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In vitro culture of endosperm and its application in plant breeding: Approaches to polyploidy breeding

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Abstract

This review article provides an overview of plant regeneration from endosperm to produce polyploid plants. We discuss the endosperm-ploidy levels and its applications in plant breeding. Representative cases of successful endosperm culture and prospects of endosperm culture are described. In the first part of the review, i.e., the background of endosperm culture, we discuss the importance of triploid plants, meaning plants derived from the endosperm of diploid plants. A brief history of endosperm culture is also provided, and a description of plant regeneration systems from the endosperm is given. The influence of culture medium on callus induction and regeneration is indicated. Finally, prospects for endosperm culture are proposed, and novel approaches for polyploidy breeding using endosperm culture are described.

Keywords: Endosperm culture, Plant regeneration, Polyploidy breeding, Triploid

Abbreviations

2,4-D	2,4-Dichlorophenoxyacetic acid
BAP (BA)	Benzylaminopurine
CH	Casein hydrolysate
CM	Coconut milk
GA_3	Gibberellic acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
MS	Murashige and Skoog (1962) medium
NAA	1-Naphthaleneacetic acid
YE	Yeast extract

1. Introduction

In diploid plants, the endosperm is a triploid (i.e., having 3 sets of chromosomes) tissue as a result of double fertilization, which is a unique process in higher plants. During the fertilization process, one of the male gametes fuses with the egg to form a zygote, which later forms the embryo; the other male gamete fuses with the central cell, which contains 2 haploid nuclei. This second fusion frequently results in a triploid structure, the endosperm. Hence, the endosperm is formed as a result of double fertilization and triple fusion (i.e., fusion between 3 different haploid nuclei, 1 from the paternal and 2 from the maternal side) and is present in all angiosperm families except Orchidaceae, Podostemaceae, and Trapaceae. Earlier studies suggested that the endosperm functions as a nutritive tissue, because growth and development of the embryo depends on the presence of the endosperm (Brink and Cooper, 1947; Raghavan, 1966). Moreover, the endosperm persists in some seeds (like cereals) as a reserve food. The endosperm represents about 60% of the world's food supply (Berger, 2003). When the endosperm fails to develop properly, abortion of the embryo results (Vijayaraghavan and Prabhakar, 1984). Endosperm may be fully utilized by the developing embryo (non-endospermous), or it may persist in mature seeds (endospermous).

In cereal crops, the endosperm shows an accumulation of seed storage proteins that are useful as food (Kawakatsu and Takaiwa, 2010). Protein synthesis and nutrient transport have been analyzed. Coconut water or coconut milk, which are media with a high nutritive value, were used to promote successful tissue culture in *Datura* embryos (Van Overbeek et al., 1941). Thereafter, plant growth regulators such as cytokinins have been found in coconut water (Letham, 1974). Thus far, coconut water has been utilized

for tissue cultures in recalcitrant plant species (Thorpe, 2007). Thus, these aspects of endosperm have been studied. In addition, a specific characteristic of endosperm is its ploidy level, which is triploid in diploid plant species.

One of the most important characteristics of triploid plants is seed sterility, and hence, the seed sterility is unfavorable for plants whose seeds are used commercially. However, triploids are of significant importance in trees and shrubs that are important for biomass and soil conservation, because they promote vegetative growth by preserving huge amounts of photosynthetic energy normally channeled to seed and fruit production. Similarly, seedlessness is used to increase the quality of several fruits, like banana, papaya, grapes, apple, etc. According to Morinaga and Fukushima (1935), triploids are more vigorous than diploids. In some plants, like *Miscanthus sinensis*, seed-sterile triploids have been grown to prevent seed dispersal in the environment (Petersen et al., 2002).

