chen, D., Shao, Q., Yin, L., Younis, A., Zheng, B., 2019. Polyamine Function in Plants: Metabolism, Regulation on Development, and Roles in Abiotic Stress Responses. Frontiers in Plant Science, 9. https://doi.org/10.3389/fpls.2018.01945

Chen, K., Chen, J., Pi, X., Huang, L. J., Li, N., 2023. Isolation, Purification, and Application of Protoplasts and Transient Expression Systems in Plants. International Journal of Molecular Sciences, 24(23). https://doi.org/10.3390/ijms242316892

Cocking, E. C., 1960. A Method for the Isolation of Plant Protoplasts and Vacuoles. Nature, 187(4741), 962–963. https://doi.org/10.1038/187962a0

Cook, T. M., Isenegger, D., Dutta, S., Sahab, S., Kay, P., Aboobucker, S. I., Biswas, E., Heerschap, S., Nikolau, B. J., Dong, L., Lübberstedt, T., 2023. Overcoming Roadblocks for In Vitro Nurseries in Plants: Induction of Meiosis. Frontiers in Plant Science, 14. https://doi.org/10.3389/fpls.2023.1204813

Cosgrove, D. J., 2024. Structure and Growth of Plant Cell Walls. Nature Reviews Molecular Cell Biology, 25(5), 340–358. https://doi.org/10.1038/s41580-023-00691-y

Cove, D. J., 1979. The Uses of Isolated Protoplasts in Plant Genetics. Heredity, 43(3), 295–314. https://doi.org/10.1038/HDY.1979.84;KWRD=BIOMEDICINE

Cui, J., Kuligowska Mackenzie, K., Eeckhaut, T., Müller, R., Lütken, H., 2019. Protoplast Isolation and Culture from Kalanchoë Species: Optimization of Plant Growth Regulator Concentration for Efficient Callus Production. Plant Cell, Tissue and Organ Culture, 138(2), 287–297. https://doi.org/10.1007/s11240-019-01624-4

Çimen, B., (2020) Efficient Protoplast Isolation from Ovule-Derived Embryogenic Callus in Citrus Volkameriana. Turkish Journal of Agriculture and Forestry: Vol. 44: No. 6, Article 3. https://doi.org/10.3906/tar-1912-16

Dambier, D., Barantin, P., Boulard, G., Costantino, G., Mournet, P., Perdereau, A., Morillon, R., Ollitrault, P., 2022. Genomic Instability in Somatic Hybridization between *Poncirus* and *Citrus* Species aiming to Create New Rootstocks. Agriculture, 12(2), 134. https://doi.org/10.3390/agriculture12020134

Davey, M. R., Anthony, P., Power, J. B., Lowe, K. C., 2005. Plant Protoplasts: Status and Biotechnological Perspectives. Biotechnology Advances, 23(2), 131–171. https://doi.org/10.1016/j.biotechadv.2004.09.008

Davey, M.R., & Power, J.B., 1988. Aspects of Protoplast Culture and Plant Regeneration. In: Puite, K.J., Dons, J.J.M., Huizing, H.J., Kool, A.J., Koornneef, M., Krens, F.A. (eds) Progress in Plant Protoplast Research. Current Plant Science and Biotechnology in Agriculture, vol 7. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-2788-9_3

Delmer, D., Dixon, R. A., Keegstra, K., Mohnen, D., 2024. The Plant Cell Wall—Dynamic, Strong, and Adaptable is a Natural Shapeshifter. Plant Cell, 36(5), 1257–1311. https://doi.org/10.1093/plcell/koad325

Deryckere, D., Eeckhaut, T., van Huylenbroeck, J., van Bockstaele, E., 2012. Low Melting Point Agarose Beads as a Standard Method For Plantlet Regeneration from Protoplasts Within The Cichorium Genus. Plant Cell Reports, 31(12), 2261–2269. https://doi.org/10.1007/s00299-012-1335-8

Du, J., Zhang, H., Li, W., Li, X., Wang, Z., Zhang, Y., Xiong, A., Li, M., 2023. Optimization of Protoplast Preparation System from Leaves and Establishment of a Transient Transformation System in Apium graveolens. Agronomy, 13(8). https://doi.org/10.3390/agronomy13082154

Eeckhaut, T., Lakshmanan, P. S., Deryckere, D., Van Bockstaele, E., Van Huylenbroeck, J., 2013. Progress in Plant Protoplast Research. Planta, 238(6), 991–1003. https://doi.org/10.1007/s00425-013-1936-7

Evans, D. A., 1983. Agricultural Applications of Plant Protoplast Fusion. Bio/Technology, 1(3), 253–261. https://doi.org/10.1038/nbt0583-253

Farrokhi, N., Burton, R. A., Brownfield, L., Hrmova, M., Wilson, S. M., Bacic, A., Fincher, G. B., 2006. Plant Cell Wall Biosynthesis: Genetic, Biochemical and Functional Genomics Approaches to the Identification of Key Genes. Plant Biotechnology Journal, 4(2), 145–167. https://doi.org/10.1111/j.1467-7652.2005.00169.x

Gamborg, O. L., 2002. Plant Tissue Culture, Biotechnology, Milestones. In Vitro Cellular and Developmental Biology - Plant, 38(2), 84–92. https://doi.org/10.1079/ivp2001281/metrics

Gamborg, O. L., Miller, R. A., Ojima, K., 1968. Nutrient Requirements of Suspension Cultures of Soybean Root Cells. Experimental Cell Research, 50(1), 151–158. https://doi.org/10.1016/0014-4827(68)90403-5

Gamborg, O. L., Shyluk, J. P., Shahin, E. A., 1981. Isolation, Fusion and Culture of Plant Protoplasts. Plant Tissue Culture, 115–153. https://doi.org/10.1016/b978-0-12-690680-6.50009-7

Grimes, H. D., & Boss, W. F., 1985. Intracellular Calcium and Calmodulin Involvement in Protoplast Fusion. Plant Physiology, 79(1), 253–258. https://doi.org/10.1104/pp.79.1.253

Grosser, J., Jr, F. G., Castle, W., Fruits, J. C., 1998. Somatic Hybridization: A New Approach to Citrus Rootstock Improvement. Revues.Cirad.FrJW Grosser, FG Gmitter Jr, WS Castle, JL ChandlerFruits, Cirad.Fr, 53, 331–334. https://revues.cirad.fr/index.php/fruits/article/download/35631/35205/38561

Grzebelus, E., Szklarczyk, M., Baranski, R., Grzebelus, D., 2012. Phytosulfokine Stimulates Cell Divisions in Sugar Beet (Beta vulgaris L.) Protoplast Cultures. Plant Growth Regulation, 67(1), 23–30. https://doi.org/10.1007/s10725-011-9654-2

Gürel, S., Gürel, E., Kaya, Z., 2002a. Protoplast Fusion in Sugar Beet (*Beta vulgaris* L.). Turkish Journal of Biology, 26, 163–170. Available at: https://journals.tubitak.gov.tr/biology/vol26/iss3/6

Gürel, E., Gürel, S., Kaya, Z., 2002b. Protoplast Isolation and Culture in Sugar Beet (*Beta vulgaris* L.). Plant Cell Biotechnology and Molecular Biology, 3: 11–20. https://ikprress.org/index.php/PCBMB/article/view/2786.

Hahn-Hägerdal, B., Hosono, K., Zaehrisson, A., Bornman, C. H., 1986. Polyethylene glycol and electric field treatment of plant protoplasts: characterization of some membrane properties. Physiologia Plantarum, 67(3), 359–364. https://doi.org/10.1111/j.1399-3054.1986.tb05748.x

Hall, R. D., Riksen-Bruinsma, T., Weyens, G., Lefèbvre, M., Dunwel, J, M., Tunen, A. V., Krens, F. A., 1997. Sugar Beet Guard Cell Protoplasts Demonstrate a Remarkable Capacity for Cell Division Enabling Applications in Stomatal Physiology and Molecular Breeding, Journal of Experimental Botany, 48(2):255–263, https://doi.org/10.1093/jxb/48.2.255

He, X. Y., Xu, L. J., Xu, X. S., Yi, D. D., Hou, S. L., Yuan, D. Y., Xiao, S. X., 2024. Embryogenic Callus Induction, Proliferation, Protoplast Isolation, and PEG Induced Fusion in Camellia oleifera. Plant Cell, Tissue and Organ Culture, 157(3), 1–14. https://doi.org/10.1007/s11240-024-02800-x/figures/9

Hida, A., Shimizu, K., Nagata, R., Yabuya, T., Adachi, T., 1999. Plant Regeneration from Protoplasts of Iris Hollandica Hort. Euphytica, 105(2), 99–102. https://doi.org/10.1023/A:1003407501918

Hoshino, Y., Nakano, M., Mii, M., 1995. Plant Regeneration from Cell Suspension-Derived Protoplasts of Saintpaulia Ionantha Wendl. Plant Cell Reports, 14(6), 341–344. https://doi.org/10.1007/BF00238593

Jayachandran, D., Smith, P., Irfan, M., Sun, J., Yarborough, J. M., Bomble, Y. J., Lam, E., Chundawat, S. P. S., 2023. Engineering And Characterization of Carbohydrate-Binding Modules for Imaging Cellulose Fibrils Biosynthesis in Plant Protoplasts. Biotechnology and Bioengineering, 120(8), 2253–2268. https://doi.org/10.1002/bit.28484

Jbir-Koubaa, R., Charfeddine, S., Ellouz, W., Saidi, M. N., Drira, M., Gargouri-Bouzid, R., Nouri-Ellouz, Q., 2015. Investigation of the Response to Salinity and to Oxidative Stress of Interspecific Potato Somatic Hybrids Grown in a Greenhouse. Plant Cell, Tissue and Organ Culture, 120, 933–947. https://doi.org/10.1007/s11240-014-0648-4

Jenes, B., & Pauk, J., 1989. Plant Regeneration from Protoplast Derived Calli in Rice (*Oryza sativa* L.) Using Dicamba. Plant Science. 63(2):187-198. https://doi.org/10.1016/0168-9452(89)90244-6

Jeong, Y. Y., Lee, H. Y., Kim, S. W., Noh, Y. S., Seo, P. J., 2021. Optimization of Protoplast Regeneration in the Model Plant Arabidopsis Thaliana. Plant Methods, 17(1). https://doi.org/10.1186/s13007-021-00720-x

Jeong, Y. Y., Noh, Y. S., Kim, S. W., Seo, P. J., 2024. Efficient Regeneration of Protoplasts from Solanum lycopersicum Cultivar Micro-Tom. Biology Methods and Protocols, 9(1). https://doi.org/10.1093/biomethods/bpae008

Jin, S. B., Lee, D. H., Park, S. M., Park, J. S., Moon, Y. E., 2025. Intergeneric Plants Developed by Protoplast Fusion between two Citrus Hybrids for Producing High-quality Fruit. Scientia Horticulturae, 343, 114086. https://doi.org/10.1016/j.scienta.2025.114086

Kakkar, R. K., Nagar, P. K., Ahuja, P. S., Rai, V. K., 2000. Polyamines and Plant Morphogenesis. Biologia Plantarum, 43(1), 1–11. https://doi.org/10.1023/A:1026582308902

Kanchanapoom, K., Boss, W. F., 1986. Osmoregulation of Fusogenic Protoplast Fusion. Biochimica et Biophysica Acta (BBA) - Biomembranes, 861(C), 429–439. https://doi.org/10.1016/0005-2736(86)90451-7

Kang, H. H., Naing, A. H., Kim, C. K., 2020. Protoplast Isolation and Shoot Regeneration from Protoplast-Derived Callus of Petunia Hybrida Cv. Mirage Rose. Biology, 9(8), 1–13. https://doi.org/10.3390/biology9080228

Kao, K. N., & Michayluk, M. R., 1974. A method for high-frequency intergeneric fusion of plant protoplasts. Planta, 115(4), 355–367. https://doi.org/10.1007/bf00388618/metrics

Kazmı, S. K., Kayani, H. A., Razıq, M., Naz, S., Khan, S., Kabır, N., 2022. Protoplast Isolation and Regeneration From Leaves and Nucellar Embryos of Kinnow Mandarin (*Citrus Reticulata Blanco*). Pak. J. Bot., 54(4): 1359-1364.

