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Review Article

An Overview of *In Vitro* Haploid Plant Production in *Citrus*

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Abstract: The main objectives of *Citrus* breeding are to have new varieties with a shorter juvenile non-fruiting period, an increased yield, a longer ripening season, regular fruit bearing, seedlessness and improved external and internal quality of the fruits. To make available new scions and rootstocks selected for resistance or tolerance to biotic and abiotic stresses is another important aim in *Citrus* improvement. *Citrus* breeding is based either on conventional methods (hybridization, selection, mutation) or biotechnological methods employing *in vitro* tissue culture, regeneration from protoplasts, somatic hybridization, *in vitro* mutant selection, genetic transformation and haploid production. An integrated approach between innovative and conventional tools is fundamental to obtaining large improvements in a short time. Haploid plants have some advantages for the plant breeding because of their one set of chromosomes, identified recessive mutations and reducing the breeding time. There are several methods to produce haploid plants such as androgenesis and gynogenesis. The pollen and microsphore cultures are used for androgenesis. The ovule and ovary culture are used for the gynogenesis. In this review we describe the principals of the haploid plant cultures and we illustrated some haploid studies in citrus.

Keywords: Haploidy, Androgenesis, Gynogenesis, Anther and Microsphore Culture, Ovule and Ovary Culture

1. Introduction

Higher plants are outbreeding, highly heterozygous and undergo a long developmental period before reaching their reproductive stage [1]. Conventional breeding programme are both unpredictable and time-consuming to overcome these problem.

Haploids originate from a single gamete therefore they are sporophytic plants with the gametophytic chromosome number. The significance of haploids in plant breeding and genetic research was recognized with the discovery of the first natural haploid in *Datura stramonium* and *Nicotiana* [2, 3, 4, 5].

Spontaneous haploid individuals have been identified in several fruit species. However, spontaneous evidence is a rare event, resulting in a limited application; hence artificial haploid induction is necessary for potential use in breeding. Haploid plants can be achieved using several methods: *in vitro* androgenesis (anther-isolated microspore culture) and gynogenesis (ovule-ovary culture), *in situ* parthenogenesis (pollen irradiation or treatment with chemicals) [6, 7].

Production of haploid progenies is of utmost importance because homozygous plants, which are very important for genetic analysis and breeding programmes, are easily obtained by doubling the chromosomes of haploid progenies. In addition, the haploids are also used for seedless triploid cultivar breeding by somatic hybridization of haploid and diploid protoplasts [8, 9, 10]; to induce new forms of interspecific and intergeneric hybrids through the fusion of haploid protoplasts [11]; to overcome incompatibility barrier [12]; gene transformation [13] and nucleus substitution [14, 15].

In the present review, we illustrated how these methods can be performed and presented some haploid studies in *Citrus*.

2. In Vitro Haploid Plant Production Techniques

2.1. Androgenesis

Androgenesis is one of the method to have haploid plants. The first androgenesis study was that Guha and Maheshwari [16] used the *Datura innoxia* anther culture [17]. In the androgenesis, pollen or isolated microspore can be used as plant material. Anther or isolated microspore culture technique is widely used method of producing haploids and doubled haploids. Haploid plants develop from the anther culture either directly or indirectly androgenesis. Direct androgenesis is similar to zygotic embriyogenesis; however, endosperm is not present. Most of the embryos are released from the pollen cell wall at the globular stage of development. Indirect androgenesis occurs callus during the irregular and asynchronous divisions [18]. In addition the basic principle of the direct or indirect androgenesis is that we should take the anthers or microspores during the first pollen mitosis which is the uninucleate phase. That principle occurs from mitosis and at the end of this stage vegetative and generative nucleus is occurred. In the androgenesis cultured uninucleus microspore divisions could make a callus. Androgenesis studies have been reported in about 200 species belonging to some families, such as Solanaceae, Cruciferae and Gramineae; many other families (Leguminosae and woody plants) [19, 20, 21, 22, 23, 24].

