Chapter 3
Gynogenesis: An Important Tool for Plant Breeders

Gynogenesis is the least adopted method to produce haploid plants, but it has been predominantly exploited in those crops that have shown a very little or no response to wide hybridization, microspore, or anther culture (Forster et al. 2007). Gynogenesis consists of in vitro culture of unfertilized gametes (female) such as ovaries or ovules, though occasionally complete flower buds have also been used for culture. It has been recommended that female gametes or flower buds for gynogenesis should be collected before anthesis (pollen shedding). However, the collection can be made at any time in case of a male sterile or self-incompatible species. For collection purposes, stage of microspores is an excellent indicator to identify the exact time with respect to female gametes. The procedures of surface sterilization are generally used, similar to androgenesis techniques, to sterilize/disinfect. The sterilization time and agent differ from one species to another. The donor plants grown in cold chambers or green houses mostly need less time to sterilize than plants grown under field conditions. Solid medium is the most commonly used medium to produce haploids via gynogenesis. It has been recommended to dry the ovules that need to be cultured prior to start the excision procedure. The irradiated pollen using cobalt-60 has also been used in some tree species to induce gynogenesis. The time of application and application dose have been termed as the most important factors leading towards gynogenic success. Recently, tetraploid (Germana and Chiancone 2001) and irradiated (Froelicher et al. 2007) pollens have been effectively used to induce gynogenesis in citrus.

The work on gynogenesis started in 1964 when Tulecke (1964) reported callus formation from female gametes for the first time. However, the advancement and improvement in gynogenesis was much slower than androgenesis. In barley, Noeum (1976) gave details of first haploid plant via gynogenesis by culturing ovaries. In few crop plants, such as wheat, barley, rice, and maize, doubled haploidy via gynogenesis is possible (Gaj 1998; Sibi et al. 2001; Tang et al. 2006; Zhou and Yang 1981), but androgenesis is a method of choice due to the reason of low embryo

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production and limited number of cells to manipulate in gynogenesis. Gynogenesis has been effectively used in sugar beet (*Beta vulgaris*) and onion (*Allium cepa*) as described by Gurel et al. (2000) and Luthar and Bohanec (1999), respectively. The frequency of haploid production via gynogenesis in angiosperms, cucumber (Gemes-Juhasz et al. 2002), sweet potato (Kobayashi et al. 1993), and in some trees (Forster et al. 2007) has shown promising results. In sugar beet and onion, gynogenesis has been achieved by culturing female gametophyte followed by their regeneration. There is no need to apply pretreatment in case of onion, but a cold shock (8 °C for 7 days) to floral organs in combination with high temperature treatment at 30 °C during culturing is needed in sugar beet to obtain desired results (Michalik et al. 2000; Wremerth and Levall 2003). Major factors that influence gynogenesis include genotype, developmental stage of female gametophyte or embryo sac, pretreatment, composition of media, and induction/cultural conditions.

3.1 Genotype

Haploid production via gynogenesis is dependent on the type of genotype being used and is highly variable from one species to another. The induction response (no. of embryos) also depends on donor plant's growth and developmental conditions and quality of female gametophytes at the time of induction. In rice (Rongbai et al. 1998), onion (Bohanec et al. 1995), and wheat (Mdarhri-Alaoui et al. 1998), genotypic variations with respect to gynogenic success of haploid production have been well documented. The variable gynogenic induction frequency has been illustrated in onion genotypes of various origins that ranged from 0 to 10 % among 10 Polish cultivars/varieties (Javornik et al. 1998), 0 to 17 % in 22 European accessions (Geoffriau et al. 1997), and 0 to 22 % among 39 Japanese and American accessions (Bohanec and Jakse 1999). Furthermore, open pollinated cultivars of *Allium cepa* showed low response to gynogenesis than inbred lines and F_1 hybrids (Bohanec and Jakse 1999). A similar genotypic variability was also noted in potato and squash cultivars that ranged from 11 to 60 (Kobayashi et al. 1993) and 0 to 49 % (Shalaby 2007), respectively. Higher embryo yield was noted in sugar beet when floral organs were collected from donor plants grown in glass cabinets/greenhouses compared to field grown plants (Lux et al. 1990). In the same way, florets that developed first on lateral branches yielded higher number of embryos as compared to florets emerged at the later stages i.e. florets developed at main stem apex (D'Halluin and Keimer 1986a, b). The planting/sowing time also affected embryoid response and it has been stated that summer planting favored embryo production in *Beta* sp., whereas autumn sowing yielded higher embryos in *Gerbera* sp. (Cappadocia and Ahmim 1988; Cappadocia et al. 1988; Doctrinal et al. 1989; Lux et al. 1990). In onion, female gametophytes collected from very large and small flowers showed a very little response to gynogenic induction than medium ones exhibiting two to four nucleate embryo sacs (Musial et al. 2005).

3.2 Developmental Stage of Female Gametophyte

The exact stage of female gametophytic (ovule or ovary) regarding its collection for induction is hard to detect. The induction of ovules/ovaries is done on the basis of microspore stage or days after anthesis; however, in few cases, flower bud's developmental stage or direct observation of ovaries/ovules has also been performed. Nearly mature or mature embryo sac is considered as a good sign to commence the process of gynogenesis (Gemes-Juhasz et al. 2002; Keller and Korzun 1996); however, a well-trained and skilled embryologist is needed to determine the exact stage of embryo sac. Yang et al. (1986) confirmed that in sunflower, a well mature embryo sac is developed just 2–3 days before anthesis, whereas an excellent response has been obtained from unfertilized ovules of "Kyoho" grape wine when they were collected/excised, 19–20 days before anthesis (Nakajima et al. 2000). In the same way, ovaries harvested 1 day prior to anthesis in *Cucurbita pepo* resulted maximum embryos (Metwally et al. 1998a, b), whereas similar findings have been reported in cucumber when excision was done only 6 h prior to anthesis (Gemes-Juhasz et al. 2002). The embryo sac consists of egg cell, two polar nuclei, antipodal cells, and synergids. In most cases, egg cell give rise to haploid embryo but sometimes production of an embryo from synergid or antipodal cells has also been documented as in the case of rice (Zhou et al. 1986) and barley (Noeum 1976). In saffron, the excision time of ovaries has been linked to stigma development. Ovaries with yellow stigma are considered one the most responsive stage for gynogenic excision (Bhagyalakshmi 1999).