Like triploid plants, polyploid plants have been incorporated into breeding programs and practical cultivation. Polyploid plants, for example, tetraploids, can be produced by the chemical treatment (colchicine, oryzalin, etc.) of diploid plants. Moreover, other ploidy levels can be obtained by crossing different ploidy levels. In this study, we propose the usefulness of triploid plants produced by endosperm culture, when further ploidy levels are produced through crossing with other ploidy levels. Furthermore, we discuss the possible endosperm culture from ovules after crossing plants with different ploidy levels.

2. Advantage of producing triploids from the endosperm

2.1. History and applications

Polyploid production has been utilized in breeding several crops. Polyploid plants are generally expected to have enlarged organs. In addition, polyploid plants exhibit disease resistance, delayed flowering, or lower fertility in some cases. These phenotypes are considered to be favorable traits. For chromosome doubling, treatment with chemicals such as colchicine, pronamide, trifluralin, oryzalin, and amiprophos methyl have been investigated (Wan et al., 1991). These chemicals inhibit cell division after chromosome doubling, which results in polyploid cell formation. Chromosome doubling has occurred as a result of these chemical treatments, and higher ploidy plants can be produced.

Triploid plants are traditionally produced by crossing a diploid plant with an induced tetraploid plant. The tetraploid plant is produced from a normal diploid plant by the chemical treatments mentioned previously. However, the cross often results in reduced seed setting compared to diploid × diploid crosses (Sikdar and Jolly, 1995). Moreover, seed germination and seedling survival are very low. Despite these difficulties, some scientists have successfully produced triploid plants through diploid × tetraploid crosses. Some plant varieties developed and released for agricultural purposes are described in the following sections.

3. Conventional triploid utility

3.1. Cassava (*Manihot esculenta*)

Cassava (*Manihot esculenta* Crantz) is an important root crop that is cultivated in tropical countries and propagated by stem cuttings. It is generally known as a poor man's crop and has become a subsidiary food in many countries. Cassava is also exploited as a raw material for starch-based industries and as cattle feed (Ghosh, 1991). There is great

potential for improving the starch yield of this industrially important crop. Cultivated cassava has a diploid number of chromosomes ($2n = 36$) and is highly heterozygous and cross-pollinated. Among artificially produced polyploids, triploids have a higher yield and higher starch potential (Jos et al., 1987; Sreekumari and Jos, 1996).

The first triploid cassava variety 'Sree Harsha' released in 1996 (Sreekumari et al., 1996) was a cross between natural diploid and induced tetraploid plants. The use of a female diploid plant yielded better results than reciprocal crosses. The characteristics include vigorous, non-branching short plants with broad, thick, dark green leaves. Compact roots yield 35–40 t/ha. Even though the crop duration is 10 months, because of its bulky nature the crop can be harvested even after 7 months without any yield loss or starch reduction in the roots.

The triploid cassava has a number of desirable features compared to its diploid counterparts. These include higher yield, a higher harvest index, increased dry matter and starch content in the roots, rapid bulking, early harvestability, shade tolerance, and tolerance to cassava mosaic virus. The triploid cassava combines a high yield with outstanding culinary quality, thereby making it a favorite both industrially and domestically.

3.2. Watermelon

Cushman et al. (2003) evaluated the field performance of various triploid (seedless) watermelon varieties. The 'Vertigo' variety produced the highest yields of marketable melons (41,000 lb/acre and 2,270 melons/acre). 'Vertigo' also produced the lowest yields of early melons (8,200 lb/acre and 450 melons/acre). Cultivar SWS 4930 produced significantly larger melons (20.6 lb) than all other varieties except 'Seedless Sangria' and SR 8026. The average weight of the 2 oval-shaped "Cooperstown" and "Triple Crown"

cultivars was lower than that of the elongated cultivars. Values for soluble solids concentration, hollowheart, and rind necrosis were not significantly different among any of the cultivars tested.