Klercker, J. A. F., 1892 Ofvers Vet.-Akad. Förhdl., 9, 43

Kotnik, T., Rems, L., Tarek, M., Miklavcic, D., 2019. Membrane Electroporation and Electropermeabilization: Mechanisms and Models. Annual Review of Biophysics, 48, 63–91. https://doi.org/10.1146/annurev-biophys-052118-115451/cite/refworks

Krens, F.A., Jamar, D., Keizer, L.C.P., Hall, R.D., 1994. The Effect of N-Propyl Gallate on the Formation of Ethylene during Protoplast Isolation in Sugarbeet (*Beta vulgaris* L.), Journal of Experimental Botany, 45(12):1899–1901, https://doi.org/10.1093/jxb/45.12.1899

Kumar, A., & Cocking, E. C., 1987. Protoplast Fusion: A Novel Approach to Organelle Genetics In Higher Plants. American Journal of Botany, 74(8), 1289–1303. https://doi.org/10.1002/j.1537-2197.1987.tb08742.x

Kyozuka, J., Kaneda, T., Shimamoto, K., 1989. Production of Cytoplasmic Male Sterile Rice (*Oryza sativa* L.) by Cell Fusion. Nature Biotechnology, 7(11), 1171–1174. https://doi.org/10.1038/nbt1189-1171;kwrd=life+sciences

Lang, I., Sassmann, S., Schmidt, B., Komis, G., 2014. Plasmolysis: Loss of Turgor and Beyond. Plants, 3(4), 583–593. https://doi.org/10.3390/plants3040583

Lee, M. H., Lee, J., Choi, S. A., Kim, Y. S., Koo, O., Choi, S. H., Ahn, W. S., Jie, E. Y., Kim, S. W., 2020. Efficient Genome Editing Using CRISPR–Cas9 RNP Delivery into Cabbage Protoplasts via Electro-Transfection. Plant Biotechnology Reports, 14(6), 695–702. https://doi.org/10.1007/s11816-020-00645-2

Li, X., Sandgrind, S., Moss, O., Guan, R., Ivarson, E., Wang, E. S., Kanagarajan, S., Zhu, L., 2021. Efficient Protoplast Regeneration Protocol and CRISPR/Cas9-Mediated Editing of Glucosinolate Transporter (GTR) Genes in Rapeseed (*Brassica napus* L.) Front. Plant Sci. 12:680859. https://doi.org/10.3389/fpls.2021.680859

Lin, C. S., Hsu, C. T., Yuan, Y. H., Zheng, P. X., Wu, F. H., Cheng, Q. W., Wu, Y. L., Wu, T. L., Lin, S., Yue, J. J., Cheng, Y. H., Lin, S. I., Shih, M. C., Sheen, J., Lin, Y. C., 2022. DNA-Free CRISPR-Cas9 Gene Editing of Wild Tetraploid Tomato *Solanum Peruvianum* Using Protoplast Regeneration. Plant Physiology, 188(4), 1917–1930. https://doi.org/10.1093/plphys/kiac022

Liu, H., Li, Y., Peng, T., Xue, S., 2023. Transmembrane Potential, an Indicator in Situ Reporting Cellular Senescence and Stress Response in Plant Tissues. Plant Methods, 19(1). https://doi.org/10.1186/s13007-023-01006-0

Maddock S. E., (1987). Suspension and Protoplast Culture of Hexaploid Wheat (*Triticum aestivum* L.). Plant cell reports, 6(1), 23–26. https://doi.org/10.1007/BF00269731

Mahalakshmi, A., Maheshwari, S.C., Khurana, P., 1993. High Frequency Divisions in Leaf Base Protoplasts of Wheat (*Triticum aestivum* L.). J. Plant Biochem. Biotechnol. 2, 61–65. https://doi.org/10.1007/BF03262924

Mahmoud, L. M., Killiny, N., Holden, P., Gmitter, F. G., Jr., Grosser, J. W., Dutt, M., 2023. Physiological and Biochemical Evaluation of Salt Stress Tolerance in a Citrus Tetraploid Somatic Hybrid. Horticulturae, 9(11), 1215. https://doi.org/10.3390/horticulturae9111215

Majewska-Sawka, A., Nakashima, H., Mori, K., 1994. Isolation and Culture of Suspension-Derived Protoplasts of *Beta vulgaris* L. Biol Plant 36, 9–13. https://doi.org/10.1007/BF02921260

Masani, M. Y. A., Noll, G., Parveez, G. K. A., Sambanthamurthi, R., Prüfer, D., 2013. Regeneration of Viable Oil Palm Plants from Protoplasts by Optimizing Media Components, Growth Regulators And Cultivation Procedures. Plant Science, 210, 118–127. https://doi.org/10.1016/j.plantsci.2013.05.021

Matsumoto, K., Duarte Vilarinhos, A., Oka, S., 2002. Somatic Hybridization by Electrofusion of Banana Protoplasts. Euphytica, 125(3), 317–324. https://doi.org/10.1023/A:1016071624090

Melchers, G., Sacristán, M. D., Holder, A. A., 1978. Somatic Hybrid Plants of Potato and Tomato Regenerated from Fused Protoplasts. Carlsberg Research Communications, 43, 203-218. https://doi.org/10.1007/BF02906548

Melikov, K. C., Frolov, V. A., Shcherbakov, A., Samsonov, A. V., Chizmadzhev, Y. A., Chernomordik, L. V., 2001. Voltage-induced Nonconductive pre-pores and Metastable Single Pores in Unmodified Planar Lipid Bilayer. Biophysical Journal, 80(4), 1829–1836. https://doi.org/10.1016/s0006-3495(01)76153-x

Mukundan, N. S., Satyamoorthy, K., Babu, V. S., 2025. Advancing Plant Protoplasts: Innovative Techniques and Future Prospects. Plant Biotechnology Reports. https://doi.org/10.1007/s11816-

025-00957-1

Murashige, T., & Skoog, F., 196). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. Physiologia Plantarum, 15(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Naing, A. H., Adedeji, O. S., Kim, C. K., 2021. Protoplast Technology in Ornamental Plants: Current Progress and Potential Applications on Genetic Improvement. Scientia Horticulturae, 283. https://doi.org/10.1016/j.scienta.2021.110043

Nassour, M., Chassériaux, G., Dorion, N., 2003. Optimization of Protoplast to Plant System for Pelargonium X Hortorum "Alain" and Genetic Stability of the Regenerated Plants. Plant Science, 165(1), 121–128. https://doi.org/10.1016/S0168-9452(03)00150-X

Navrátilová, B., 2004. Protoplast Cultures and Protoplast Fusion Focused on *Brassicaceae*: A Review. Horticultural Science, 31(4), 140–157. https://doi.org/10.17221/3809-hortsci

Ochatt, S. J., & Power J. B., 1992. Plant Regeneration from Cultured Protoplast of Higher Plants. In: Fowler, M. W., Warren, G. S., Moo-Young, M., (eds), Plant Biotechnology: Comprehensive Biotechnology, Second Supplement, pp: 99-127, Pergamın Press.

Ozyigit, I. I., Dogan, I., Hocaoglu-Ozyigit, A., Yalcin, B., Erdogan, A., Yalcin, I. E., Cabi, E., Kaya, Y., 2023. Production of Secondary Metabolites Using Tissue Culture-Based Biotechnological Applications. Frontiers in Plant Science, 14. https://doi.org/10.3389/fpls.2023.1132555

Pasternak, T., Paponov, I. A., & Kondratenko, S., 2021. Optimizing Protocols for Arabidopsis Shoot and Root Protoplast Cultivation. Plants, 10(2), 375. https://doi.org/10.3390/plants10020375

Pearce, R. S., & Cocking, E. C., 1973. Behaviour in Culture of Isolated Protoplasts from "Paul's Scarlet" Rose Suspension Culture Cells. Protoplasma: An International Journal of Cell Biology, 77(2), 165–180. https://doi.org/10.1007/BF01276755

Power, J. B., & Cocking, E. C., 1970. Isolation of Leaf Protoplasts: Macromolecule Uptake and Growth Substance Response. Journal of Experimental Botany, 21(1), 64–70. https://doi.org/10.1093/jxb/21.1.64

Pulz, O., & Gross, W., 2004. Valuable Products from Biotechnology of Microalgae. Applied Microbiology and Biotechnology, 65(6), 635–648. https://doi.org/10.1007/s00253-004-1647-x/tables/7

Qiu, L., Wang, Y., Qu, H., 2020. Loading Calcium Fluorescent Probes into Protoplasts to Detect Calcium in The Flesh Tissue Cells of Malus Domestica. Horticulture Research, 7(1). https://doi.org/10.1038/s41438-020-0315-3

Rahmani, M. S., Pijut, P. M., Shabanian, N., 2016. Protoplast Isolation and Genetically True to Type Plant Regeneration From Leaf and Callus-Derived Protoplasts of Albizia Julibrissin. Plant Cell, Tissue and Organ Culture, 127(2), 475–488. https://doi.org/10.1007/s11240-016-1072-8

Rákosy-Tican, L., Hornok, M., Menczel, L., 2001. An Improved Procedure for Protoplast Microelectrofusion and Culture of Nicotiana tabacum Intraspecific Somatic Hybrids: Plant Regeneration and Initial Proofs on Organdie Segregation. Plant Cell, Tissue and Organ Culture, 67(2), 153–158. https://doi.org/10.1023/a:1011971222826/metrics

Reed, K. M., & Bargmann, B. O. R., 2021. Protoplast Regeneration and Its Use in New Plant Breeding Technologies. Frontiers in Genome Editing, 3. https://doi.org/10.3389/fgeed.2021.734951

Ren, R., Gao, J., Yin, D., Li, K., Lu, C., Ahmad, S., Wei, Y., Jin, J., Zhu, G., Yang, F., 2021. Highly Efficient Leaf Base Protoplast Isolation and Transient Expression Systems for Orchids and Other Important Monocot Crops. Frontiers in Plant Science, 12. https://doi.org/10.3389/fpls.2021.626015

Reyna-Llorens, I., Ferro-Costa, M., Burgess, S. J., 2023. Plant Protoplasts in the Age of Synthetic Biology. Journal of Experimental Botany, 74(13), 3821–3832. https://doi.org/10.1093/jxb/erad172

Roest, S., & Gilissen, L. J. W., 1989. Plant Regeneration from Protoplasts: A Literature Review. Acta Botanica Neerlandica, 38(1), 1–23. https://doi.org/10.1111/j.1438-8677.1989.tb01907.x

Ruesink, A. W., 1978. Leucine Uptake and Incorporation by Convolvulus Tissue Culture Cells and Protoplasts under Severe Osmotic Stress. Physiologia Plantarum, 44(1), 48–56. https://doi.org/10.1111/j.1399-3054.1978.tb01612.x

Salazar-González, J. A., Castro-Medina, M., Bernardino-Rivera, L. E., Martínez-Terrazas, E., Casson, S. A., Urrea-López, R., 2023. In-Planta Transient Transformation of Avocado (*Persea Americana*) by Vacuum Agroinfiltration of Aerial Plant Parts. Plant Cell, Tissue and Organ Culture, 152(3), 635–646. https://doi.org/10.1007/s11240-022-02436-9

Seidel, T., Artmann, P. J., Gkekas, I., Illies, F., Baack, A. L., Viefhues, M., 2024. Microfluidic Single-Cell Study on Arabidopsis thaliana Protoplast Fusion—New Insights on Timescales and Reversibilities. Plants, 13(2), 295. https://doi.org/10.3390/plants13020295/s1

Shapka, N., & Nagy, P. D., 2004. The AU-Rich RNA Recombination Hot Spot Sequence of Brome Mosaic Virus Is Functional in Tombusviruses: Implications for the Mechanism of RNA Recombination . Journal of Virology, 78(5), 2288–2300. https://doi.org/10.1128/jvi.78.5.2288-2300.2004/asset/1c48f02c-1436-4bdd-a3f3-ad203b00e833/assets/graphic/zjv0050415380008.jpeg

Sharma, M., Yediyur Gavisiddaiah, S., Rao, A. M., Ramesh, S., 2022. Utilization of Wild Species for Diversifying the Cytoplasmic Male Sterility Source of Sunflower (*Helianthus annuus* L.) hybrids. Helia, 45(76), 71–98. https://doi.org/10.1515/helia-2022-0004/downloadasset/suppl/j_helia-2022-0004_suppl_002.doc

Shrestha, S., Rahman, M. S., Qin, W., 2021. New Insights in Pectinase Production Development and Industrial Applications. Applied Microbiology and Biotechnology, 105(24), 9069–9087. https://doi.org/10.1007/s00253-021-11705-0

Sinha, A., Wetten, A. C., Caligari, P. D. S., (2003a). Effect of Biotic Factors on the Isolation of Lupinus Albus Protoplasts. Australian Journal of Botany, 51(1), 103–109. https://doi.org/10.1071/BT01104

Sinha, A., Wetten, A. C., Caligari, P. D. S.,(2003b). Optimisation of Protoplast Production in White Lupin. Biologia Plantarum, 47(1), 21–25. https://doi.org/10.1023/A:1027316511865

Srivastava, V., McKee, L. S., Bulone, V., 2017. Plant Cell Walls. Encyclopedia of Life Sciences, 1–17. https://doi.org/10.1002/9780470015902.a0001682.pub3

Stajič, E., (2023). Improvements in Protoplast Isolation Protocol and Regeneration of Different Cabbage (*Brassica oleracea* var. *capitata* L.) Cultivars. Plants (Basel, Switzerland), 12(17), 3074. https://doi.org/10.3390/plants12173074

Subburaj, S., Chung, S. J., Lee, C., Ryu, S. M., Kim, D. H., Kim, J. S., Bae, S., Lee, G. J., 2016. Site-Directed Mutagenesis in Petunia × Hybrida Protoplast System Using Direct Delivery of Purified Recombinant Cas9 Ribonucleoproteins. Plant Cell Reports, 35(7), 1535–1544. https://doi.org/10.1007/s00299-016-1937-7

Takebe, I., Otsuki, Y., Aoki, S., 1968 Isolation of Tobacco Mesophyll Cells in Intact and Active State. Plant and Cell Physiol. 9, 115–124.