In androgenesis, microspore development is critical for induction, because it depends on genetic capacity of male gamete. Male gametes become competent to differentiate in a different way from the gametophytic pathway with continued growth and division in the period around the first haploid mitosis (late uninucleate or early bicellular pollen stage). Moreover, external factors such as stresses are necessary for transformation the microspores to embryogenic development. The stress can be physical (also wounding connected to the anther excision and culture), thermal (heat, cold) or chemical (water stress, starvation) [5].

Some factors such as genotype, age and physiological condition of donor plants, the stage of microspore improvement, media effects androgenesis success. Genotype plays a major role in determining the success or failure of an experiment. Some species as well as some genotype have the capability to produce haploid plants at much higher ratio than others. It is a general rule to culture anthers from buds collected as early as possible during the process of flowering. Affecting the donor plants, diverse environmental factors such as photoperiod, temperature and light intensity also influence the production of haploid plant like healthier and vigorously plants than others. The stage of microspore improvement is a crucial factor that affects haploid production from anther and isolated microspore culture; anthers should be collected during the uninucleate stage of pollen development for many species so as to achieve. For some species, it has been found that pretreatment is beneficial. While temperature from 4 to 10 ºC and durations from 3 days to 3 weeks have been utilized for particular species, more than one optimum temperature and length of treatment combination may be used for other kind of species [18].

2.2. Gynogenesis

In vitro gynogenesis is used as an alternate technique in species where anther/pollen culture is inaccessible or unsuccessful [1]. As is the case in androgenesis, haploids plants produced by gynogenesis may develop directly or indirectly via regeneration from callus [25]. The first cell division of gynogenesis are equal to those of zygotic embryogenesis. The egg cell, synergid or antipodals with organized cell division are the main parts of direct gynogenesis leading first to the formation of proembryos and then to well-differentiated embryos. On the other hand; callus may be formed directly from the egg cell, synergids, polar nuclei, antipodals or may develop from proembryos in indirect gynogenesis [18]. Culturing is divided in two ways: an induction medium containing hormones and a regeneration medium with reduce or without hormones [26].

There are many factors which effected gynogenesis experiments including plant genotype, medium, donor plant conditions and pretreatments. Also physical conditions of the plants and pretreatment methods are important factors which effected achievement of the gynogenesis studies.

2.3. Irradiated Pollen Technique

Pollen irradiation (UV, gamma rays, and X-rays) is the most widely used technique to induce *in situ* parthenogenetic haploid plants. Gamma rays are commonly used in haploid programmes because of their simple application, good penetration, reproducibility, high mutation frequency, and low disposal (lethal) problems [27]. This technique was used firstly with embryo culture on different species of Nicotiana [28]. Irradiated pollen can germinate on the stigma, grow within the style and reach the embryo sac, but cannot fertilize the egg-cell and the polar nuclei [29]. Genetically inactive but germinable pollen can be used to stimulate the division of the egg cell, and thus induce parthenogenesis or development of parthenocarpic fruit, including gynogenic haploid production [30].

3. Haploidy Studies in Citrus

Many studies have been performed related to haploidy in *Citrus* via anther/microspore culture, ovul/ovary culture and irradiated pollen techniques. Though anther culture technique has been utilized in order to obtain haploid calli, embryoids and plantlets with restricted struggles in few *Citrus* species like *C. madurensis*, *C. limon*, *C. deliciosa* × *C. paradise* [31, 32, 33], extensive research has been accomplished on *C. clementina*. There have been many studies to be conducted to induce embryogenic calli and haploid plantlet development in *C. clementina* Hort. Ex Tan. cv Nules [34, 35, 36, 37, 38, 39]. Germena et al [39] reported that regenerants from anther culture of *C. clementina* Hort. ex Tan. cv. Nules, SRA 63, and Monreal were obtained in different experiments from 1994 to 2002. Genetic analysis of 37 such regenerants was carried out using 4 microsatellite markers that were heterozygous in the

parental genotypes. The results showed that in all cases but one the regenerants carried only one or the other allele of the parental genotype, and were therefore homozygous and produced through a process of gametophytic embryogenesis.