3.3. Little gourd (*Coccinia grandis*)

A promising triploid variety of *Coccinia grandis* was developed by Suresh Babu and Rajan (2001). The tender fruits of this crop are cooked as a vegetable. The plant has several medicinal properties also. The fruit contains appreciable amounts of iron, vitamin A, and vitamin C. The triploid plants were produced by crossing colchicine-induced tetraploid with a normal diploid parent. Although the fruit set was observed in all such crosses, the seeds per fruit were 2.4%. Morphologically, the triploid plants more or less resembled the diploid, but the significant features of the triploid were its increased fruit size, lower astringency, vigorous growth, and higher yield. Moreover, the fruits of the triploid had less polyphenols, they were tastier, and hence they could be promoted as a salad crop. The fruit length, weight, and fruit yield/plant/year of the triploid plants were 7.50 cm, 44.2 g, and 15.25 kg, respectively, which were comparatively higher than those of diploid and tetraploid *C. grandis* plants.

As mentioned previously, triploid plants are very useful for agricultural production. To produce triploid plants, we focus on the tissues of endosperm, indicating a triploid level in a diploid plant species. By using tissue culture techniques, one can regenerate triploid plants from the endosperm. In the following section, we describe previous successful plant regeneration from the endosperm.

4. Endosperm culture for the production of triploids and polyploids

As compared to conventional methods, endosperm culture provides an easy 1-step protocol for triploid plant production (Thomas and Chaturvedi, 2008). In responding systems, the time needed for triploid plant production is lower than that needed for production using conventional methods. Hence, this method is preferred over conventional techniques. The parenchymatous nature of the endosperm and the absence of vascular tissues make it a unique and excellent experimental system for in vitro culture studies. The first report on endosperm culture was on maize by Lampe and Mills (1933). They cultured maize endosperm on a medium fortified with extracts of potato and young corn kernels. This was followed by detailed maize endosperm culture studies by La Rue and his coworkers at University of Michigan in 1947 (La Rue, 1947). They cultured maize endosperm on an artificial medium and obtained regenerated roots in few cultures and a root-shoot axis in 1 instance. However, they failed to obtain differentiation of roots and shoots in subsequent reports (Straus and La Rue, 1954; Straus, 1960). Later, maize endosperm culture studies were carried out in other research labs (Faranda et al., 1994; Felker and Goodwin, 1988; Manzocchi, 1991; Sehgal, 1969; Tamaoki and Ullstrup, 1958; Zhu et al., 1988).

Following the maize endosperm culture, some other Gramineae members were also investigated for endosperm culture. These included *Triticum aestivum* (Sehgal, 1974), *Hordeum vulgare* (Sehgal, 1974; Sun and Chu, 1981), and *Oryza sativa* (Bajaj et al., 1980; Nakano et al., 1975).

Early researchers failed to achieve regeneration from cultured endosperm probably because of the improper selection of explant stage, nutrient media, and additives, especially plant growth regulators. However, the Murashige and Skoog (1962) medium, which contains a rich amount of nitrogen, is now routinely used for endosperm culture,

proliferation, and regeneration.

The first demonstration of direct shoot formation from cultured mature endosperm of *Exocarpos cupressiformis* by Johri and Bhojwani (1965) paved the way for successful studies on endosperm-derived plantlets in several systems (Table 1). Now the potential of endosperm cells for unlimited growth and differentiation in vitro has been well documented. Differentiation of plantlets from cultured endosperm has been reported in several systems, including *Citrus* spp. (Gmitter et al., 1990), *Acacia nilotica* (Garg et al., 1996), *Mallotus philippensis* (Sehgal and Abbas, 1996), *Actinidia* spp. (Machno and Przywara, 1997), *Morus alba* (Thomas et al., 2000), *Azadirachta indica* (Chaturvedi et al., 2003), *Actinidia deliciosa* (Góralski et al., 2005), and *Lonicera caerulea* (Miyashita et al., 2009).

