

Chapter

5

DOUBLED HAPLOID PRODUCTION IN MELON

Nebahat SARI¹
İlknur SOLMAZ²

INTRODUCTION

Melon (*Cucumis melo* L.; $2n = 2x = 24$) is a member of the Cucurbitaceae family having high monetary value. This cucurbit crop is sensitive to frost and mostly cultivated in tropic and subtropic climate zones throughout the world. Melon total production is 27.349.214 tonnes worldwide and China is the leading producer country (12.788.218 tonnes) followed by Turkey (1.753.942 tonnes) (FAO, 2018). Among the genus *Cucumis*, melon is known to be the most polymorphic species commonly in terms of fruit traits. According to recent taxonomic data two subspecies has been classified within *C. melo*, as subsp. *melo* and subsp. *agrestis*. Nineteen different groups have been identified within these species as: *acidulus*, *agrestis*, *ameri*, *cantalupensis*, *chandalak*, *chate*, *chinensis*, *chito*, *conomon*, *cassaba*, *dudaim*, *flexuosus*, *ibericus*, *inodorus*, *indicus*, *kachri*, *makuwa*, *momordica* and *tibish*. (Pitrat, 2016; Hoogvorst et al., 2020). These groups have high levels of diversity in fruit shape, fruit size, fruit and rind color, fruit texture, flavour, aroma and ripening behaviour.

Commercial melon production is done with OP and hybrid cultivars mainly belong to *cantalupensis*, *reticulatus* and *inodorus* groups. Hybrid melons are developed by traditional breeding methods however, Pitrat et al. (1999) reported that recovery of new melon varieties using classical hybridisation techniques are usually slow due to narrow gene pool (Thakur et al., 2019). Several hybrid

¹ Prof. Dr., Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana, Turkey (Retired), nesari@cu.edu.tr

² Assoc. Prof., Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana, Turkey, isolmaz@cu.edu.tr

for the diagnosis of human cancer cells, has started to be used for the diagnosis of haploid plants in the 1980s. Flow cytometry has been used successfully in determining ploidy in carrot, melon, watermelon, and then this device has been used for rapid chromosome counts in many haploid plant species (Sari et al., 1999; Weber et al., 2005; Li et al., 2020).

CONCLUSIONS

Melon is one of the most produced Cucurbit crops with an increasing monetary value. It is extensively cultivated in most parts of the world in open fields or in greenhouses. Commercial OP and hybrid (F1) melon cultivars mostly belong to inodorus, reticulatus and cantalupensis groups which are generally achieved by traditional breeding methods. Hybrid cultivars are developed by crossing two homozygous lines which are obtained by several selfing cycles. For the development of inbred pure line in melon at least 7-8 generations of selfing is needed which is time consuming and labor cost. Nevertheless, this process can be shortened by using haploid (H) and doubled haploid (DH) technology. Induced haploid plants can be derived from androgenesis, gynogenesis and parthenogenesis pathways. Early attempts for haploid melon induction via anther or ovule culture were not found successful, however few studies reported gynogenic plants recovered by ovary culture. So far irradiated pollen technique (in situ induced parthenogenesis) is the most efficient technique to generate haploid lines in melon and currently used in breeding programmes.

ACKNOWLEDGEMENTS

Melon haploidy works in Cukurova University were supported by Turkish Scientific and Technological Council (TUBITAK), Turkish Ministry of Industry and Technology, Cukurova University Scientific Research Unit and some private seed companies. The author thanks all for their contributions.

REFERENCES

1. Abak, K., Sari, N., Paksoy, M., Yilmaz, H., Aktas, H., & Tunali, C. (1996). Genotype response to haploid embryo induction with pollination by irradiated pollens in melon, obtaining of dihaploid lines, determination of haploid and diploid plants by different techniques. *Tr. J. Agric. For.*, 20, 425-430.
2. 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.
3. Ari, E., Ikten, H., Gocmen, M., Coskun, R., & Eren, A. (2010). Comparative evaluation of different embryo rescue techniques on parthenogenetic melon (*Cucumis melo* L.) fruits induced with irradiated pollen. *African Journal of Biotechnology*, 9 (33), 5347-5356.