Tang, K., Hu, Q., Zhao, E., & Wu, A. (2000). Factors influencing plant regeneration from protoplasts isolated from long-term cell suspension culture of recalcitrant indica rice cultivar IR36. In Vitro Cellular and Developmental Biology - Plant, 36(4), 255–259. https://doi.org/10.1007/s11627-000-0047-x

Tomiczak, K., Sliwinska, E., Rybczyński, J. J., 2017. Protoplast Fusion in the Genus Gentiana: Genomic Composition and Genetic Stability of Somatic Hybrids Between *Gentiana kurroo Royle* and *G. cruciata* L. Plant Cell, Tissue and Organ Culture, 131, 1–14. https://doi.org/10.1007/s11240-017-1256-x

Tu, W., Dong, J., Zou, Y., Zhao, Q., Wang, H., Ying, J., Wu, J., Du, J., Cai, X., Song, B., 2021. Interspecific Potato Somatic Hybrids between Solanum malmeanum and S. tuberosum Provide Valuable Resources for Freezing-Tolerance Breeding. Plant Cell, Tissue and Organ Culture, 147, 73–83. https://doi.org/10.1007/s11240-021-02106-2

Usui, H., Maeda, M., Ito, M. 1974. High Frequency of Spontaneous Fusion in Protoplasts from Various Plant Tissues. The Botanical Magazine Tokyo, 87(2), 179–182. https://doi.org/10.1007/

bf02489068/metrics

Van Wert, S. L., & Saunders, J. A., 1992. Electrofusion and Electroporation of Plants. Plant Physiology, 99(2), 365–367. https://doi.org/10.1104/PP.99.2.365

Vasil, I. K., 1988. Progress in the Regeneration and Genetic Manipulation of Cereal Crops. Bio/Technology, 6(4), 397–402. https://doi.org/10.1038/nbt0488-397

Vasilkoski, Z., 2006. The Effect of Electric Fields on Lipid Membranes. https://arxiv.org/pdf/physics/0701013

Vienken, J., Zimmermann, U., Ganser, R., Hampp, R. 1983. Vesicle Formation During Electro-fusion of Mesophyll Protoplasts of Kalanchoë daigremontiana. Planta, 157(4), 331–335. https://doi.org/10.1007/bf00397404/metrics

Waara, S., & Glimelius, K., 1995. The Potential of Somatic Hybridization in Crop Breeding. Euphytica, 85:1, 85(1), 217–233. https://doi.org/10.1007/bf00023951

Wang, L., Liu, H., Liu, P., Wu, G., Shen, W., Cui, H., & Dai, Z., 2023. Cotyledon Peeling Method for Passion Fruit Protoplasts: a Versatile Cell System for Transient Gene Expression in Passion Fruit (*Passiflora edulis*). Frontiers in Plant Science, 14, 1236838. https://doi.org/10.3389/fpls.2023.1236838

Wang, Y., Zhang, Y., Dong, Y., Li, D., Shi, S., Li, S., Li, L., He, Y., Li, J., Chen, H., Ge, H., Liu, Y., 2022. A Highly Efficient Mesophyll Protoplast Isolation and PEG-Mediated Transient Expression System in Eggplant. Scientia Horticulturae, 304. https://doi.org/10.1016/j.scienta.2022.111303

Weaver, J. C., Chizmadzhev, Y. A., 1996. Theory of Electroporation: A Review. Bioelectrochemistry and Bioenergetics, 41(2), 135–160. https://doi.org/10.1016/S0302-4598(96)05062-3

Willison, J. H. M., & Cocking, E. C., 1975. Microfibril Synthesis at The Surfaces of Isolated Tobacco Mesophyll Protoplasts, a Freeze-Etch Study. Protoplasma, 84(1–2), 147–159. https://doi.org/10.1007/BF02075950

Withers, L. A., & Cocking, E. C., 1972. Fine-structural Studies on Spontaneous and Induced Fusion of Higher Plant Protoplasts. Journal of Cell Science, 11(1), 59–75. https://doi.org/10.1242/jcs.11.1.59

Wu, S. C., & Kuniyuki, A. H., 1985. Isolation and Culture of Almond Pprotoplasts. Plant Science, 41(1), 55–60. https://doi.org/10.1016/0168-9452(85)90065-2

Wu, S., Zhu, H., Liu, J., Yang, Q., Shao, X., Bi, F., Hu, C., Huo, H., Chen, K., Yi, G., 2020. Establishment of a PEG-mediated Protoplast Transformation System Based on DNA and CRISPR/Cas9 Ribonucleoprotein Complexes for Banana. BMC Plant Biology, 20(1), 1–10. https://doi.org/10.1186/s12870-020-02609-8/figures/6

Xu, H., Chang, Q., Huang, L., Wei, P., Song, Y., Guo, Z., Peng, Y.-L., Fan, J. (2023). An Agrobacterium-Mediated Transient Expression Method for Functional Assay of Genes Promoting Disease in Monocots. International Journal of Molecular Sciences, 24(8), 7636. https://doi.org/10.3390/ijms24087636

Yang, D., Zhao, Y., Liu, Y., Han, F., Li, Z., 2022. A high-efficiency PEG-Ca2+-Mediated Transient Transformation System for Broccoli Protoplasts. Frontiers in Plant Science, 13, 1081321. https://doi.org/10.3389/fpls.2022.1081321/bibtex

Yang, S. H., Kim, S. W., Lee, S., Koo, Y., 2024. Optimized Protocols for Protoplast Isolation, Transfection, and Regeneration in the Solanum Genus for the CRISPR/Cas-Mediated Transgene-Free Genome Editing. Applied Biological Chemistry, 67(1). https://doi.org/10.1186/s13765-024-00870-1

Yu, J., Tu, L., Subburaj, S., Bae, S., Lee, G. J., 2021. Simultaneous Targeting of Duplicated Genes in Petunia Protoplasts for Flower Color Modification via CRISPR-Cas9 Ribonucleoproteins. Plant Cell Reports, 40(6), 1037–1045. https://doi.org/10.1007/s00299-020-02593-1

Zhang, B., Gao, Y., Zhang, L., Zhou, Y., 2021. The Plant Cell Wall: Biosynthesis, Construction, and Functions. Journal of Integrative Plant Biology, 63(1), 251–272. https://doi.org/10.1111/jipb.13055

Zhang, D., Wang, R., Han, S., Li, Z., Xiao, J., Li, Y., Wang, L., Li, S., 2022. Transcriptome Analysis of Sugarcane Young Leaves and Protoplasts after Enzymatic Digestion. Life, 12(8). https://doi.org/10.3390/life12081210

Zhou, A., Xia, G., Chen, H., Hu, H. 2001. Comparative Study of Symmetric and Asymmetric Somatic Hybridization between Common Wheat and Haynaldia villosa. Science in China, Series C: Life Sciences, 44(3), 294–304. https://doi.org/10.1007/bf02879336/metrics

Bölüm 15

BİTKİ DOKU KÜLTÜRLERİNDE BİYOREAKTÖR SİSTEMLERİ

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15.1 GİRİŞ

Bitki doku kültüründe biyoreaktör sistemleri, geleneksel *in vitro* kültür yöntemlerine göre daha yüksek verim ve otomasyon imkânı sunan modern üretim platformlarıdır. Bu sistemler özellikle nadir, ekonomik değeri yüksek veya tıbbi öneme sahip bitkilerin hızlı çoğaltılması, sekonder metabolit üretimi ve klonal materyal elde edilmesinde önemli avantajlar sağlamaktadır. Kontrollü ortam koşulları sayesinde besin maddeleri, oksijen ve büyüme düzenleyicilerinin etkin dağılımı mümkün olurken, iş gücü ve maliyet gereksinimi de önemli ölçüde azaltılmaktadır. Son dönemlerde sıklıkla kullanılmaya başlanan geçici daldırma biyoreaktör sistemleri hem hiperhidrisite sorununu azaltmaları hem de besin ortamının daha verimli kullanımını sağlamalarıyla bitki doku kültürü çalışmalarında dikkat çeken bir yaklaşım haline gelmiştir.

15.2 BİYOREAKTÖR SİSTEMLERİ

Biyoreaktörler, kültür ortamındaki kimyasal ve fiziksel faktörlerin düzenlenmesine, steril ortamın bozulmadan yeni besin ortamı eklenmesine olanak sağlayan, büyük ölçekli hüc-

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KAYNAKLAR

Aka Kaçar, Y. A., Dönmez, D., Biçen, B., Erol, M. H., Şimsek, Ö., Mendi, Y. Y., 2020. Micropropagation of Spathiphyllum with temporary immersion bioreactor system. Turkish Journal of Agriculture - Food Science and Technology, 8(5), 1195–1200. https://doi.org/10.24925/turjaf.v8i5.1195-1200.3364

Aka Kaçar, Y., Biçen, B., Şimşek, Ö., Dönmez, D., Erol, M. H., 2020. Evaluation and comparison of a new type of temporary immersion system (tis) bioreactors for myrtle (*Myrtus communis* L.). Applied Ecology & Environmental Research, 18(1).

Akita, M., Takayama, S., 1994. Stimulation of potato (*Solanum tuberosum* L.) tuberization by semi-continuous liquid medium surface level control. Plant Cell Reports, 13, 184–187.

Alves, G. L., Pinheiro, M. V. M., Marinho-Dutra, T. R., da Silva Vieira, K., de Assis Figueiredo, F. A. M. M., Ferraz, T. M., de Oliveira Reis, F., 2024. Photoautotrophic potential and photosynthetic competence in *Ananas comosus* [L]. Merr. cultivar Turiaçu in *in vitro* culture systems. *In Vitro* Cellular & Developmental Biology - Plant, 60(1), 131–146.

Arano-Avalos, S., Gómez-Merino, F. C., Mancilla-Álvarez, E., Sánchez-Páez, R., Bello-Bello, J. J., 2020. An efficient protocol for commercial micropropagation of malanga (*Colocasia esculenta* L. Schott) using temporary immersion. Scientia Horticulturae, 261, 108998.

Arigundam, U., Variyath, A. M., Siow, Y. L., Marshall, D., Debnath, S. C., 2020. Liquid culture for efficient *in vitro* propagation of adventitious shoots in wild *Vaccinium vitis*-idaea ssp. minus (lingonberry) using temporary immersion and stationary bioreactors. Scientia Horticulturae, 264, 109199.

Ávila-Hernández, J. G., Aguilar-Zárate, P., Carrillo-Inungaray, M. L., Michel, M. R., Wong-Paz, J. E., Muñiz-Márquez, D. B., Martínez-Ávila, G. C. G., 2022. The secondary metabolites from Beauveria bassiana PQ2 inhibit the growth and spore germination of *Gibberella moniliformis* LIA. Brazilian Journal of Microbiology, 53(1), 143–152.

Bello-Bello, J. J., Schettino-Salomón, S., Ortega-Espinoza, J., Spinoso-Castillo, J. L., 2021. A temporary immersion system for mass micropropagation of pitahaya (*Hylocereus undatus*). 3 Biotech, 11(10), 437.

Bulbarela-Marini, J. E., Gómez-Merino, F. C., Galindo-Tovar, M. E., Pastelín-Solano, M. C., Murguía-González, J., Núñez-Pastrana, R., Castañeda-Castro, O., 2023. Ratio of somaclonal variation and the phytohormonal content of Citrus × latifolia in three *in vitro* culture systems. Journal of Plant Growth Regulation, 42(6), 3356–3364.

Cao, Y., Qian, X., Yu, T., Jia, Q., Sarsaiya, S., Chen, J., 2024. Improving biomass and dendrobine-type total alkaloids (DTTAs) production of *Dendrobium nobile* through combining temporary immersion bioreactor system (TIBS) with endophyte MD33 elicitation. Plant Cell, Tissue and Organ Culture (PCTOC), 156(1), 9.

Cengiz, M., Aka Kaçar, Y., 2019. Micropropagation of some citrus rootstocks with classical and new generation tissue culture techniques. Turkish Journal of Agriculture—Food Science and Technology, 7(9), 1469–1478.

Cristina, M. A. M., Eucario, M. Á., Luis, S. C. J., Jabín, B. B. J., 2023. Evaluation of the effect of different culture systems on photomixotrophic capacity during *in vitro* multiplication of pitahaya (*Hylocereus undatus*). South African Journal of Botany, 159, 396–404.

Dewir, Y. H., Habib, M. M., Alaizari, A. A., Malik, J. A., Al-Ali, A. M., Al-Qarawi, A. A., Alwahibi, M. S., 2023. Promising application of automated liquid culture system and arbuscular mycorrhizal fungi for large-scale micropropagation of red dragon fruit. Plants, 12(5), 1037.

Erol, M. H., 2025. Micropropagation of banana using SETIS™ bioreactor: impact of nutrient medium replacement intervals on plant growth and medium stability. Plant Cell, Tissue and Organ Culture (PCTOC), 163(2), 1-14.

Erol, M. H., Dönmez, D., Biçen, B., Şimşek, Ö., Kaçar, Y. A., 2023. Modern approaches to *in vitro* clonal banana production: Next-generation tissue culture systems. Horticulturae, 9(10), 1154.

Espinosa-Leal, C., Puente-Garza, C. A., Gracia-Lara, S., 2018. *In vitro* plant tissue culture: means for production of biological active compounds. Planta, 248, 1–18.

Ferri, M., Dipalo, S. C., Bagni, N., Tassoni, A., 2011. Chitosan elicits mono-glucosylated stilbene production and release in fed-batch bioreactor cultures of grape cells. Food Chemistry, 124(4), 1473–1479.

Gago, D., Sánchez, C., Aldrey, A., Christie, C. B., Bernal, M. Á., Vidal, N., 2022. Micropropagation of plum (*Prunus domestica* L.) in bioreactors using photomixotrophic and photoautotrophic conditions. Horticulturae, 8(4), 286.

Garcia-Ramirez, Y., 2023. Temporary immersion system for *in vitro* propagation via organogenesis of forest plant species. Trees, 37(3), 611-626.

Georgiev, V., Schumann, A., Pavlov, A., Bley, T., 2014. Temporary immersion systems in plant biotechnology. Engineering in Life Sciences, 14, 607–621.

Gianguzzi, V., Sottile, F., 2024. Temporary immersion system as an innovative approach for *in vitro* propagation of *Sorbus domestica* L. Horticulturae, 10(2), 164.

Grzegorczyk-Karolak, I., Staniewska, P., Lebelt, L., Piotrowska, D. G., 2022. Optimization of cultivation conditions of *Salvia viridis* L. shoots in the Plantform bioreactor to increase polyphenol production. Plant Cell, Tissue and Organ Culture (PCTOC), 149(1), 269–280.

Jafernik, K., Kubica, P., Dziurka, M., Kulinowski, Ł., Korona-Głowniak, I., Elansary, H. O., Szopa, A., 2024. Comparative assessment of lignan profiling and biological activities of *Schisandra henryi* leaf and *in vitro* PlantForm bioreactor-grown culture extracts. Pharmaceuticals, 17(4), 442.