Both immature and mature endosperms have been used for endosperm culture. However, the growth stage of the endosperm at the time of culture is an important factor that determines plant regeneration. This is crucial in the case of immature endosperm culture. The stages of endosperm at the time of culture were normally expressed as days after pollination (Thomas et al., 2000). However, some researchers estimated the endosperm stage as the stage of developing embryo (Walia et al., 2007). In the case of immature endosperm, the addition of a nitrogen source is essential for endosperm proliferation. For this purpose, yeast extract (Bajaj et al., 1980; Tulecke et al., 1988; Zhu et al., 1988), casein hydrolysate (Kin et al., 1990; Liu and Liu, 1980; Wang and Chang, 1978), coconut milk (Garg et al., 1996; Gayatri, 1978), or tomato juice (Straus and La Rue, 1954) were routinely added to the medium. Regeneration of plantlets from immature endosperm has been reported in several systems, including *Pyrus communis* (Zhao, 1988), *Coffea* sp. (Raghuramulu, 1989), *Actinidia* sp. (Kin et al., 1990), *Acacia nilotica* (Garg et

al., 1996), *Morus alba* (Thomas et al., 2000), *Azadirachta indica* (Chaturvedi et al., 2003), and *Carthamus tinctoriuss* (Walia et al., 2007). In all of these reports, the addition of plant growth regulators in one or other combination was indispensable for inducing organogenesis.

The composition of the culture medium is very important for the success of plant regeneration from the endosperm. Representative culture conditions for endosperm cultures are shown in Table 2. As a basal medium, the Murashige and Skoog (1962) medium was frequently used. In most cases, callus was induced from cultured endosperm tissues. For callus induction, plant growth regulators (2,4-dichlorophenoxyacetic acid, 1-naphthaleneacetic acid, or indole-3-butyric acid as auxins; benzylaminopurine or kinetin as cytokinins) were added to culture media. In *Actinidia chinensis* (Gui et al., 1982), *Diospyros kaki* (Tao et al., 1997), and *Azadirachta indica* (Chaturvedi et al., 2003), the addition of casein hydrolysate to the culture medium was effective for callus induction. Subsequently, adventitious shoots were stimulated by changing the culture medium. Occasionally, regenerated shoots were rooted on media containing indole-3-butyric acid, 1-naphthaleneacetic acid, or indole-3-acetic acid. These regeneration processes were precisely controlled by culture media. As shown in Table 2, optimal culture media should be examined in plant materials and genotypes. These case studies will contribute to the further development of endosperm culture for plant regeneration in other plant species.

The suitable stages of endosperm as an explant for culture are shown in Table 2. In the case of mature endosperm, the time required for callus proliferation varied from 10 (Johri and Srivastava, 1973) to 25 (Rangaswamy and Rao, 1963) days. However, in some systems, like *Ricinus*, this time could be reduced by presoaking the endosperm in

gibberellic acid (Johri and Srivastava, 1973). Mature endosperm-derived plantlets were reported in *Lycium barbarum* (Gu et al., 1985), *Emblica officinalis* (Sehgal and Khurana, 1985), *Annona squamosa* (Nair et al., 1986), *Asparagus officinalis* (Liu et al., 1987), *Citrus* spp. (Gmitter et al., 1990), *Mallotus philippensis* (Sehgal and Abbas, 1996), and *Actinidia* spp. (Góralski et al., 2005; Machno and Przywara, 1997). In parasitic plants, the mature endosperm was normally used for culture. Here, the endosperm bypasses the callus phase and produces plantlets directly, as reported for *Exocarpos cupressiformis* (Johri and Bhojwani, 1965) and *Taxillus vestitus* (Nag and Johri, 1971). Usually, the endosperm needs an early association with the embryo to form the callus. However, in some other systems, the endosperm proliferates independent of the presence or absence of an embryo (Chaturvedi et al., 2003; Thomas et al., 2000).

Despite the successful studies on endosperm culture in several systems, plant regeneration frequencies vary in the literature, as shown in Table 2. These techniques still need a lot of refinement. Even though endosperm-derived plants have vigorous growth and greater biomass than their diploid counterparts, they are still not used commercially or for agricultural purposes as conventional triploid plants are. This may be because scientists have paid more attention to achieving callus regeneration from various systems rather than examining the field performance of endosperm-derived plants. The field performance of endosperm-derived plants should be given greater priority in future research.