4. Baktemur, G., Taskin, H., & Buyukalaca, 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*). *Sci. World J.*, 10, 1-7.
5. Beharav, A., & Cohen, Y. (1995). Effect of kinetin and GA₃ on in vitro ovule embryo culture of *Cucumis melo* L. *Plant Growth Regul.*, 16 (3), 267-269.
6. 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.
7. Bohanec, B. (2003). Ploidy determination using flow cytometry. In: Doubled Haploid Production in Crop Plants, 397-403.
8. Chambonnet, D., & Dumas De Vault, R. (1985). Obtention of embryos and plants from in vitro culture of unfertilized ovules of *Cucurbita pepo*. *Cucurbit Genetics Coop Rep*, 8, 66.
9. Cuny, F., & Roudot, A. C. (1991). Germination et croissance pollinique in vitro du pollen de melon (*Cucumis melo* L.) apres irradiations gamma. *Environ. Exp. Bot.*, 31 (3), 277-283.
10. Cuny, F., Dumas de Vault, R., Longhi, B., & Siadous, R. (1992). Analyse des plantes de melon (*Cucumis melo* L.) issues de croisements avec du pollen irradié a differentes doses. *Agronomie*, 12 (8), 623-630.
11. Cuny, F., Grotte, M., Dumas de Vault, 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.
12. Dal, B., Sari, 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.
13. Dong, Y. Q., Zhao ,W. X., Li, X. H., Liu, X. C., 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.
14. Dumas de Vault, R. (1979). Obtention de plantes haploides chez le melon (*Cucumis melo* L.) après pollinisation par *Cucumis ficifolius* A. Rich. CR Acad. Sci. (Paris) 289, 875-878.
15. FAO, (2018). FAOSTAT Statistical Databases. <http://www.fao.org/faostat/en/#data/QC>.
16. Ficcadenti, N., Sestili, S., Annibali, S., Marco, M. D., & Schiavi, M. (1999). In vitro gynogenesis to induce haploid plants in melon (*Cucumis melo* L.). *J. Genet. Breed.*, 53 (3), 255-257.
17. Forster, B.P., & Thomas, W. T. B. (2005). Doubled haploids in genetics and plant breeding. *Plant Breed. Rev.*, 25, 57-88.
18. Gałazka, J., & Niemirowicz-Szczytt, K. (2013). Review of research on haploid production in cucumber and other cucurbits. *Folia Hort.*, 25 (1), 67-78.
19. Germaná, M. A. (2011). Gametic embryogenesis and haploid technology as valuable support to plant breeding. *Plant Cell Rep.*, 30 (5), 839-857.
20. Godbole, M., & Murthy, H. N. (2012). Parthenogenetic haploid plants using gamma irradiated pollen in snapmelon (*Cucumis melo* var. *momordica*). *Plant Cell Tissue Organ. Cult.*, 109, 167-170.
21. Gonzalo, M. J., Claveria, E., Monforte, A. J., & Dolcet-Sanjuan, R. (2011). Parthenogenic haploids in melon: generation and molecular characterization of a doubled haploid line population. *J. Am. Soc. Hort. Sci.*, 136, 145-154.
22. Gursoz-Sari, N. (1990). Kavun (*Cucumis melo* var. *inodorus* ve *reticulatus*) ve karpuzda (*Citrullus lanatus* (Thunb.)Mansf.) ışınlanmış 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 (in Turkish).
23. 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, Article 378.
24. Kasapoğlu, S., Gürsoy, I., Sari, N., & Solmaz, İ. (2011). The effects of different harvest time and storage temperatures on embryo yield and transformation to plant in Kirkagac and Yuva-Hasanbey melons obtained by pollination with irradiated pollen. Turkey IVth Seed Congress, Ondokuz Mayıs Univ. Fac. of Agric., 14-17 June 2011, Samsun, Proceeding book-1, 243-248.

25. Koksall, N., Yetisir, H., Sari, N., & Abak, K. (2002). Comparison of different in vivo methods for chromosome duplication in muskmelon (*Cucumis melo* L.). Proc. 2nd International Symposium on Cucurbits, *Acta Horticulturae*, 588, 293-298.
26. Koli, S. P., & Murthy, H. N. (2013). Haploid plant regeneration from unpollinated ovules of *Cucumis melo* L. var. *conomon* cv. Mudicode. *Br. Biotechnol. J.*, 3 (4), 605-613.
27. Malik, A.A., Li, C., Zhang, S. X., & Chen, J. F. (2011). Efficiency of SSR markers for determining the origin of melon plantlets derived through unfertilized ovary culture. *Horticultural Science*, 38, 27-34.
28. Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant*, 15(3):473-497.
29. 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 (Nature Publisher Group)*, London, 10 (1), 1-10.
30. Lim, W., & Earle, E. D. (2008). Effect of in vitro and in vivo colchicines treatments on pollen production and fruit set of melon plants obtained by pollination with irradiated pollen. *Plant Cell Tissue Organ Cult.*, 95, 115-124.
31. Lim, W., & Earle, E. D. (2009) Enhanced recovery of doubled haploid lines from parthenogenetic plants of melon (*Cucumis melo* L.). *Plant Cell Tiss Org Cult.*, 98, 351-356.
32. Lotfi, M., Alan, A. R., Henning, M. J., Jahn, M. M., & Earle, E. D. (2003). Production of haploid and doubled haploid plants of melon (*Cucumis melo* L.) for use in breeding for multiple virus resistance. *Plant Cell Rep.*, 2, 1121-1128.
33. Maluszynska, J. (2003). Cytogenetic tests for ploidy level analyses - chromosome counting. In: Maluszynski M., Kasha K. J., Forster B. P., Szarejko I. (eds) *Doubled Haploid Production in Crop Plants*. Springer, Dordrecht.
34. 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.
35. Ochatt, S. J., Patat-Ochatt, E. M., & Moessner, A. (2011). Ploidy level determination within the context of in vitro breeding. *Plant Cell Tissue Organ Cult.*, 104, 329-341.
36. Pitrat, M. (2016). Melon genetic resources: phenotypic diversity and horticultural taxonomy. In *Genetics and Genomics of Cucurbitaceae*, eds R. Grumet, N. Katzir, and J. Garcia-Mas, (Cham: Springer International Publishing), 25-60.
37. Pitrat, M., Chauvet, C., & Foury, C. (1999). Diversity, history and production of cultivated Cucurbits. In: Proc. 1st Int. Symp. on Cucurbits. (Eds. K. Abak & S. Büyükalaca) *Acta Horticulturae*, 492, 21-28.
38. Sauton, A. (1987). Recherche d'haploïdes chez le melon (*Cucumis melo* L.): Etude et application à la sélection parthenogénèse induite par du pollen irradié. These (Docteur Nouveau Régime). Spécialité: Biologie et Physiologie Végétales, Université des Sciences et Techniques du Languedoc, Montpellier, 123 pp.
39. Sauton, A., & Dumas de Vaulx, R. (1988). Doubled haploid production in melon (*Cucumis melo* L.). In: Proceedings of EUCARPIA meeting on Cucurbit Genetics and Breeding. Avignon Monfavet, France, pp. 119-128.
40. Sauton A., & Dumas de Vaulx, R. (1987). Production of haploid plants in melon (*Cucumis melo* L.) as a result of gynogenesis induced by irradiated pollen. *Agronomie*, 7, (2), 141-147.
41. Sari, 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). *Doğa Turkish Journal of Agric. and Forestry*, 16, 302-314.
42. Sari, N., Ekiz, H., Yuçel, S., Yetisir, H., Ekbic, E., & Abak, K. (1999a). Investigation of new protected cultivation melon lines resistant to *Fusarium oxysporum* f. sp. *melonis* using dihaploidization. In: Proceedings of the Third Turkish National Horticultural Congress, September 14-17, Ankara, Turkey, pp. 498-503.