Germana and Chiancone [6] reported that haploid plantlet regeneration through gynogenesis in *C. clementina* Hort. ex Tan., cv. Nules, induced by *in vitro* pollination with pollen grains of Oroblanco, a triploid cultivar of grapefruit. It indicates that parthenogenesis induced *in vitro* by triploid pollen can be an alternative method to obtain haploids in monoembryonic cultivars of *Citrus*. Actually, despite considerable efforts, androgenesis has not been yet successful in many genotypes of *Citrus*. Pollination and mature stage of pistils was necessary for gynogenic embryo regeneration. Fourteen haploid gynogenic embryos of Nules clementine were obtained. Embryo conversion was high (85.7%) and embryos vigorously germinated producing twelve plantlets.

Froelicher et al [40] researched that haploid induction in mandarin through *in situ* gynogenesis by pollination with irradiated pollen of ‘Meyer’ lemon. They performed pollination in three genotypes of mandarin with four levels of gamma-ray-irradiated pollen (150, 300, 600, and 900 Gy). In that study embryos were rescued *in vitro* and the ploidy level of the plantlets was determined by flow cytometry analysis. As a result of study, haploid, diploid, triploid plantlets were obtained. Kundu et al [15] investigated that induction of haploid plants in *C. grandis* through *in situ* parthenogenesis by pollination with gamma irradiated pollen of *C. limetta* and *C. sinensis*, treated with 50, 100, 200, 300 and 400 gray (Gy) gamma ray doses followed by *in vitro* ovule culture. Researcher revealed that two haploid plants with nine chromosome number were induced in *C. grandis* from the ovule culture which was cultured at 50 days after pollination (DAP) following pollination with irradiated pollen of *C. sinensis* at 400 Gy and *C. limetta* pollen at 300 Gy. Triploidy of pollen, like irradiation, does not hinder pollen germination, but prevents pollen fertilization and stimulates the development of haploid embryoids from ovules. Some haploid plants from the crossed of the triploid hybrid of ‘Kawano natsudaidai’ (*C. natsudaidai*) and two monoembryonic diploid (Clementine and ‘Lee’) have been provided by parthenogenesis induced by triploid pollen [41]. Successful haploidy studies in *Citrus* and haploidy technique were presented in Table 1.

Table 1. *Haploidy studies in Citrus.*

Genotype	Haploidy Technique	Reference
Poncirus trifoliata L. Raf.	Androgenesis	[42]
C. madurensis Lour.	Androgenesis	[31]
Hybrid No. 14 of *C. ichangensis* × *C. reticulata*	Androgenesis	[43]
C. clementina Hort. ex Tan	Gynogenesis	[41]
(*C. clementina* Hort. ex Tan.), cv. SRA 63	Irradiated Pollen	[44]
C. maxima	Triploid Pollen	[45]
C. clementina Hort. ex Tan., cv. Nules	Gynogenesis	[6]
C. clementina Hort. ex Tan. cv. Nules	Androgenesis	[46]
C. clementina Hort. ex Tan. cvv. Nules, SRA 63, and Monreal	Androgenesis	[39]
Fortune’ (*C. clementina* Hort ex Tan. · *C. tangerina* Hort ex Tan.) and ‘Ellendale’ (*C. reticulata* Blanco · *C. sinensis* L. Osb)	Irradiated Pollen	[40]
Clemenules	Irradiated Pollen	[47]
C. grandis	Irradiated Pollen	[15]

4. Conclusion

Citrus is one of the most important fruit crop in the world. The genus *Citrus* possesses several undesirable characteristics including salt and cold sensitivity; they are also susceptible to diseases caused by fungi, bacteria and viruses, such as Citrus exocortis viroid (CEV), Citrus infectious variegation virus (CIVV), Citrus cachexia viroid (CCaV) and Citrus tristeza closterovirus (CTV) Traditional citrus breeding programme is unpredictable and time-consuming [48, 49, 50]. Therefore an immediate need is felt to integrate biotechnology to speed up the crop improvement programs [51]. Haploids are important because of their undergone chromosome duplication represent a particularly attractive biotechnological method to accelerate plant breeding. The great potential of employing haploidy, doubled haploidy and gametic embryogenesis in *Citrus* breeding is clearly evident. Haploids can improve the efficiency and the speed of the usually cumbersome, time consuming, laborious and sometimes rather inefficient conventional breeding methods. On the other hand, haploid and doubled haploid lines are very interesting since they allow to fix traits in homozygous state in a single step. These homozygous individuals are very useful for genome mapping, providing reliable information about the location of major genes and quantitative trait loci for economically important traits [52, 53].