5. Prospects for endosperm culture

As described previously, plant regeneration from endosperm has been developed in several plant species. The regenerated plants showed the endosperm ploidy levels. This

indicates that triploid plants can be produced from diploid plants. One of the advantages of endosperm culture is to produce triploids quickly from diploid plants. For example, when triploid plants are produced from diploid plants using conventional procedures, tetraploid plants must first be produced by colchicine and other chemical treatments. Then, these tetraploid plants are grown for flowering. Furthermore, both the diploid and tetraploid flowers are cross-pollinated. Finally, triploid plants are obtained. Thus, the production of triploid plants takes time. In particular, tree crops need more years for triploid production because they take a long time to flower.

Endosperm culture is a useful procedure for the production of triploids from diploid plants. Compared with conventional chemical treatments, endosperm culture saves a great deal of time. This time-saving aspect will play an important role in the quick production of various polyploid plants, especially tree crops, which take several years to flower. Figure 1 presents a schematic model of the production of different polyploid plants using endosperm culture. Thus, further polyploid series will be produced rapidly using endosperm culture. In addition to conventional polyploid production by chemical treatment, endosperm culture can be incorporated into polyploid breeding programs.

Endosperm culture is a novel procedure for producing polyploid plants with endosperm ploidy level. However, researchers need to pay attention to the differences between endosperm culture-derived plants and colchicine treatment-derived polyploid plants. The significant difference is the genome composition. The endosperm genome is composed of a maternal:paternal genome ratio of 2:1. This affects allelic variations in gene expression, and the differences must occur in endosperm culture-derived plants. Moreover, unique character of endosperm-derived plants is newly produced nuclear-cytoplasmic interactions. The endosperm genome is composed of a

maternal:paternal genome ratio of 2:1, and the cytoplasm originates from the maternal side only. On the other hand, colchicine treatment-derived polyploid plants maintain same nuclear-cytoplasmic interactions. This is the distinct difference affecting nuclear-cytoplasmic interactions. However, no comparative studies have been undertaken on endosperm culture-derived plants and colchicine treatment-derived polyploid plants.

Another aspect of endosperm culture concerns the specific function and origin of endosperm. One of the important functions of endosperm is nourishing the embryo. In principle, the endosperm undergoes degeneration and disappears after supporting the embryo. Because of this specific characteristic, plant regeneration from endosperm might be difficult. As mentioned previously, recent studies have proven that the endosperm retains plant regeneration ability. However, the number of plant species successfully regenerated from the endosperm is limited. To apply endosperm culture to other plant species, researchers must determine the precise process and machinery of plant regeneration from the endosperm, especially in terms of conditioning culture media and other factors such as the developmental stage of the endosperm.

Recently, studies on genomic imprinting using the endosperm have been undertaken (Kinoshita, 2007; Kinoshita et al., 2008). The phenomenon of genomic imprinting involves a characterization of the endosperm. The unequal expression of maternal and paternal genes in endosperm is observed. The imprinting genes are differentially regulated between paternal and maternal genomes by DNA methylation. During endosperm development, genes of genomic imprinting may affect morphogenesis. The endosperm regeneration systems described in this study are beneficial for genomic imprinting by comparing gene expression patterns between in vivo and cultured

endosperm.