Kim, J. H., Han, J. E., Murthy, H. N., Kim, J. Y., Kim, M. J., Jeong, T. K., Park, S. Y., 2023. Production of secondary metabolites from cell cultures of *Sageretia thea* (Osbeck) MC Johnst. using balloon-type bubble bioreactors. Plants, 12(6), 1390.

Kumokita, R., Yoshida, T., Shirai, T., Kondo, A., Hasunuma, T., 2023. Aromatic secondary metabolite production from glycerol was enhanced by amino acid addition in Pichia pastoris. Applied Microbiology and Biotechnology, 107(24), 7391–7401.

Le, K. C., Johnson, S., Aidun, C. K., Egertsdotter, U., 2023. *In vitro* propagation of the blueberry 'blue suede™' (*Vaccinium* hybrid) in semi-solid medium and temporary immersion bioreactors. Plants, 12(15), 2752.

Lee, J. S., 1999. Micropropagation of Phalaenopsis by flower stalk-derived axillary bud culture. M.Sc. Thesis, Chungbuk National University, Korea.

Lopes, T., Correia, M., Pedrosa, A., Baltazar, E., Canhoto, J., Correia, S., 2022. Improved micropropagation of *Prunus* spp. rootstocks using temporary immersion systems. XXXI International Horticultural Congress (IHC2022): International Symposium on *In Vitro* Technology and Micropropagated Plants, 1359, 249–254.

Luna, C. V., Duarte, M. J., Brugnoli, E. A., Ayala, P. G., Espasandin, F. D., Bernardis, A. C., Sansberro, P. A., 2024. *In vitro* plantlet production of Ilex paraguariensis adult plants using BIT bioreactors. Plant Cell, Tissue and Organ Culture (PCTOC), 157(1), 1–12.

Maene, P., Debergh, P., 1985. Liquid medium additions to established tissue cultures to improve elongation and rooting in vivo. Plant Cell, Tissue and Organ Culture, 67, 25–35.

Makowski, W., Królicka, A., Tokarz, B., Szopa, A., Ekiert, H., Tokarz, K. M., 2023. Temporary immersion bioreactors as a useful tool for obtaining high productivity of phenolic compounds with strong antioxidant properties from *Pontechium maculatum*. Plant Cell, Tissue and Organ Culture (PCTOC), 153(3), 525–537.

Mamun, N. H., Egertsdotter, U., Aidun, C. K., 2015. Bioreactor technology for clonal propagation of plants and metabolite production. Frontiers in Biology, 10, 177–193.

Mancilla-Álvarez, E., Pérez-Sato, J. A., Núñez-Pastrana, R., Spinoso-Castillo, J. L., Bello-Bello, J. J., 2021. Comparison of different semi-automated bioreactors for *in vitro* propagation of taro (*Colocasia esculenta* L. Schott). Plants, 10(5), 1010.

Mancilla-Álvarez, E., Spinoso-Castillo, J. L., Schettino-Salomón, S. S., Bello-Bello, J. J., 2024. Temporary immersion systems induce photomixotrophism during *in vitro* propagation of agave Tobalá. 3 Biotech, 14(3), 74.

Méndez-Hernández, H. A., Galaz-Ávalos, R. M., Quintana-Escobar, A. O., Pech-Hoil, R., Collí-Rodríguez, A. M., Salas-Peraza, I. Q., Loyola-Vargas, V. M., 2023. *In vitro* conversion of *Coffea* spp. somatic embryos in SETIS™ bioreactor system. Plants, 12(17), 3055.

Mohammadpour Barough, A., Dianati Daylami, S., Fadavi, A., Vahdati, K., 2024. Enhancing Phalaenopsis orchid production: a comparative study of permanent and temporary immersion bioreactors. *In Vitro* Cellular & Developmental Biology - Plant, 1–15.

Murthy, H. N., Joseph, K. S., Paek, K. Y., Park, S. Y., 2023. Bioreactor systems for micropropagation of plants: present scenario and future prospects. Frontiers in Plant Science, 14, 1159588.

Orozco-Ortiz, C., Sánchez, L., Araya-Mattey, J., Vargas-Solórzano, I., Araya-Valverde, E., 2023. BIT® bioreactor increases *in vitro* multiplication of quality shoots in sugarcane (*Saccharum* spp. variety LAICA 04-809). Plant Cell, Tissue and Organ Culture (PCTOC), 152(1), 115–128.

Ozudogru, E. A., Karlik, E., Elazab, D., Lambardi, M., 2022. Establishment of direct organogenesis protocol for *Arachis hypogaea* cv. Virginia in liquid medium by temporary immersion system (TIS). Horticulturae, 8(12), 1129.

Paek, K. Y., Chakrabarty, D., Hahn, E. J., 2005. Application of bioreactor systems for large scale production of horticultural and medicinal plants. In: Liquid culture systems for *in vitro* plant propagation, Springer, Dordrecht, 95–116.

Paek, K. Y., Hahn, E. J., Son, S. H., 2001. Application of bioreactors for large-scale micropropagation systems of plants. *In Vitro* Cellular & Developmental Biology - Plant, 37(2), 149–157.

Peña-Rojas, G., Carhuaz-Condori, R., Andía-Ayme, V., Leon, V. A., Herrera-Calderon, O., 2022. Improved production of mashua (*Tropaeolum tuberosum*) microtubers MAC-3 morphotype in liquid medium using temporary immersion system (TIS-RITA®). Agriculture, 12(7), 943.

Ramírez-Mosqueda, M. A., Bello-Bello, J. J., 2021. SETIS™ bioreactor increases *in vitro* multiplication and shoot length in vanilla (*Vanilla planifolia* Jacks. ex Andrews). Acta Physiologiae Plantarum, 43(4), 52.

Ramos-Castellá, A., Iglesias-Andreu, L. G., Bello-Bello, J., Lee-Espinosa, H., 2014. Improved propagation of vanilla (*Vanilla planifolia* Jacks. ex Andrews) using a temporary immersion system. *In Vitro* Cellular & Developmental Biology - Plant, 50, 576–581.

Rico, S., Garrido, J., Sánchez, C., Ferreiro-Vera, C., Codesido, V., Vidal, N., 2022. A temporary immersion system to improve *Cannabis sativa* micropropagation. Frontiers in Plant Science, 13, 895971.

Roels, S., Escalona, M., Cejas, I., Noceda, C., Rodriguez, R., Canal, M. J., Debergh, P., 2005. Optimization of plantain (*Musa* AAB) micropropagation by temporary immersion system. Plant Cell, Tissue and Organ Culture, 82, 57–66.

Rout, G. R., Samantharay, S., Das, P., 2000. *In vitro* manipulation and propagation of medicinal plants. Biotechnology Advances, 18, 91–120.

Saptari, R. T., Esyanti, R. R., Putranto, R. A., 2022. Daminozide enhances the vigor and steviol glycoside yield of stevia (*Stevia rebaudiana* Bert.) propagated in temporary immersion bioreactors. Plant Cell, Tissue and Organ Culture (PCTOC), 149(1), 257–268.

Shahid, M., Shahzad, A., Malik, A., Sahai, A. (Eds.), 2013. Recent trends in biotechnology and therapeutic applications of medicinal plants. Springer Science+Business Media, Dordrecht.

Şimşek, Ö., Dönmez, D., Sarıdaş, M. A., Acar, E., Kaçar, Y. A., Kargı, S. P., İzgü, T., 2023. *In vitro* and *ex vitro* propagation of Turkish myrtles through conventional and plantform bioreactor systems. PeerJ, 11, e16061.

Son, S. H., Choi, S. M., Choi, K. B., Lee, Y. H., Lee, D. S., Choi, M. S., Park, Y. G., 1999. Selection and proliferation of rapid growing cell lines from embryo derived cell cultures of yew tree (*Taxus cuspidata* Sieb. et Zucc). Biotechnology and Bioprocess Engineering, 4, 112–118.

Sota, V., Benelli, C., Çuko, B., Papakosta, E., Depaoli, C., Lambardi, M., & Kongjika, E. (2021). Evaluation of ElecTIS bioreactor for the micropropagation of *Malus sylvestris* (L.) Mill., an important autochthonous species of Albania. Horticultural Science, 48(1).

Szewczyk, A., Marino, A., Taviano, M. F., Cambria, L., Davì, F., Trepa, M., Miceli, N., 2023. Studies on the accumulation of secondary metabolites and evaluation of biological activity of *in vitro* cultures of *Ruta montana* L. in temporary immersion bioreactors. International Journal of Molecular Sciences, 24(8), 7045.

Takahashi, S., Matsubara, H., Yamagata, H., Morimoto, T., 1992. Micropropagation of virus free bulblets of *Lilium longiflorum* by tank culture. 1. Development of liquid culture method and large-scale propagation. Acta Horticulturae, 319, 83–88.

Takayama, S., Akita, M., 1994. The types of bioreactors used for shoots and embryos. Plant Cell, Tissue and Organ Culture, 39(2), 147–156.

Takayama, S., Misawa, M., 1981. Mass propagation of Begonia × hiemalis plantlets by shake culture. Plant and Cell Physiology, 22(3), 461–467.

Takloo, S. M., Kargar, S., Jalili, H., Babaei, A., Amrane, A., 2024. Novel approaches to improve lovastatin production in membrane gradostat bioreactor. Biocatalysis and Agricultural Biotechnology, 103273.

Tarraf, W., İzgü, T., Şimşek, Ö., Cicco, N., Benelli, C., 2024. Saffron *in vitro* propagation: An innovative method by temporary immersion system (TIS), integrated with machine learning analysis. Horticulturae, 10(5), 454.

Teisson, C., Alvard, D., 1995. A new concept of plant *in vitro* cultivation liquid medium: Temporary immersion. In: Current Issues in Plant Molecular and Cellular Biology. Current Plant Science and Biotechnology in Agriculture. Eds. M. Terzi, R. Cella, A. Falavigna. Dordrecht: Springer, 105–110.

Tisserat, B., Vandercook, C. E., 1985. Development of an automated plant culture system. Plant Cell, Tissue and Organ Culture, 5, 107–117.

Topcu, Ş., Çölgeçen, H., 2015. Bitki sekonder metabolitlerinin biyoreaktörlerde üretilmesi. Türk Bilimsel Derlemeler Dergisi, (2), 9–29.

Uma, S., Karthic, R., Kalpana, S., Backiyarani, S., 2023. Evaluation of temporary immersion bioreactors for *in vitro* micropropagation of banana (*Musa* spp.) and genetic fidelity assessment using flow cytometry and simple-sequence repeat markers. South African Journal of Botany, 157, 553–565.

Uma, S., Karthic, R., Kalpana, S., Backiyarani, S., Saraswathi, M. S., 2021. A novel temporary immersion bioreactor system for large scale multiplication of banana (Rasthali AAB—Silk). Scientific Reports, 11(1), 20371.

Van Minh, T., 2022. Micropropagation of Mokara orchid by temporary immersion system technique. International Journal of Research and Innovation in Applied Science, 7(5), 54–58.

Vendrame, W. A., Xu, J., Beleski, D. G., 2023. Micropropagation of *Brassavola nodosa* (L.) Lindl. using SETIS™ bioreactor. Plant Cell, Tissue and Organ Culture (PCTOC), 153(1), 67–76.

Vilariño, S., Florido, M. D. C., García, J. L., Cantos, M., 2023. Effects of culture system and substrate composition on micropropagated plantlets of two varieties of Stevia rebaudiana Bert. Physiologia, 3(1), 74–85.

Watt, M. P., 2012. The status of temporary immersion system (TIS) technology for plant micropropagation. African Journal of Biotechnology, 11, 14025–14035.

Zhang, B., Niu, Z., Li, C., Hou, Z., Xue, Q., Liu, W., Ding, X., 2022. Improving large-scale biomass and total alkaloid production of Dendrobium nobile Lindl. using a temporary immersion bioreactor system and MeJA elicitation. Plant Methods, 18(1), 10.

Bölüm 16

BİTKİ DOKU KÜLTÜRÜNDE KARŞILAŞILAN SORUNLAR VE ÇÖZÜM ÖNERİLERİ

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Fildaus NYİRAHABİMANA [2]
İlknur SOLMAZ [3]

16.1 DOKU KÜLTÜRÜNDE KARŞILAŞILAN SORUNLAR VE ÇÖZÜM ÖNERİLERİ

Günümüzde küresel nüfus baskısının artmasına paralel olarak gıda talebi de hızla yükselmektedir. Bu bağlamda sürdürülebilir tarımı destekleyen, kitlesel ve homojen üretime olanak tanıyan bitki doku kültürü, modern biyoteknolojinin umut vaat eden ve sürekli gelişim gösteren yöntemlerinden biri olarak önem kazanmıştır (Thorpe, 2007; Permadi ve ark., 2023; Pasternak ve Steinmacher, 2024).

Bitki doku kültürü, aseptik koşullar altında, bir bitki parçasından hatta tek bir bitki hücresinden bütün bir bitki oluşumu ilkesine dayanmaktadır (Soumare ve ark., 2021).

Yüksek ölçekli ve sürdürülebilir bitkisel üretime yönelik artan gereksinimler doğrultusunda, bitki doku kültürü teknikleri; bitki büyümesinin optimize edilmesi, biyolojik aktivitelerinin artırılması, genetik transformasyonun kolaylaştırılması ve sekonder metabolit üretiminin iyileştirilmesi amacıyla yaygın biçimde kullanılmaya başlanmıştır (Twaij ve ark., 2020). Bitki doku kültürü teknikleri, hem temel araştırmalar hem de pratik uygulamalar için yaygın olarak kullanılan biyoteknolojik araçlardır. Bitki gelişimi ve gen fonksiyonunun anlaşılması, ticari mikroçoğaltım uygulamaları, istenen özelliklere sahip

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KAYNAKLAR

Abadie, J. C., Puttsepp, U., Gebauer, G., Faccio, A., Bonfante, P., and Selosse, M. A., 2006. *Cephalanthera longifolia* (Neottieae, *Orchidaceae*) Ismixotrophic: A Comparative Study Between Green and Nonphotosynthetic in-Dividuals. Can. J. Bot., 84: 1462-1477.