References

[1] V. K. Mishra, and R. Gowswami, “Haploid production in higher plant,” Int. J. Chem. Biol. Sci., 1: 26-45, 2014.

[2] A. F. Blakeslee, J. Belling, M. E. Farnham, and A. D. Bergner, “A haploid mutant in the Jimson weed, *Datura stramonium*,” Sci., 55: 646–647, 1922.

[3] A. F. Blakeslee, and J. Belling, “Chromosomal mutations in the Jimson weed, *Datura stramonium*,” J. Hered., 15: 195–206, 1924.

[4] D. Kostoff, “An androgenic Nicotiana haploid. Zeitschrift für Zellforschung und Mikroskopische,” Anatomie, 9: 640–642, 1929.

[5] M. A. Germena, “Haploidy,” in: Citrus genetics, breeding and biotechnology, I. A. Khan, Ed. CABI, 167-196, 2007.

[6] M. A. Germanà, and B. Chiancone, “Gynogenetic haploids of Citrus after *in vitro* pollination with triploid pollen grains,” Plant Cell Tiss Org., 66 (1): 59-66, 2001.

[7] M. Blasco, M. L. Badenes, and M. del Mar Naval, “Induced parthenogenesis by gamma-irradiated pollen in loquat for haploid production,” Breed. Sci., 66 (4): 606-612, 2016.

[8] S. Kobayashi, T. Ohgawara, W. Saito, Y. Nakamura, and M. Omura, “Production of triploid 14 somatic hybrids in Citrus,” J. Jpn. Soc. Hort. Sci., 66: 453-458, 1997.

[9] P. Ollitrault, D. Dambier, F. Sudahono Mademba-Sy, F. Vanel, F. Luro, and B. Aubert, “18 Biotechnology for triploid mandarin breeding,” In Fifth International Congress of Citrus 19 Nurserymen, ISCN, Montpellier, 20: 337, 1997.

[10] P. Ollitrault, D. Dambier, F. Vanel, and Y. Froelicher, “Creation of triploid Citrus hybrids by 21 electrofusion of haploid and diploid protoplasts,” Acta Hort., 535 (22): 191-197, 2000.

[11] J. W. Grosser, P. Ollitrault, and O. Olivares-Fuster, “Somatic hybridization in citrus: an effective 18 tool to facilitate variety improvement,” In Vitro Cell Dev., 36: 434-449, 2000.

[12] K. K. Pandey, “Overcoming interspecific pollen incompatibility through the use of ionising 1 radiation,” J. Hered., 33: 279-287, 1974.

[13] K. K. Pandey, “Gametic gene transfer in Nicotiana by means of irradiated pollen,” Genetica, 49 (3): 53-69, 1978.

[14] C. Raquin, A. Cornu, E. Farcy, D. Maizonnier, G. Pelletier, and F. Vedel, “Nucleus substitution 15 between *Petunia* species using gamma ray induced androgenesis,” Theor Appl Genet., 78 (16): 337-341, 1989.

[15] M. Kundu, A. Dubey, M. Srivastav, and S. Malik, “Induction of haploid plants in citrus through gamma irradiated pollen and ascertain of ovule age for maximum recovery of haploid plantlets,” Turk J Biol., ISSN: 1300-0152, 2017. DOI: 10.3906/biy-1606-28.

[16] S. Guha, and S. C. Maheshwari, “*In vitro* production of embryos from anthers of Datura,” Nature, 204: 497–498, 1964.

[17] M. A. Germanà, “Anther culture for haploid and doubled haploid production,” Plant Cell Tiss Org., 104 (3): 283-300, 2011.

[18] S. M. Reed, “Haploid Cultures,” in: Plant Development and Biotechnology, R. N. Trigiano, and D. J. Gray, Eds. 225-234, 2005.

[19] J. M. Dunwell, “Pollen, ovule and embryo culture, as tools in plant breeding,” In: Plant tissue culture and its agricultural applications, L. A. Withers, and P. G. Alderson Eds. Butterworths, London, 375–404, 1986.