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Table 1. Selected reports on the successful production of endosperm-derived plants

Serial No.	Plant species	Family	Reference
1	*Acacia nilotica*	Mimosaceae	Garg et al. (1996)
2	*Actinidia deliciosa*	Actinidiaceae	Góralski et al. (2005)
3	*Annona squamosa*	Annonaceae	Nair et al. (1986)
4	*Asparagus officinalis*	Liliaceae	Liu et al. (1987)
5	*Azadirachta indica*	Meliaceae	Chaturvedi et al. (2003)
6	*Carthamus tinctorius*	Asteraceae	Walia et al. (2007)
7	*Citrus grandis*	Rutaceae	Wang and Chang (1978)
8	*Citrus* spp.	Rutaceae	Gmitter et al. (1990)
9	*Coffea* sp.	Rubiaceae	Raghuramulu (1989)
10	*Codiaeum variegatum*	Euphorbiaceae	Gayatri (1978)
11	*Emblica officinale*	Euphorbiaceae	Sehgal and Khurana (1985)
12	*Hordeum vulgare*	Gramineae	Sun and Chu (1981)
13	*Juglans regia*	Juglandaceae	Tulecke et al. (1988)
14	*Lonicera caerulea*	Caprifoliaceae	Miyashita et al. (2009)
15	*Lycium barbarum*	Solanaceae	Gu et al. (1985)
16	*Mallotus philippensis*	Euphorbiaceae	Sehgal and Abbas (1996)
17	*Morus alba*	Moraceae	Thomas et al. (2000)
18	*Passiflora foetida*	Passifloraceae	Mohamed et al. (1996)
19	*Petroselinum hortense*	Umbelliferae	Masuda et al. (1977)
20	*Pyrus communis*	Rosaceae	Zhao (1988)
21	*Santalum album*	Santalaceae	Lakshmi Sita et al. (1980)
22	*Zea mays*	Gramineae	Zhu et al. (1988)

Table 2. Representative culture conditions for successful plant regeneration from endosperm tissues, suitable explant stages, and regeneration frequency

Plant species	Culture condition	Suitable explant stage	Best regeneration frequency	Reference
Acacia nilotica	Basal medium: MS + 3% sucrose + 0.8% agar Embryogenic callus induction: 2,4-D (10 μM)＋BAP (25 μM) ＋ CH (1000 $mg \cdot l^{-1}$) Germination of somatic embryo: modified MS containing B5 major salts, inositol, glutamine, CW, CH, and 0.2% phytagel	Immature cellular endosperm from seeds containing 0.5–3 mm long dicotyledonous embryo	<Embryogenic callus induction> 7%	Garg et al. (1996)
Actinidia chinensis	Callus induction: MS + Zeatin (3 ppm) + 2,4-D (0.5 ppm) + CH (400 ppm) Embryoid regeneration: MS + Zeatin (1 ppm) + CH (400 ppm)	–	–	Gui et al. (1982)
Actinidia deliciosa	Callus induction: MS + 2,4-D (2 $mg \cdot l^{-1}$) + kinetin (5 $mg \cdot l^{-1}$) Shoot regeneration: MS + TDZ (0.5 $mg \cdot l^{-1}$) Rooting: Shoots were excised from callus → soaked in solution of IBA (100 $mg \cdot l^{-1}$) → 1/2 MS	Mature endosperm	<Callus induction> 80% <Shoot regeneration> 6.2 shoots per culture	Góralski et al. (2005)
Annona squamosa	Basal medium: White's medium + 3% sucrose + 0.8% agar or Nitsch's medium + 2% sucrose + 0.8% agar Callus induction: White's medium + kinetin (0.1 $mg \cdot l^{-1}$) + BAP (0.2 $mg \cdot l^{-1}$) + NAA (1 $mg \cdot l^{-1}$) + GA_3 (1 $mg \cdot l^{-1}$) Shoot regeneration: Nitsch's medium + BAP (2 $mg \cdot l^{-1}$) + NAA (0.5 $mg \cdot l^{-1}$)	Mature endosperm excised from germinated seeds (2–4 days after radicle emergence)	<Shoot regeneration (no root regeneration)> 25%	Nair et al. (1986)
Azadirachta indica	Basal medium: MS + 3% sucrose + 0.8% agar Callus induction: NAA (5 μM) + BAP (2 μM) + CH (500 $mg \cdot l^{-1}$) Shoot regeneration: BAP (5 μM) Shoot elongation: BAP (0.5 μM) Shoot multiplication: BAP (1 μM) + CH (250 $mg \cdot l^{-1}$) Rooting: 1/2 MS + IBA (0.5 μM)	Whole seeds at the early dicotyledonous stage of the embryo	<Callus induction> 53% <Shoot regeneration> 95% calli differentiated more than 14 shoots per	Chaturvedi et al. (2003)