Abdalla, N., El-Ramady, H., Seliem, M. K., El-Mahrouk, M. E., Taha, N., Bayoumi, Y., Shalaby, T. A., & Dobránszki, J., 2022. An Academic and Technical Overview on Plant Micropropagation Challenges. Horticulturae, 8(8), 677.

Abdelwahd, R., Hakamu, N., Labhilili, M., & Udupa, S. M., 2008. Use of an Adsorbent and Antioxidants to Reduce the Effects of Leached Phenolics *in Vitro* Plantlet Regeneration of Faba Bean. African Journal of Biotechnology, 7(8), 997-1002.

Ahmad, I., Hussian, T., Ashraf, I., Nefees, M., Marayam, M. R., & Iqbal, M., 2013. Lethal Effects of Secondary Metabolites on Plant Tissue Culture. American-Eurasian Journal of Agricultural & Environmental Sciences, 13(4), 539-547.

Ahmad, I., Jaskani, M. J., Nafees, M., Ashraf, I., & Qures, R., 2016. Control of Media Browning in Micro Propagation of Guava (*Psidium guajava* L.). Pakistan Journal of Botany, 48(2), 713-716.

Ak, B. E., & Yücel, N. K., 2025. *In Vitro* Bitki Doku Kültüründe Temel Yaklaşımlar, Problemler ve Yeni Teknolojiler. Livre de Lyon.

Aliyu, O. M., 2005. Application of Tissue Culture to Cashew (*Anacardiumoccidentale*. P. L.) Breeding: An Appraisal. African Journal of Biotechnology, 4(13), 1485-1489.

Alkhateeb, A.A., 2008. A Review The Problems Facing The Use of Tissue Culture Technique in Date Palm (*Phoenix dactylifera* L.). Sci. J. King Faisal Univ. (Basic Applied Sci.)., 9: 85-104.

Armijos-González, R., Espinosa-Delgado, L., & Cueva-Agila, A., 2021. Indirect Shoot Regeneration Using 2, 4-D Induces Somaclonal Variations in *Cinchona officinalis*. Floresta e Ambiente, 28(3), e20210017.

Babaei, N., Abdullah, N. A. P., Saleh, G., & Abdullah, T. L. 2013. Control of Contamination and Explant Browning in *Curculigo Latifolia in vitro* Cultures. Journal of Medicinal Plants Research, 7(8), 448-454.

Beckman, C. H., 2000. Phenolic-Storing Cells: Keys To Programmed Cell Death And Periderm Formation in Wilt Disease Resistance and in General Defence Responses in Plants? Physiological and Molecular Plant Pathology, 57(3), 101-110.

Benson, E. E., 2004. Plant Conservation and Micropropagation: Tools for Resolving Environmental Resistance. Plant Cell, Tissue and Organ Culture, 77(3), 263-275.

Bhojwani, S. S., Razdan, M. K., 1986. Plant Tissue Culture: Theory and Practice. Elsevier.

Bidabadi, S. S., Sharifi, P., & Jain, S. M., 2021. Plant Breeding Integrated with Genomic-Enabled Prediction. OBM genetics, 5(3), 1-35.

Block, R., & Lankes, C. 1996. Measures to Prevent Tissue Browning of Explants of The Apple Rootstock M9 During *In Vitro* Establishment.

Cai, X., Wei, H., Liu, C., Ren, X., Thi, L. T., & Jeong, B. R. 2020. Synergistic Effect of NaCl Pretreatment and PVP on Browning Suppression and Callus Induction From Petal Explants of *Paeonia Lactiflora* Pall. 'Festival Maxima'. Plants (Basel, Switzerland), 9(3), 346.

Campbell, R., 1985. Plant Microbiology. Arnold, London pp. 191.

Cassells, A.C., 1991. Problems in Tissue Culture: Culture Contamination. In: Debergh, P.C., Zimmerman, R.H. (eds) Micropropagation. Springer, Dordrecht.

Cassells, A.C., 2000.Contamination and its Impact in Tissue Culture. In IV International Symposium on *In Vitro* Culture and Horticultural Breeding. 560, (pp. 353-359).

Cassells, A. C., 2012. Pathogen and Biological Contamination Management in Plant Tissueculture: Phytopathogens, Vitro Pathogens, and Vitro Pests. Plant Cell Culture Protocols. 57-80.14.

Chae, S. C., Kim, H. H., & Park, S. U., 2012. Ethylene Inhibitors Enhance Shoot Organogenesis of Gloxinia (*Sinningia speciosa*). The Scientific World Journal, 2012(1), 859381.

Chavan, S. S., Ranade, S. S., Deore, A. C., Deshpande, R. S., & Dhonukshe, B. L. 2000., Cloning of Alphonso mango through vegetative explants. Annals of Plant Physiology, 14(2), 178-181.

Dewir, Y. H., Murty,H. N., Ammar, M. H., Alghandi, S. S., Al-Suhaibani, N. A., Aldason, A. A., and Paek, K. Y., 2016. *In vitro* Rooting of Leguminous Plants: Difficulties, Alternatives, And Strategies For Improvement. Horticulture, Environment, and Biotechnology, 54 (4), 311-322.

Dineshbabu, K., Sathiamoorthy, N., Chezhiyan, N., Kapil, D., & Singh, N., 2002. *In Vitro* Establishment of Gynodioecious Papaya Variety CO-7 Influenced By Age of Mother Plants. Orissa Journal of Horticulture, 30(1), 5-7.

Dobránszki, J., & Teixeira da Silva, J. A., 2010. Micropropagation of Apple-A Review. Biotechnology Advances, 28(4), 462–488.

Duta-Cornescu, G., Constantin, N., Pojoga, D. M., Nicuta, D., & Simon-Gruita, A., 2023. Somaclonal Variation Advantage or Disadvantage in Micropropagation of The Medicinal Plants. International Journal of Molecular Sciences, 24(1), 838.

Ersali, Y., 2024. Control of Hyperhydricity of *Pistacia khinjuk* Stocks *in vitro* Shoots. BMC Biotechnology, 24(1), 97.

García-Gonzáles, R., Quiroz, K., Carrasco, B., Caligari, P., 2010. Plant Tissue Culture: Currentstatus, Opportunities and Challenges. International Journal of Agriculture and Natural Resources. 37(3), 5-30.15.

Gaspar, T., 1991. Vitrification in Micropropagation. In High-tech and micropropagation I (pp. 116-126). Berlin, Heidelberg: Springer Berlin Heidelberg. L.

George, E. F., & Sherington, P. D., 1984. Plant Propagation By Tissue Culture (pp. 690). England: Exegentics Limited.

George, E. F., 1993. Plant Propagation By Tissue Culture. Part 1: The Technology (No. Ed. 2, pp. viii+-574).

George, E. F., Hall, M. A., De Klerk, G. J., 2007. Plant Propagation By Tissue Culture: Volume 1. The Background.Springer Science & Business Media;2007;1.

George, E. F., & Davies, W., 2008. Effects of the Physical Environment. Plant Propagation by Tissue Culture 1, 423-464. Dordrecht, The Netherlands: Wiley-Blackwell.

Germana, M. A. 2011. Anther Culture for Haploid and Doubled Haploid Production. Plant Cell, Tissue and Organ Culture (PCTOC), 104(3), 283-300.

Gerszberg, A., Hnatuszko-Konka, K., & Kowalczyk, T., 2015. *In Vitro* Regeneration of Eight Cultivars of *Brassica oleracea* var. *capitata*. *In vitro* Cellular & Developmental Biology. Plant, 51(1), 80–87.

Gerszberg, George, D., Gonzalez, M., & Jones, S., 2017. Microbial Contamination in Plant Tissue Culture: Sources, Detection, And Prevention. Plant Cell Reports, 32(3), 331-345.

Haque, M., Siddique, A. B., & Islam, S. S., 2015. Effect of Silver Nitrate and Amino Acids on High Frequency Plants Regeneration in Barley (*Hordeum vulgare* L.). Plant Tissue Culture and Biotechnology, 25(1), 37-50.

Hassanein, A.M., Salem, J.M., Faheed, F.A. El-nagish, A., 2018. Effect of Anti-Ethylene Compounds on Isoenzyme Patterns and Genome Stability During Long Term Culture of *Moringa oleifera*. Plant Cell Tissue Organ Cult. 132, 201-212.

Herman, E. B. 2015., Plant Tissue Culture Contaminastion: Challenges And Opportunities. In IV Internnational Symposium on Production and Establishment of Micropropated Plants, 1155, 231-238.

Hoque, M. T., & Khatun, M., 2014. Contamination in Plant Tissue Culture: Sources, Types, Detection and Control. Journal of Microbiology and Biotechnology Research, 4(2), 16-23.

Ikenganyia, E.E., Anikwe, M.A.N., Omeje T.E. and Adinde, J.O., 2017. Plant Tissue Culture Regeneration And Aseptic Techniques. Asian J. Biotechnol. Bioresour. Technol., 1: 1-6.

Irshad, M., Rizwan, H. M., Debnath, B., Anwar, M., Li, M., Liu, S., He, B., & Qiu, D. 2018., Ascorbic Acid Controls Lethal Browning And Pluronic F-68 Promotes High-Frequency Multiple Shoot

Regeneration From Cotyldonary Node Explant of Okra (*Abelmoschus esculentus* L.). Hortscience, 53(1), 183-190.

Jain, A.K., Shanker, R., Kumar, S., Vishwakarma, R.K., 2017. Contamination in Plant Tissue Culture: Sources, Types and Solutions. Biotechnology Reports, 15: 34-41.

Jones, A. M., & Saxena, P. K., 2013. Inhibition of Phenylpropanoid Biosynthesis in *Artemisia annua* L.: A Novel Approach To Reduce Oxidative Browning in Plant Tissue Culture. PloS One, 8(10), e76802.

Kabir, K. M. R., Kwon, S.W., & Park, Y. J., 2013. Application of Cobalt Chloride And Silver Nitrate For Efficient Microspore Culture of *Brassica Rapa* ssp. Plant Tissue Culture and Biotechnology, 23(1), 1-10.

Karakaş, İ., 2023. Bitkilerin Totipotensi Yeteneklerinin Bitki Islahında Kullanılması: Doku Kültürü Teknikleri. Acta Natura & Scientia, 4(2).

Kastrop, P. M., de Graaf-Miltenburg, L. A., Gutknecht, D. R., Weima, S. M., 2007. Microbial Contamination of Embryo Cultures in Anart Laboratory: Sources and Management. Human Reproduction. 2007; 22(8):2243-2248.

Khalid, U., Kainat, W., Anjum, M. N., Mehmood, M., & Joyia, F. A., 2024. Effect of Varying Concentrations of Phytagel, Sorbitol and Mannitol on Hyperhydricity of *In Vitro* Regenerated Tobacco Shoots. Trends Biotech Plant Sci, 2(1), 11-19.

Kneifel, W. and Leonhardt, W., 1992. Testing of Different Antibiotics Against Gram-Positive and Gram-Negative Bacteria isolated From Plant Tissue Culture. Plant Cell Tissue Organ Cult., 29: 139-144.

Kotomory, S., & Murashige, T., 1965. Some Aspects of Aseptic Propagation of Orchids. American Orchid Society Bulletin, 34, 484-489.

Krishna, H., Sairam, R. K., Singh, S. K., Patel, V. B., Sharma, R. R., Grover, M., Nain, L., & Sachdev, A., 2008. Mango Explants Browning: Effect of Ontogenic Age, Mycorrhization and Pre-Treatments. Scientia Horticulturae, 118(2), 132-138.

Kumar, K., Gambhir, G., Dass, A., Tripathi, A. K., Singh, A., Jha, A. K., Rakshit, S., 2020. Genetically Modified Crops: Current Status and Future Prospects. Planta. 251:1-27.16.

Kumari, M., Clarke, H. J., Small, I., & Siddique, K. H., 2009. Albinism in Plants: A Major Bottleneck İn Wide Hybridization, Androgenesis and Doubled Haploid Culture. Critical Reviews in Plant Science, 28(6), 393-409.

Kyesmu, P. M., Omaliko, C. P. E. and Maduekwe, A., 2004. Basic Facts on Plant Tissue Culture: A Guide to Plant Tissue Culture Practice. Dolio-B Press, Abuja, Nigeria.

Lainé, E., & David, A., 1994. Regeneration of Plants From Leaf Explants of Micropropagated Clonal Eucalyptus Grandis. Plant Cell Reports, 13(8), 473-476.

Laukkanen, H., Rautiainen, L., Taulavuori, E., & Hohtola, A., 2000. Changes in Cellular Structures and Enzymatic Activities During Browning Of Scots Pine Callus Derived From Mature Buds. Tree Physiology, 20(7), 467-475.

Leelavathy, S. and Sankar, P. D., 2016. Curbing the Menace of Contamination in Plant Tissue Culture. J. Pure Applied Microbiol., 10: 2145-2152

Liu, J., Cao, L., & Dou, S., 2017. Bioaccumulation of Heavy Metals And Health Risk Assessment in Three Benthic Bivalves Along The Coast of Laizhou Bay, China. Marine Pollution Bulletin, 117(1-2), 98-110.

Lorenzo, J. C., de los Angeles Blanco, M., Pelaez, O., Gonzalez, A., Cid, M., Iglesias, A., Gonzalez, B., Escalona, M., Espinosa, P., & Borroto, C., 2001. Sugarcane Micropropagation and Phenolic Excretion. Plant Cell, Tissue and Organ Culture, 65, 1-8.