[20] H. Hu, and H. Y. Yang, “Haploids of higher plants *in vitro*,” China Academic/Springer, Beijing/Berlin Heidelberg New York, 1986.

[21] B. S. Sangwan-Norreel, R. S. Sangwan, and J. Pare, “Haploidie et embryogenèse provoquée *in vitro*,” Bull Soc Bot Fr Actual Bot., 133: 7–39, 1986.

[22] Y. P. S. Bajaj, “*In vitro* production of haploids and their use in cell genetics and plant breeding. In: Haploids in crop improvement, Biotechnology in agriculture and forestry,” Y. P. S. Bajaj, Ed. vol 12. Springer, Berlin Heidelberg New York, 1–44, 1990.

[23] V. Raghavan, “From microspore to embryoid: faces of the angiosperm pollen grain,” In: Progress in plant cellular, H. J. J. Nijkamp, L. H. W. van der Plas, and J. van Hartrigik, Eds. Mol Biol. Kluwer, Dordrecht, 213–221, 1990.

[24] G. Wenzel, U. Frei, A. Jahoor, A. Graner, and B. Foroughi-Wehr, “Haploids—an integral part of applied and basic research,” In Current Issues in Plant Molecular and Cellular Biology, Springer Netherlands, 127-135, 1995.

[25] E. J. Keller, and L. Korzun, “Ovary and ovule culture for haploid production,” In *In vitro* haploid production in higher plants, Springer Netherlands, 217-235, 1996.

[26] B. P. Forster, E. Herberle-Bors, K. J. Kasha, and A. Touraev, “The resurgence of haploids in higher plants,” Trends Plant Sci., 12 (8): 368–375, 2007.

[27] G. S. Chahal, and S. S. Gosal, “Principles and procedures of plant breeding,” Alpha Science, Oxford, 2002.

[28] K. K. Pandey, and M. Phung, “Hertwig effect in plants: induced parthenogenesis through the use of irradiated pollen,” Theor. Appl. Genet., 62: 295–300, 1982.

[29] F. Cuny, “Processus d’induction d’embryons haploides par du pollen irradie´ chez le melon (*Cucumis melo* L.) responses du pollen a` l’irradiation gamma,” The`se de Docteur, Universite´ d’Avignon et des Pays de Vaucluse, Avignon, 1992.

[30] E. S. Kurtar, and A. Balkaya, “Production of *in vitro* haploid plants from in situ induced haploid embryos in winter squash (*Cucurbita maxima* Duchesne ex Lam.) via irradiated pollen,” Plant Cell Tiss. Org., 102 (3): 267-277, 2010.

[31] Z. Chen, H. Wang, and H. Liao, “The induction of Citrus pollen plants in artificial media,” Acta Genetica Sinica, 7: 189–192, 1980.

[32] M. A. Germanà, F. G. Crescimanno, F. De Pasquale, and W. Yu Ying, “Androgenesis in 5 cultivars of *Citrus limon* L. Burm. f.,” *In Vitro* Culture, XXIII IHC, 300: 315-324, 1990.

[33] M. A. Germanà, and G. R. Recupero, “Haploid embryos regeneration from anther culture of'Mapo'tangelo (*Citrus deliciosa× C. paradisi*),” Advances Hortic. Sci., 147-152, 1997.

[34] H. C. Chaturvedi, and A. K. Sharma, “Androgenesis in *Citrus aurantifolia* (Christm.) swingle”. Planta, 165 (1): 142-144, 1985.

[35] G. Geraci, A. Starrantino, “Attempts to regenerate haploid plants from *in vitro* cultures of *Citrus* anthers,” Acta Hortic., 280: 315-320, 1990.

[36] M. A. Germanà, Y. Y. Wang, M. G. Barbagallo, G. Iannolino and F. G. Crescimanno, “Recovery of haploid and diploid plantlets from anther culture of *Citrus clementina* Hort. Ex Tan. and *Citrus reticulata* Blanco,” J. Hort. Sci., 69: 473-480, 1994.