3.3 Pretreatment

The floral organs are pretreated in few species which is also considered as an important factor to stimulate the process of sporophytic development in female gametophytes. Similar to androgenic methods, starvation, heat, and/or cold shocks are given alone or in combination with each other to induce stress conditions. However, duration, time, type, and level of pretreatment vary considerably from one species to another. In rice, cold treatment at 8 °C for 6–14 days enhanced embryogenic response to a greater extent (Rongbai et al. 1998) and similar effect of cold treatment has been seen in sugar beet, wheat, and *Salvia sclarea* (Bugara and Rusina 1989a, b; Gurel et al. 2000; Sibi et al. 2001). Yang et al. (1986) reported that a rapid cold shock for 1–2 days at 4 °C also improved embryogenic efficiency in *Helianthus* sp. On the other hand, heat treatment for 2–4 days at 33 °C seemed to be efficient to promote sporophytic development of female gametophyte in *Picea sitchensis* (Baldursson et al. 1993). Gemes-Juhasz et al. (2002) described that heat shock (32 °C) is also effective in cucumber to obtain an effective gynogenic response provided that heat treatment is given during cultural/induction phase. In few species such as *Cucurbita pepo* (Metwally et al. 1998a, b), niger (Bhat and Murthy 2007),

and rice (Zhou et al. 1986), there is no need to apply any pretreatment and heat/cold shocks have shown detrimental effects in these species. In the same way, high illumination favors onion gynogenesis (Puddephat et al. 1999), whereas dark incubation during induction phase is required in saffron (Bhagyalakshmi 1999) and cucumber (Gemes-Juhasz et al. 2002).

3.4 Composition of Media

The composition of media is also a critical factor that affects success rate of gynogenesis to a considerable degree. Media constituents differ not only for regeneration and induction phases but also among crops. The media of regeneration phase require growth hormones/regulators to promote growth, whereas low concentration or sometime even no growth regulators are needed for induction medium. MS, Millers, N6, and B5 with minor changes/modifications in the sources of growth regulators, carbohydrates, and nitrogen are among the most widely adapted media.

Sucrose is the most extensively used carbohydrate and its concentration in media vary from 58 to 348 mM or 2 to 12 % (Juhasz et al. 1997; Mdarhri-Alaoui et al. 1998). The sucrose in a concentration of 6 % in the media enhanced number of embryos and hampered somatic tissue growth in wheat gynogenesis (Mukhambetzhanov 1997), but its high concentration believed to be beneficial in carnation and has an adverse effect on the frequency of embryos in squash (Sato et al. 2000). On the other hand, in few species, maltose has been exploited/used rather than sucrose (Cordewener et al. 1995). For gynogenic haploid production, Cytokinin and Auxin have been mainly used in various crop species, but it has been observed that novel polyamines gave much better response as compared to growth hormones/regulators in onion and their substitution has indicated better results (Martinez et al. 2000). Several other growth hormones such as indole-3-acetic acid (IAA) in onion (Bohanec et al. 1995) and carrot (Kielkowska and Adamus 2010), naphthalene acetic acid (NAA) in rice (Rongbai et al. 1998), coconut water in barley (Castillo and Cistue 1993), dimethyl sulfoxide (DMSO) in rice (Rongbai et al. 1998), and Thidiazuron (TDZ) in cucumber (Diao et al. 2009) alone or in combination with each other have given promising gynogenic results. In wheat, solid medium is preferred over liquid because it improves callus growth (Gusakovskaya and Najar 1994).

A significant amount of research work has been conducted in the area of doubled haploidy via gynogenesis over the past few decades. The research papers have addressed various aspects of gynogenesis; however, the major focal point has been to improve the methodology using responsive genotypes and altering donor plant's conditions, pretreatments, and media composition. Besides many recent gynogenesis publications, a very little information exists with respect to molecular and genetics of gynogenesis in crop plants. As described earlier, gynogenesis is considered a method of choice where other methods of doubled haploidy are not available or species are irresponsive to other methods. This is especially true in case of onion, melon, and sugar beet where significant achievements have been made with

gynogenesis because androgenesis via anther culture or IMC is not successful. Furthermore, gynogenesis has also proved to be beneficial in case of male sterile plants e.g. haploids via gynogenesis have been effectively developed in photosensitive male sterile line in rice by culturing unfertilized ovaries (Cai et al. 1988). The problem of albinism in cereals can also be tackled with gynogenesis. In petunia, the percentage of nonhaploids were more than haploids using androgenesis (anther culture) as a method of haploid production, but DeVerna and Collins (1984) reported that 93 % plants were haploids when produced through gynogenesis. Similar findings in rice with the use of gynogenesis have been reported. It is suggested that future gynogenesis work should be conducted to improve our knowledge to track gynogenic sporophytic pathway and to identify new QTLs or genes associated with it. The development and identification of genetic/molecular markers associated with higher frequency of embryoids and green plants through gynogenesis will definitely improve gynogenic doubled haploidy in crop plants to a considerable degree.

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