Loannidis, K., Tomprou, I., Mitsis, V., & Koropouli, P., 2022. Genetic Evaluation of *in vitro* Micropropagated and Regenerated Plants of *Cannabis Sativa* L. Using SSR Molecular Markers. Plants, 11(19), 2569.

Loyola-Vargas, V.M., Ochoa-Alejo, N., 2018. An Introduction to Plant Tissue Culture: Advances and Perspectives. In: Loyola-Vargas, V., Ochoa-Alejo, N. (eds) Plant Cell Culture Protocols. Methods in Molecular Biology, vol 1815. Humana Press, New York, NY.

Makowska, K., and Oleszczuk, S., 2014. Albinism in Barley Androgenesis. Plant Cell Rep. 33, 385-392.

Manole-Paunescu A., 2014. Biotechnology For Endangered Plant Conservation. Biotechnology and Biodiversity. 181-202.

Mookkan, M., & Andy, G., 2014. $AgNO_3$ Boosted High-Frequency Shoot Regeneration in *Vigna mungo* (L.) Hepper. Plant Signaling & Behavior, 9(10), e972284.

Murkute, A. A., Shanti Patil, S. P., & Mayakumari, M., 2003. Exudation and Browning in Tissue Culture of Pomegranate. Agricultural Science Digest, 23(1), 29-31.

Naaz, A., Shahzad, A., Anis, M., 2014. Effect of Adenine Sulphate Interaction on Growth and Development of Shoot Regeneration and inhibition of Shoot Tip Necrosis Under *in vitro* Condition in Adult *Syzygium cumini* L.-A Multipurpose Tree. Appl. Biochem. Biotechnol. 173, 90-102.

Nassar, M. A., El-Kader, E. M. A., El-Sheikh, M. A., & Saber, G. A., 2018. Microbial Contamination Assessment of Plant Tissue Culture Facilities in Sohag University, Egypt. Plant Cell Biotechnology and Molecular Biology, 19(25-26), 817-823.

Neves, M., Correia, S., & Canhoto, J., 2023. Ethylene Inhibition Reduces De Novo Shoot Organogenesis and Subsequent Plant Development from Leaf Explants of *Solanum betaceum* Cav. Plants, 12(9), 1854.

Ngomuo, M., Mneney, E., & Ndakidemi, P., 2014. Control of Lethal Browning By Using Ascorbic Acid On Shoot Tip Cultures of A Local *Musa* s*pp*. (Banana) cv. Mzuzu in Tanzania. African Journal of Biotechnology, 13(16), 1721-1725.

Ochoa-Alejo, N., & Ramirez-Malagon, R., 2001. *In vitro* Chili Pepper Biotechnology. *In Vitro* Cell Dev Biol Plant. *In Vitro* Cellular & Developmental Biology-Plant, 37(6), 701-729.

Onuoha, I. C., Eze, C. J., & Unamba, C. I., 2011. *In vitro* Prevention of Browning in Plantain Culture. On Line Journal of Biological Sciences, 11(1), 13-17.

Orlikowska, T. and Zawadzka, M., 2006. *In vitro* Bacteria in Plant Tissue Cultures. Biotechnologia, 4: 64-77

Ozyigit, I. I., 2008. Phenolic Changes During *in vitro* Organogenesis of Cotton (*Gossypium hirsutum* L.) Shoot tips. African Journal of Biotechnology, 7(8), 1145-1150.

Panaia, M., Senaratna, T., Bunn, E., Dixon, K. W., & Sivasithamparam, K., 2000. Micropropagation of the Critically Endangered Western Australian Species, *Symonanthus bancroftii* (F. Muell.) L. Haegi (*Solanaceae*). Plant Cell, Tissue and Organ Culture, 63(1), 23-29.

Parthasarathy, V. A., Keshavachandran, R., Nazeem, P., Girija, D., John, P. S Peter, K. V., 2007. High Tech Propagation of Horticultural Crops-Accent on Recalcitrance. Recent Trends in Horticultural Biotechnology, Vol. I and II (pp. 85-91). ICAE national Symposium on Biotechnological Interventions for Improvement of Horticultural Crops: Issues and Strategies. Vellanikkara, Kerala, India: New India Publishing Agency.

Pasternak, T. P., & Steinmacher, D., 2024. Plant Growth Regulation in Cell and Tissue Culture *in vitro*. Plants, 13(2), 327.

Peng, X., Zhang, T.T., Zhang, J., 2015. Effect of Subculture Times on Genetic Fidelity, Endogenous Hormone Level And Pharmaceutical Potential of *Tetrastigma hemsleyanum* Callus. Plant Cell Tissue Organ Cult. 122, 67-77.

Permadi, N., Nurzaman, M., Alhasnawi, A. N., Doni, F., & Julaeha, E., 2023. Managing Lethal Browning and Microbial Contamination in Musa spp. Tissue Culture: Synthesis And Perspectives. Horticulturae, 9(4), 453.

Pierik, R., 1999. *In vitro* Culture of Higher Plants. Kluwer Academic Publishers, Dordrecht.

Polivanova, O. B., & Bedarev, V. A., 2022. Hyperhydricity in Plant Tissue Culture. Plants, 11(23), 3313.

Ranghoo-Sanmukhiya, V. M., 2021. Somaclonal Variation and Methods Used for Its Detection. In

Propagation and Genetic Manipulation of Plants; Siddique, I., Ed.; Springer Nature: Singapore, 1-18.

Reed, B.M. & Bryce, J.H., 2014. Contamination in Plant Tissue Culture. in: Plant Biotechnology and Agriculture: Prospects for the 21st Century, ed. by A. Altman & P.M. Hasegawa, Chapter 1, pp. 3-21. Elsevier.

Sara, H. C., René, G. H., Rosa, U. C., Angela, K. G., Clelia, D. P., 2020. Agave Angustifolia Albino Plantlets Lose Stomatal Physiology Function by Changing The Development of The Stomatal Complex Due to a Molecular Disruption. Mol. Genet Genom. 295, 787-805.

Selvarajan, E., Veena, R., & Kumar, N. M., 2008. Polyphenol Oxidase, Beyond Enzyme Browning, A Review. In J. Singh, D. Sharma, G. Kumar & N. Sharma (Eds.),

Shen, H. L., 2005. Plant Tissue Culture, 3, 256-261. Beijing, China: China's Forestry Press.

Singh, P., & Patel, R. M., 2016. Factors Affecting *in vitro* Degree of Browning and Culture Establishment of Pomegranate. African Journal of Plant Science, 10(2), 43-49.

Sluis, C. J., 2008. Integrating Automation Technologies with Commercial Micropropagation. Plant Tissue Culture Engineering. 231-251.

Smith, J., Johnson, A., & Williams, R., 2018. Preventing Contamination in Plant Tissue Culture Laboratories: Implementing Aseptic Techniques and Standard Operating Procedures. Journal of Plant Science, 25(2), 45-56.

Solano, M.C.P., Ruız, J.S., Arnao, M.R., Castro, O.C., Tovar, M.E.G., Bello, J. J. B., 2019. Evaluation of *in vitro* Shoot Multiplication And ISSR Marker-Based Assessment Of Somaclonal Variants At Different Subcultures Of Vanilla (*Vanilla planifolia* Jacks). Physiol. Mol. Biol. Plants. 25, 561-567.

Soumare, A., Diédhiou, A. G., Arora, N. K., Tawfeeq Al-Ani, L. K., Ngom, M., Fall, S., & Sy, M. O., 2021. Potential Role and Utilization of Plant Growth Promoting Microbes in Plant Tissue Culture. Frontiers in Microbiology, 12, 649878.

Sreelekshmi, R., & Siril, E. A., 2020. Influence of Polyamines On Hyperhydricity Reversion and Its Associated Mechanism During Micropropagation of China pink (*Dianthus chinensis* L.). Physiology and Molecular Biology of Plants, 26(10), 2035-2045.

Sreelekshmi, R., & Siril, E. A., 2021. Effective Reversal of Hyperhydricity Leading to Efficient Micropropagation of *Dianthus chinensis* L. 3 Biotech, 11(2), 95.

Sahin, E. C., Aydin, Y., & Uncuoglu, A. A., 2024. Molecular Marker Applications in the Selection of Elite Genotypes for Plant Stress Tolerance and Genetic Fidelity. OBM Genetics, 8(3), 1-25.

Tabiyeh, D. T., Bernard, F., & Shacker, H., 2006. Investigation of Glutathione, Salicylic Acid and GA3 Effects on Browning in *Pistacia vera* Shoot Tips Culture. Acta Horticulturae, 726, 201-204.

Tazeb, A., 2017. Plant Tissue Culture Technique As A Novel Tool in Plant Breeding: A review article. Am.-Eurasian J. Agric. Environ. Sci., 17: 111-118.

Teixeira da Silva, J.A., Nezami-Alanagh, E., Barreal, M. E., Kher, M. M., Wicaksono, A., Gulyás, A., Hidvégi, N., Magyar-Tábori, K., Mendler-Drienyovszki, N., Márton, L., 2020. Shoot Tip Necrosis of *In vitro* Plant Cultures: A Reappraisal of Possible Causes And Solutions. Planta, 252, 47

Tersi, M., Xynias, I. N., Gouli-Vavdinoudi, E., and Roupakias, D. G., 2005. Effect of Genome, induction Medium and Temperature Pre-Treatment on Green Plant Production in Durum (*Triticum turgidum* var. *durum*) x bread wheat (*Triticum aestivum* L. em Thell) Crosses. Acta Physiol. Plant. 27: 641-649.

Thomas, C. W., 2002. Bureaucratic landscapes:Interagency Cooperation and The Preservation of Biodiversity. Mit Press; 2002.

Thomas, T. D., 2008. The Role of Activated Charcoal in Plant Tissue Culture. Biotechnology Advances, 26(6), 618-631.

Thorpe, T. A., 2007. History of Plant Tissue Culture. Molecular Biotechnology, 37(2), 169-180.

Titov, S., Bhowmik, S. K., Mandal, A., Alam, M. S., & Uddin S. N., 2006. Control of Phenolic Compound Secretion And Effect of Growth Regulators For Organ Formation From *Musa* spp. cv.

Kanthali Floral Bud Explants. American Journal of Biochemistry and Biotechnology, 2(3), 97-104.
Twaij, B. M., Jazar, Z. H., & Hasan, M. N., 2020. Trends in The Use of Tissue Culture, Applications and Future Aspects. International Journal of Plant Biology, 11(1).
Umarusman, M. A., Şimşek, Ö., Biçen, B., Serçe, S., & Aka Kaçar, Y., 2020. Investigation of Micropropagation Opportunities of Different Blackberry (*Rubus fruticosus* L.) Cultivars with Classical and New Generation Tissue Culture Techniques. Alatarım, 19 (2): 75-84.
Yao, J. L. and Cohen, D., 2000. Multiple Gene Control of Plastome-Genome Incompatibility and Plastid DNA Inheritance in Interspecific Hybrids of Zantedeschia. Theor. Appl. Genet. 101: 400-406.
Yonas, B., 2023. Elimination of Contamination in Plant Tissue Culture Laboratory. Acta Botanica Plantae. https://doi.org/10.51470/ABP.2023.02.03.22

Bölüm 17

BİTKİ DOKU KÜLTÜRÜNDE YAPAY ZEKÂ VE MAKİNE ÖĞRENMESİ UYGULAMALARI

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17. 1 GİRİŞ

Bitki doku kültürü, yirminci yüzyılın başlarından itibaren bitki biyoteknolojisinin gelişiminde temel bir dönüm noktası olmuş ve hem temel araştırmalarda hem de tarım ve bahçeciliğe yönelik ticari uygulamalarda geniş bir kullanım alanı bulmuştur (Chaudhari, 2023). Hücre totipotensisi ilkesinin deneysel olarak ortaya konulmasıyla birlikte, bitkilerin kontrollü koşullar altında yeniden oluşturulabilmesi mümkün hâle gelmiş ve bu durum bitki gelişiminin yönlendirilmesine yönelik yeni biyoteknolojik yaklaşımların önünü açmıştır (Thorpe, 2007). Zaman içerisinde doku kültürü teknikleri; üstün genotiplerin çoğaltılması, genetik kaynakların korunması, virüssüz bitki üretimi ve sekonder metabolit sentezinin artırılması gibi pek çok alanda etkin biçimde kullanılmaya başlanmıştır (Mahato ve ark., 2023). Günümüzde mikroçoğaltım, germplazm muhafazası ve moleküler ıslah gibi uygulamaları kapsayan geniş kullanım alanı sayesinde bitki doku kültürü hem araştırma hem de ticari üretim açısından vazgeçilmez bir teknoloji hâline gelmiştir (Hussain ve ark., 2012).

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ölçeklenebilir bir yapıya dönüştürmektedir. Yapay zekâ, otomasyon ve veri temelli üretim yaklaşımlarının önümüzdeki yıllarda hem akademik araştırmalarda hem de ticari uygulamalarda standart hâle gelmesi beklenmektedir.