43. Sari, N., Abak, K., & Pitrat, M. (1999b). Comparison of ploidy level screening methods in watermelon: *Citrullus lanatus* (Thunb.) Matsum. and Nakai. *Sci. Hort.*, 82, 265-277.
44. Sari, N., Solmaz, I., Kasapoglu, S., Gursoy, I., Szamosi, C., Unlu, H., & Park, K.S. (2010). Effect of different pollination dates with irradiated pollens on fruit set, haploid embryo induction and plant obtention in Turkish (Kirkagac, Yuva and Hasanbey) melons. *Acta Horticulturae*, 871, 639-648.
45. Sari, N., Solmaz, İ., Kacar, Y., & Dal, B. (2018). Altınbaş grubu kavunların dihaploidizasyon yöntemi ile saflaştırılması ve heterotik grupların oluşturularak F₁ hibritlerin geliştirilmesi. TÜBİTAK TOGTAG 114O230 Nolu Proje Sonuç Raporu, 111 s.
46. Sari, N., Solmaz, İ., Pamuk, S., Dal, B., Mancak, İ., & Alsaleh, A. (2019). Galia tipi kavunlarda yüksek verimli ve raf ömrü uzun saf hatlar geliştirme. SAN-TEZ 0807.STZ.2014 Nolu Proje Sonuç Raporu, 85 s.
47. Solmaz, İ., Sari, N., Gürsoy, I., & Kasapoğlu, S. (2011). Comparison of in vivo and in vitro colchicine application for production of dihaploid 'Kirkagac' and 'Yuva Hasanbey' melons. *African Journal of Biotechnology*, 10 (70), 15717-15724.
48. Thakur, H., Sharma, S., & Thakur, M. (2019). Recent trends in muskmelon (*Cucumis melo* L.) research: an overview. *The Journal of Horticultural Science and Biotechnology*, 94, (4), 533-547.
49. Weber, S., Ünker, F., & Friedt, W. (2005). Improved doubled haploid production protocol for Brassica napus using microsporecolchicine treatment in vitro and ploidy determination by flow cytometry. *Plant Breeding*, 124, 511-513.
50. Yashiro, K., Hosoya, K., Kuzuya, M., & Tomita, K. (2002). Efficient production of doubled haploid melon plants by modified colchicine treatment of parthenogenetic haploids. *Acta Hort.*, 588, 335-338.
51. Yetisir, H., & Sari, N. (2003). A new method for haploid muskmelon (*Cucumis melo* L.) dihaploidization. *Scientia Horticulturae*, 98, 277-283.
52. 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 chloroplast in stomatal guard cells in *Brassica oleracea*. *Agricultural Sciences in China*, 8 (8), 939-946.
53. Zhang, Y. B., Chen, J. F., Yi, H. P., Lei, C., & Wu, M. Z. (2006). Induction of haploid melon (*Cucumis melo*) plants by pollination with irradiated pollens. *J. Fruit Sci.*, 23, (6), 892-895.
54. Zhu, Y. C., Sun, D. X., Deng, Y., An, G. L., Li, W. H., Si, W. J., Liu, J. P., & Sun, X. P. (2020). Comparative transcriptome analysis of the effect of different heat shock periods on the unfertilized ovule in watermelon (*Citrullus lanatus*). *Journal of Integrative Agriculture*, 19 (2), 528-540.