[37] M. A. Germanà, F. G. Crescimanno, A. Motisi, “Factors affecting androgenesis in *Citrus clementina* Hort. ex Tan.”, Advances Hortic. Sci., 14: 49-58, 2000.

[38] M. A. Germanà, F. G. Crescimanno, G. Reforgiato, M. P. Russo, “Preliminary characterization of several doubled haploids of Citrus clementina cv. Nules,” Acta Hortic., 535: 183-190, 2000.

[39] M. A. Germanà, B. Chiancone, O. Lain, and R. Testolin, “Anther culture in Citrus clementina: a way to regenerate tri-haploids,” Crop and Pasture Sci., 56 (8): 839-845, 2005.

[40] Y. Froelicher, J. B., Bassene, E. Jedidi-Neji, D. Dambier, R. Morillon, G. Bernardini, G. Costantino and P. Ollitrault, “Induced parthenogenesis in mandarin for haploid production: induction procedures and genetic analysis of plantlets,” Plant Cell Reports, 26 (7): 937, 2007.

[41] I. Oiyama, and S. Kobayashi, “Haploids obtained from diploid × triploid crosses of citrus,” J. Jpn. Soc. Hortic. Sci., 62 (1): 89-93, 1993.

[42] T. Hidaka, Y. Yamada, and T. Shichijo, “In vitro differentiation of haploid plants by anther culture in *Poncirus trifoliata (*L.) Raf,” Japan J. Breed., 29: 248–254, 1979.

[43] X. X. Deng, Z. A. Deng, S. Y. Xiao and W. C. Zhang, “Pollen derived plantlets from anther culture of Ichang papeda hybrids No. 14 and Trifoliate orange,” in: Proceedings of the International Society of Citriculture. Acireale, Italy, 190–192, 1992.

[44] P. Ollitrault, V. Allent, and F. Luro, “Production of haploid plants and embryogenic calli of clementine (*Citrus reticulata* Blanco) after in situ parthenogenesis induced by irradiated pollen,” Proc Int Soc Citriculture, 2: 913–917, 1996.

[45] P. Toolapong, H. Komatsu, and M. Iwamasa, “Triploids and haploid progenies derived from small seeds of 'Banpeiyu', a pummelo, crossed with 'Ruby Red 'grapefruit,” J. Jpn. Soc. Hortic. Sci., 65 (2): 255-260, 1996.

[46] M. A. Germanà, and B. Chiancone, “Gynogenetic haploids of Citrus after in vitro pollination with triploid pollen grains,” Plant Cell Tiss. Org., 66 (1): 59-66, 2001.

[47] P. Aleza, J. Juárez, M. Hernández, J. A. Pina, P. Ollitrault, and L. Navarro, “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 (1): 110, 2009.

[48] P. Garcia-Agustin and E. Primo-Millo, “Selection of a NaCl tolerant Citrus plant,” Plant Cell Rep., 14 (5): 314–318, 1995.

[49] B. Van Le, N. Thanh Ha, L. T. Anh Hong, and K. Trân Thanh Vân, “High frequency shoot regeneration from trifoliate orange (*Poncirus trifoliata* L. Raf.) using the thin cell layer method,” Comptes Rendus de l’Academie des Sciences, 322 (12): 1105–1111, 1999.

[50] D. Donmez, O. Simsek, T. Izgu, Y. A. Kacar, and Y. Y. Mendi, “Genetic Transformation in Citrus,” The Scientific World Journal, 1-8, 2013.

[51] D. Dönmez, Ö. Şimşek, and Y. A. Kaçar, “Genetic Engineering Techniques in Fruit Science,” Int. J. Environ. Agric. Res., 2 (12): 115-128, 2016.

[52] G. S. Kush, and S. S. Virmani, “Haploids in plant breeding,” In: *In vitro* haploid production in higher plants, S. M. Jain, S. K. Sopory and R. E. Veilleux, Eds. Kluwer, Dordrecht, 11–33, 1996.

[53] M. Blasco, M. L. Badenes, and M. del Mar Naval, “Induced parthenogenesis by gamma-irradiated pollen in loquat for haploid production,” Breed. Sci., 66 (4): 606-612, 2016.