17.9 SONUÇ

Bu bölümde, yapay zekâ ve makine öğrenmesi uygulamalarının bitki doku kültürü alanındaki güncel kullanım alanları, sağladığı bilimsel ve teknik kazanımlar ile geleceğe yönelik potansiyeli bütüncül bir bakış açısıyla ele alınmıştır. Yapay zekâ temelli yaklaşımların mikroçoğaltım veriminin artırılmasından besin ortamı ve hormon optimizasyonuna, stres fizyolojisi analizlerinden sekonder metabolit üretiminin tahminine kadar geniş bir uygulama alanına sahip olduğu görülmektedir. Ayrıca görüntü tabanlı analizler ve akıllı biyoreaktör sistemleri sayesinde *in vitro* üretim süreçlerinin daha kontrollü, tekrarlanabilir ve verimli hâle geldiği açıkça ortaya konulmuştur. Elde edilen veriler, yapay zekâ destekli sistemlerin klasik doku kültürü yöntemleri için bir alternatiften çok, güçlü bir tamamlayıcı teknoloji olduğunu göstermektedir. Bu sistemler sayesinde deneysel süreçler daha kısa sürede, daha düşük maliyetle ve daha yüksek doğrulukla yönetilebilmekte; karmaşık biyolojik ilişkiler daha güvenilir şekilde modellenebilmektedir. Bununla birlikte, bu teknolojilerin etkin biçimde uygulanabilmesi için yüksek kaliteli veriye, güçlü bir teknik altyapıya ve disiplinler arası uzmanlığa duyulan gereksinim de göz ardı edilmemelidir. Sonuç olarak, yapay zekâ ve makine öğrenmesi tabanlı yaklaşımlar bitki doku kültürü alanında yalnızca mevcut uygulamaları geliştirmekle kalmamakta, aynı zamanda doku kültürü tekniklerinin gelecekte daha akıllı, öngörülebilir ve sürdürülebilir bir yapıya kavuşmasının da önünü açmaktadır. Önümüzdeki yıllarda bu teknolojilerin hem akademik araştırmalarda hem de ticari üretim sistemlerinde standart birer araç hâline gelmesi beklenmektedir.

KAYNAKLAR

Acharjee, A., Larkman, J., Xu, Y., Cardoso, V., Gkoutos, G., 2020. A random forest based biomarker discovery and power analysis framework for diagnostics research. *BMC Medical Genomics*, 13(1). https://doi.org/10.1186/s12920-020-00826-6

Aliabad, F., Aliabad, K., Habbab, E., Bello, A., 2023. Evaluation of the ability to measure morphological structures of plants obtained from tissue culture applying image processing techniques. https://doi.org/10.21203/rs.3.rs-3153365/v1

Amato, G., Falchi, F., Gennaro, C., 2015. Fast image classification for monument recognition. *Journal on Computing and Cultural Heritage*, 8(4), 1–25. https://doi.org/10.1145/2724727

Arab, M., Yadollahi, A., Ahmadi, H., Eftekhari, M., Maleki, M., 2017. Mathematical modeling and optimizing of in vitro hormonal combination for g × n15 vegetative rootstock proliferation using artificial neural network-genetic algorithm (ANN-GA). *Frontiers in Plant Science*, 8. https://doi.org/10.3389/fpls.2017.01853

Arab, M., Yadollahi, A., Eftekhari, M., Ahmadi, H., Akbari, M., Sarikhani, S., 2018. Modeling and optimizing a new culture medium for in vitro rooting of g × n15 *Prunus* rootstock using artificial neural network-genetic algorithm. *Scientific Reports*, 8(1). https://doi.org/10.1038/s41598-018-27858-4

Arab, M., Yadollahi, A., Shojaeiyan, A., Ahmadi, H., 2016. Artificial neural network genetic algorithm as powerful tool to predict and optimize in vitro proliferation mineral medium for g × n15 rootstock. *Frontiers in Plant Science*, 7. https://doi.org/10.3389/fpls.2016.01526

Arteta, T., Hameg, R., Landín, M., Gallego, P., Barreal, M., 2022. Artificial neural networks elucidated the essential role of mineral nutrients versus vitamins and plant growth regulators in achieving healthy micropropagated plants. *Plants*, 11(10), 1284. https://doi.org/10.3390/plants11101284

Bektaş, Ü., Isak, M., Bozkurt, T., Dönmez, D., İzgü, T., Tütüncü, M., Şimşek, Ö., 2024. Genotype-specific responses to in vitro drought stress in myrtle (*Myrtus communis* L.): integrating machine learning techniques. *PeerJ*, 12, e18081. https://doi.org/10.7717/peerj.18081

Bozkurt, T., İnan, S., Dündar, İ., Isak, M., Şimşek, Ö., 2024. Optimizing the in vitro propagation of tea plants: a comparative analysis of machine learning models. *Horticulturae*, 10(7), 721. https://doi.org/10.3390/horticulturae10070721

Chapu, I., Chandel, A., Sie, E., Okello, D., Oteng-Frimpong, R., Okello, R., Balota, M., 2024. Comparing regression and classification models to estimate leaf spot disease in peanut (*Arachis hypogaea* L.) for implementation in breeding selection. *Agronomy*, 14(5), 947. https://doi.org/10.3390/agronomy14050947

Chaudhari, V., 2023. Tissue culture: aeon of micro propagation in vegetable crops. *International Journal of Environment and Climate Change*, 13(10), 1989–1999. https://doi.org/10.9734/ijecc/2023/v13i102857

Davidson, S., Saggese, T., Krajňáková, J., 2024. Deep learning for automated segmentation and counting of hypocotyl and cotyledon regions in mature *Pinus radiata* D. Don somatic embryo images. *Frontiers in Plant Science*, 15. https://doi.org/10.3389/fpls.2024.1322920

Fan, Z., Zhang, K., Wang, F., Zhao, X., Bai, R., Liu, B., 2020. Effects of rare earth elements on growth and determination of secondary metabolites under in vitro conditions in *Salvia miltiorrhiza*. *HortScience*, 55(3), 310–316. https://doi.org/10.21273/hortsci14661-19

Gago, J., Martínez-Núñez, L., Landín, M., Gallego, P., 2010. Artificial neural networks as an alternative to the traditional statistical methodology in plant research. *Journal of Plant Physiology*, 167(1), 23–27. https://doi.org/10.1016/j.jplph.2009.07.007

Gago, J., Pérez-Tornero, O., Landín, M., Burgos, L., Gallego, P., 2011. Improving knowledge of plant tissue culture and media formulation by neurofuzzy logic: a practical case of data mining using apricot databases. *Journal of Plant Physiology*, 168(15), 1858–1865. https://doi.org/10.1016/j.jplph.2011.04.008

Galatalı, S., Balcı, M., Akguller, O., Kaya, E., 2021. Production of disease-free olive seedlings with artificial intelligence and biotechnological methods. *European Journal of Biology and Biotechnology*, 2(3), 79–84. https://doi.org/10.24018/ejbio.2021.2.3.172

Grevenstuk, T., Romano, A., 2013. Micropropagation for the production of high quality phytochemicals. *Acta Horticulturae*, (988), 15–29. https://doi.org/10.17660/actahortic.2013.988.1

Hesami, M., Alizadeh, M., Naderi, R., Tohidfar, M., 2020. Forecasting and optimizing *Agrobacterium*-mediated genetic transformation via ensemble model–fruit fly optimization algorithm: a data mining approach using chrysanthemum databases. *PLOS ONE*, 15(9), e0239901. https://doi.org/10.1371/journal.pone.0239901

Hesami, M., Condori-Apfata, J., Valencia, M., Mohammadi, M., 2020. Application of artificial neural network for modeling and studying in vitro genotype-independent shoot regeneration in wheat. *Applied Sciences*, 10(15), 5370. https://doi.org/10.3390/app10155370

Hesami, M., Naderi, R., Tohidfar, M., Yoosefzadeh-Najafabadi, M., 2019. Application of adaptive neuro-fuzzy inference system–non-dominated sorting genetic algorithm-II (ANFIS-NSGAII) for modeling and optimizing somatic embryogenesis of chrysanthemum. *Frontiers in Plant*

Science, 10. https://doi.org/10.3389/fpls.2019.00869

Hussain, A., Qarshi, I. A., Nazir, H., Ullah, I., 2012. Plant tissue culture: current status and opportunities. In *Recent advances in plant in vitro culture*. IntechOpen. https://doi.org/10.5772/50568

Isak, M., Bozkurt, T., Tütüncü, M., Dönmez, D., İzgü, T., Şimşek, Ö., 2024. Leveraging machine learning to unravel the impact of cadmium stress on goji berry micropropagation. *PLOS ONE*, 19(6), e0305111. https://doi.org/10.1371/journal.pone.0305111

Jafari, M., Daneshvar, M., 2023. Prediction and optimization of indirect shoot regeneration of *Passiflora caerulea* using machine learning and optimization algorithms. *BMC Biotechnology*, 23(1). https://doi.org/10.1186/s12896-023-00796-4

Jafari, M., Daneshvar, M., Jafari, S., Hesami, M., 2022. Machine learning-assisted in vitro rooting optimization in *Passiflora caerulea*. *Forests*, 13(12), 2020. https://doi.org/10.3390/f13122020

Jamshidi, S., Yadollahi, A., Ahmadi, H., Arab, M., Eftekhari, M., 2016. Predicting in vitro culture medium macro-nutrients composition for pear rootstocks using regression analysis and neural network models. *Frontiers in Plant Science*, 7. https://doi.org/10.3389/fpls.2016.00274

Jamshidi, S., Yadollahi, A., Arab, M., Soltani, M., Eftekhari, M., Sabzalipoor, H., Shiri, J., 2019. Combining gene expression programming and genetic algorithm as a powerful hybrid modeling approach for pear rootstocks tissue culture media formulation. *Plant Methods*, 15(1). https://doi.org/10.1186/s13007-019-0520-y

Jamshidi, S., Yadollahi, A., Arab, M., Soltani, M., Eftekhari, M., Shiri, J., 2020. High throughput mathematical modeling and multi-objective evolutionary algorithms for plant tissue culture media formulation: case study of pear rootstocks. *PLOS ONE*, 15(12), e0243940. https://doi.org/10.1371/journal.pone.0243940

Ji, B., Xuan, L., Zhang, Y., Mu, W., Paek, K., Park, S., Gao, W., 2023. Application of data modeling, instrument engineering and nanomaterials in selected medicinal plant tissue culture. *Plants*, 12(7), 1505. https://doi.org/10.3390/plants12071505

Kakani, A., Li, G., Peng, Z., 2008. Role of AUX1 in the control of organ identity during in vitro organogenesis and in mediating tissue specific auxin and cytokinin interaction in *Arabidopsis*. *Planta*, 229(3), 645–657. https://doi.org/10.1007/s00425-008-0846-6

Kaushik, P., Rani, M., Khurana, N., Pandey, P., Payal, P., Kapoor, S., 2025. Revolutionizing plant tissue culture: harnessing artificial intelligence for precision propagation and optimization. *The Natural Products Journal*, 15(3). https://doi.org/10.2174/0122103155302871240527094915

Khan, I., Akbar, F., Ali, M., Ahmad, N., 2025. Advances in tissue culture and regeneration of canola (*Brassica napus* L.): explant responses, hormone-free shock treatments, and emerging biotechnological applications. https://doi.org/10.20944/preprints202508.2195.v1

Ma, J., Zhou, X., Xie, B., Wang, C., Chen, J., Zhu, Y., Huang, F., 2023. Application for identifying the origin and predicting the physiologically active ingredient contents of *Gastrodia elata* Blume using visible–near-infrared spectroscopy combined with machine learning. *Foods*, 12(22), 4061. https://doi.org/10.3390/foods12224061

Mahato, A., Chaudhari, G., Banjade, G., Uprety, S., 2023. Tissue culture and its application in modern agriculture. *Inwascon Technology Magazine*, 5, 09–11. https://doi.org/10.26480/itechmag.05.2023.09.11

Malabadi, R., TL, N., Kolkar, K., Chalannavar, R., Mudigoudra, B., Lavanya, L., Baijnath, H., 2023. *Cannabis sativa*: applications of artificial intelligence (AI) and plant tissue culture for micropropagation. *International Journal of Research and Innovation in Applied Science*, VIII(VI), 117–142. https://doi.org/10.51584/ijrias.2023.8614

Mamun, N., Egertsdotter, U., Aidun, C., 2015. Bioreactor technology for clonal propagation of plants and metabolite production. *Frontiers in Biology*, 10(2), 177–193. https://doi.org/10.1007/s11515-015-1355-1

Mehrotra, S., Prakash, O., Mishra, B., Dwevedi, B., 2008. Efficiency of neural networks for prediction of in vitro culture conditions and inoculum properties for optimum productivity. *Plant Cell,*

Tissue and Organ Culture, 95(1), 29–35. https://doi.org/10.1007/s11240-008-9410-0
Melnyk, C., 2023. Quantitative regeneration: Skoog and Miller revisited. *Quantitative Plant Biology*, 4. https://doi.org/10.1017/qpb.2023.9
Moore, S., Zhang, X., Liu, J., Lindsey, K., 2015. Modelling plant hormone gradients, 1–10. https://doi.org/10.1002/9780470015902.a0023733
Munroe, S., Sandoval, K., Martens, D., Sipkema, D., Pomponi, S., 2019. Genetic algorithm as an optimization tool for the development of sponge cell culture media. *In Vitro Cellular & Developmental Biology – Animal*, 55(3), 149–158. https://doi.org/10.1007/s11626-018-00317-0
Nas, M., Bölek, Y., Sevgin, N., 2013. Shortcut to long-distance developing of a tissue culture medium: micropropagation of mature almond cultivars as a case study. *Turkish Journal of Botany*, 37, 1134–1144. https://doi.org/10.3906/bot-1302-49
Nezami-Alanagh, E., Garoosi, G., Landín, M., Gallego, P., 2018. Combining DOE with neurofuzzy logic for healthy mineral nutrition of pistachio rootstocks in vitro culture. *Frontiers in Plant Science*, 9. https://doi.org/10.3389/fpls.2018.01474
Niedz, R., Evens, T., 2007. Regulating plant tissue growth by mineral nutrition. *In Vitro Cellular & Developmental Biology – Plant*, 43(4), 370–381. https://doi.org/10.1007/s11627-007-9062-5
Padilha, J., Ribas, L., Amano, É., Quoirin, M., 2015. Somatic embryogenesis in *Acrocomia aculeata* Jacq. (Lodd.) ex Mart using the thin cell layer technique. *Acta Botanica Brasilica*, 29(4), 516–523. https://doi.org/10.1590/0102-33062015abb0109
Paek, K., Chakrabarty, D., Hahn, E., 2005. Application of bioreactor systems for large scale production of horticultural and medicinal plants. *Plant Cell, Tissue and Organ Culture*, 81(3), 287–300. https://doi.org/10.1007/s11240-004-6648-z
Pepe, M., Hesami, M., Small, F., Jones, A., 2021. Comparative analysis of machine learning and evolutionary optimization algorithms for precision micropropagation of *Cannabis sativa*: prediction and validation of in vitro shoot growth and development based on the optimization of light and carbohydrate sources. *Frontiers in Plant Science*, 12. https://doi.org/10.3389/fpls.2021.757869
Ram, P., Singh, S., Ali, S., Aasım, M., Rajendran, A., Govindan, S., Ramesh, M., 2025. Machine learning-driven screening of promising mutants in *Nilgirianthus ciliatus* callus cultures for enhanced phytochemicals production. *Physiologia Plantarum*, 177(5). https://doi.org/10.1111/ppl.70536
Rezaei, H., Mirzaie-Asl, A., Abdollahi, M., Tohidfar, M., 2023. Enhancing petunia tissue culture efficiency with machine learning: a pathway to improved callogenesis. *PLOS ONE*, 18(11), e0293754. https://doi.org/10.1371/journal.pone.0293754
Sadat-Hosseini, M., Arab, M., Soltani, M., Eftekhari, M., Soleimani, A., Vahdati, K., 2022. Predictive modeling of Persian walnut (*Juglans regia* L.) in vitro proliferation media using machine learning approaches: a comparative study of ANN, KNN and GEP models. *Plant Methods*, 18(1). https://doi.org/10.1186/s13007-022-00871-5
Shukla, M., Singh, A., Piunno, K., Saxena, P., Jones, A., 2017. Application of 3D printing to prototype and develop novel plant tissue culture systems. *Plant Methods*, 13(1). https://doi.org/10.1186/s13007-017-0156-8
Su, R., Sujarani, M., Shalini, P., Prabhu, N., 2019. A review on bioreactor technology assisted plant suspension culture. *Asian Journal of Biotechnology and Bioresource Technology*, 1–13. https://doi.org/10.9734/ajb2t/2019/v5i330062
Şimşek, Ö., Şekerci, A., Isak, M., Bulut, F., İzgü, T., Tütüncü, M., Dönmez, D., 2024. Optimizing micropropagation and rooting protocols for diverse lavender genotypes: a synergistic approach integrating machine learning techniques. *Horticulturae*, 10(1), 52. https://doi.org/10.3390/horticulturae10010052
Thorpe, T., 2007. History of plant tissue culture. *Molecular Biotechnology*, 37(2), 169–180. https://doi.org/10.1007/s12033-007-0031-3

Ukey, N., Yang, Z., Li, B., Zhang, G., Hu, Y., Zhang, W., 2023. Survey on exact KNN queries over high-dimensional data space. *Sensors*, 23(2), 629. https://doi.org/10.3390/s23020629

Wijerathna-Yapa, A., Hiti-Bandaralage, J., 2023. Tissue culture—a sustainable approach to explore plant stresses. *Life*, 13(3), 780. https://doi.org/10.3390/life13030780

Yasemin, S., Beruto, M., 2024. A review on flower bulb micropropagation: challenges and opportunities. *Horticulturae*, 10(3), 284. https://doi.org/10.3390/horticulturae10030284

Yue, M., Jong, M., Dai, Y., 2022. Pedagogical design of K–12 artificial intelligence education: a systematic review. *Sustainability*, 14(23), 15620. https://doi.org/10.3390/su142315620

Zhang, X., Liu, Y., Wang, J., 2025. Optimization of crop planting strategies based on improved genetic algorithm, 17. https://doi.org/10.1117/12.3073367

Zielińska, S., Kępczyńska, E., 2014. Neural modeling of plant tissue cultures: a review. *Biotechnologia*, 94(3), 253–268. https://doi.org/10.5114/bta.2013.46419

Bölüm 18

BİTKİ DOKU KÜLTÜRÜ ÜRÜNLERİNİN TİCARİLEŞMESİ

Buhara YÜCESAN [1]

18.1 TİCARİLEŞMEDEKİ ARKA PLANI

Kitabımızın birinci bölümünde bitki doku kültürünün (BDK) tarihsel gelişimi ayrıntılı olarak ele alınmıştır. Bu bölümde ise konunun ticarileşme boyutuna odaklanılmıştır. Ticarileşmeyi mümkün kılan başlıca unsurlar; farklı bitki türlerinin hızlı, düşük maliyetli ve sağlıklı şekilde üretilebilmesi, Ar-Ge çalışmalarının sürekliliği ve sistemin işlerliğini sağlayan ekipman ile sarf malzemelerinin geliştirilmesidir. Dolayısıyla ticarileşme sürecinin temelini hem üretim verimliliği hem de teknolojik ve kurumsal altyapıyı besleyen bu çok yönlü faktörleri çeşitli yönleriyle incelenmesi faydalı olacaktır.

Bu bölümde ticarileşmenin arka plan süreci iki ayrı dönemde ele alınmıştır: ilki Murashige ve Skoog (MS) ortamının (Murashige ve Skoog, 1962) önceki dönemi (1900'ler-1962 arası) ve MS ortamının yaygınlaşmasıyla birlikte 1962 sonrası dönem şeklindedir (Tablo 18.1). Özellikle 1960'lı yıllara kadar BDK çalışmaları sınırlı ölçüde devam eden çalışmalar olup, bugünkü gibi üretim anlamında verimli değildi. Örneğin 1902'de Gottlieb Haberlandt, bitki hücrelerinin teorik olarak totipotent olduğunu, yani tek bir hücrenin tüm bitkiyi oluşturabileceğini öne sürmüş; ancak ispatını ele alan çalışmalar bunu izleyen yaklaşık 40 yıl içinde yürütülen araştırmalarla kayda değer gelişme göstermiştir (Krikorian ve Berquam, 2003). Çoğunlukla hijyenik ortamda yapılan kültür denemelerinde basit mineral çözeltiler (Knop, White medium gibi) kullanıldığından, karbon kaynağı eklenmediği için bitkiler fotosenteze bağımlı kalıyordu. Benzer biçimde, kallus oluşumu bazı türlerde sağlanabiliyor, ancak sürgün ve kök gelişimi oldukça sınırlı kalıyordu. Bu

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sektörde kalıcı başarı ancak teknik mükemmeliyetin, ekonomik sürdürülebilirliğin ve düzenleyici uyumun bütüncül biçimde yönetilmesiyle mümkün olacaktır.

KAYNAKLAR

Abdalla, N., El-Ramady, H., Seliem, M. K., El-Mahrouk, M. E., Taha, N., Bayoumi, Y., Shalaby, T. A., Dobránszki, J., 2022. An academic and technical overview on plant micropropagation challenges. Horticulturae, 8(8), 677. https://doi.org/10.3390/horticulturae8080677

Altman, D. W., 2011. Biotechnology entrepreneurship in the 21st century. In: Trigiano, R. N., Gray, D. J. (eds) Plant Tissue Culture, Development, and Biotechnology. CRC Press, Taylor and Francis Group, NY, USA.

Baranwal, V. K., Kapoor, R., Shukla, S. K., 2019. Banana tissue culture in India – a success story. Asia-Pacific Association of Agricultural Research Institutions. https://apari.org.in/banana-tissue-culture-in-india

BÜGEM (T.C. Tarım ve Orman Bakanlığı) 2025. Doku kültürü. https://www.tarimorman.gov.tr/BUGEM/Belgeler/doku%20kültürü.pdf (erişim tarihi 23 Ağustos, 2025).

Chandra, S., Lata, H., Elsohly, M., 2017. Cannabis sativa L.- Botany and Biotechnology, Springer Nature, DOI 10.1007/978-3-319-54564-6

Coherent Market Insights., 2025. Plant tissue culture market expands with growing demand. OpenPR. https://www.openpr.com/news/4101157/plant-tissue-culture-market-expands-with-growing-demand

Custom Market Insights., 2025. Latest global plant tissue culture market size & share worth USD 1,205.94 million by 2034 at an 8.67% CAGR – Custom Market Insights analysis, outlook, leaders, report, trends, forecast, segments & growth drivers. GlobeNewswire. https://www.globenewswire.com/news-release/2025/07/22/3119763/0/en/Latest-Global-Plant-Tissue-Culture-Market-Size-Share-Worth-USD-1-205-94-Million-by-2034-at-a-8-67-CAGR-Custom-Market-Insights-Analysis-Outlook-Leaders-Report-Trends-Forecast-Segmen.html

Çetiner, S., 2015. Biyoteknoloji stratejisi Mi dediniz? Tarlasera Düşünceler, 22–23. https://www.tarlasera.com/makale-9708-biy oteknoloji-stratejisi-mi-dediniz

Çetiner, S., 2024. The impact of regulatory oversight on the development and adoption of plant biotechnology. 2nd Conference of CopyTree, 173–177.

Dogra, S., 2023. Plant tissue culture industry in India: Trends and scope. Int. J. Adv. Biochem. Res., 7(1S), 28-33. https://doi.org/10.33545/26174693.2023.v7.i1Sa.176

Eisenschenk, C. D. W., 2011. Intellectual property protection for plants. In: Trigiano, R. N., Gray, D. J. (eds) Plant Tissue Culture, Development, and Biotechnology. CRC Press, Taylor and Francis Group, NY, USA.

FAO - Food and Agriculture Organization., 2001. Seed policy and programmes for the Central and Eastern European countries, Commonwealth of Independent States and other countries in transition: Proceedings of the Regional Technical Meeting, Budapest, Hungary, 6–10 March 2001. https://www.fao.org/4/y2722e/y2722e00.htm (erişim tarihi 23 Ağustos, 2025).

FAO/IPPC., 2012. ISPM 36: Integrated measures for plants for planting, *in vitro* produced plants. Rome: FAO.

Global Growth Insights., 2025. Plant tissue culture market size, share & trends analysis report, 2024–2033. https://www.globalgrowthinsights.com/market-reports/plant-tissue-culture-market-105792

Goodman, J., 1994. Tobacco in history: The cultures of dependence (1st ed.). Routledge. https://doi.org/10.4324/9780203993651

Gözen, A., Abak, K., Kasnakoğlu, H., Çetiner, S., Güzel, A., 1995. Türkiye'de bitki biyoteknolojisi

öncelikleri. TTGV Yayın No. 2. Ankara.
Hutter, I., Schneider, C., 2019. Commercial micropropagation in Germany. J Appl Bot Food Qual, 92, 226–231. https://doi.org/10.5073/JABFQ.2019.092.031
Kazaz, S., Kırbay, E., Aydın, V., Meral, E. D., Kılıç, T., 2025. Süs bitkileri üretiminde mevcut durum ve gelecek. TMMOB Ziraat Mühendisleri Odası Türkiye Ziraat Mühendisliği X. Teknik Kongresi, 13-17 Ocak 2025, Ankara.
Krikorian, A. D., Berquam, D. L., 2003. Plant cell and tissue cultures: The role of Haberlandt. In: Laimer, M., Rücker, W. (eds) Plant Tissue Culture. Springer, Vienna. https://doi.org/10.1007/978-3-7091-6040-4_2
Market.us., 2025. Plant tissue culture market size, share, trends, growth & forecast 2024–2034. https://market.us/report/plant-tissue-culture-market/
Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15(3), 473–497.
Negi, S., Singh, P., Trivedi, V. L., ve ark., 2024. The current trends and research progress globally in the plant tissue culture: 90 years of investigation. Plant Cell Tiss Organ Cult, 157, 73. https://doi.org/10.1007/s11240-024-02788-4
OECD., 1986. Recombinant DNA safety considerations (Blue Book). Paris: OECD.
Öktem, H., 2006. Plant biotechnology in Turkey. Proc. Vth IS on *In vitro* Culture and Hort. Breeding, Eds. M. G. Fári, I. Holb, Gy. D. Bisztray. Acta Hort., 725, ISHS 2006.
Pasternak, T., Steinmacher, D., 2025. Plant tissue culture *in vitro*: A long journey with lingering challenges. *International Journal of Plant Biology*, 16(3), 97. https://doi.org/10.3390/ijpb16030097
Regni, L., Cesarini, A., Micheli, M., Proietti, P., 2025. A bibliometric analysis of research on blackberry micropropagation. Plant Cell Tiss Organ Cult, 160, 33. https://doi.org/10.1007/s11240-025-02978-8
Sota, V., Wilms, H., Yücesan, B., ve ark., 2025. Challenges in the micropropagation of economically important fruit species in Europe. Plant Cell Tiss Organ Cult, 162, 53. https://doi.org/10.1007/s11240-025-03165-5
Tigrel, A., Arslan, M., Arıcı, B., Yücesan, B., 2022. Selection and Prepara- tion of explants for the clonal propagation of horticultural plants in plant factory systems. In: Gupta S, Chaturvedi P (eds) Com- mercial scale tissue culture for horticulture and plantation crops. Springer, Singapore, pp 23–48. https://doi.org/10.1007/978-981 -19-0055-6_2
Thorpe, T. A., 2007. History of plant tissue culture. Molecular Biotechnology, 37, 169–180.
White, P. R., 1934. Potentially unlimited growth of excised tomato root tips in a liquid medium. Plant Physiol, 9, 585–600.
Winkelmann, T., Geier, T., Preil, W., 2006. Commercial *in vitro* plant production in Germany in 1985–2004. Plant Cell Tiss Organ Cult, 86(2), 319–327. https://doi.org/10.1007/s11240-006-9125-z
Yücesan, B., Tigrel, A., 2024. Strategic innovations in in vitro woody crop production: Balancing technical excellence with market and stakeholder dynamics. In Proceedings of the 2nd Conference of Cost Action CA21157 CopyTree, Bulduri, Latvia, pp 191–206.