			culture <Shoot elongation> More than 80% of shoots elongated <Rooting> 100%	
Diospyros kaki	Basal medium: modified MS (1/2 strength of nitrates) Callus induction: Zeatin (10 μM) + IAA (10 μM) + CH (500 mg·l^{-1}) Subculture: Zeatin (10 μM) + IAA (1 μM) Adventitious bud formation: Zeatin (10 μM) + IAA (0.1 μM) Shoot growth: Zeatin (5 μM) + IBA (1 μM) Rooting: Shoots were excised from callus → dipped in aqueous ethanol solution of IBA (1.5 mM) → 1/2 MS	Endosperm of 80 days after anthesis (Endosperm had an elastic soft texture with slight opaque white color.)	<Callus formation> 24% <Adventitious bud formation> 37% <Develop into shoot> More than two thirds <Rooting> About half of shoot	Tao et al. (1997)
Emblica officinale	Basal medium: MS + 2% sucrose + 0.8% agar Callus induction: IAA (1 mg·l^{-1}) + BAP (1 mg·l^{-1}) or 2,4-D (1 mg·l^{-1}) + kinetin (1 mg·l^{-1}) Shoot or embryo-like structure regeneration: IAA (0.1 mg·l^{-1}) + BAP (0.2 mg·l^{-1}) Shoot elongation and rooting: NAA (0.002 mg·l^{-1}) (liquid) filter paper bridges immersed in this liquid medium	Endosperm from mature fruits	<Regeneration of shoot> 50% <Regeneration of embryo-like structure> 8%	Sehgal and Khurana (1985)
Lonicera caerulea	Basal medium: MS + 3% sucrose + 0.2% gellan gum Callus induction: BA (2.22 μM) + IBA (0.49 μM) Shoot primordial regeneration: BA (2.22 μM) + IBA (0.49 μM) Shoot primordial proliferation: 1/2 MS + GA_3 (2.89 μM) Shoot elongation and rooting: 1/2 MS + BA (0.44 μM) + GA_3 (2.89 μM) Development of plantlets: 1/2 MS	Endosperm at the globular to early torpedo-stage of the embryo	<Callus induction> 63% <Shoot primordial regeneration> 10%	Miyashita et al. (2009)
Morus alba	Basal medium: MS + 3% sucrose + 0.8% agar Callus induction: BAP (5 μM) + NAA (1 μM) + CM (15%) or YE (1000 mg·l^{-1}) Callus multiplication: 2,4-D (5 μM) Shoot regeneration: TDZ (1 μM) or BAP (5 μM) + NAA (1 μM) Shoot multiplication: 5 or 7 μM BAP	Endosperm 17–20 days after pollination (with or	<Callus induction> 70–72% <Callus multiplication>	Thomas et al. (2000)

	Rooting: 1/2 MS + IBA (7 μM)	without the embryo)	92% <Shoot regeneration> 63–75% <Shoot multiplication> 88–92% <Rooting> 89% of the shoot developed 4–5 roots	
Oryza sativa	Callus induction: MS + 2,4-D (2 $mg \cdot l^{-1}$) Subculture: MS + 2,4-D (1 $mg \cdot l^{-1}$) Shoot regeneration: MS + IAA (4 $mg \cdot l^{-1}$) + kinetin (2 $mg \cdot l^{-1}$)	Mature endosperm and immature endosperm (4–8 days after pollination)	–	Bajaj et al. (1980)
Passiflora foetida	Basal medium: MS + 0.09 M sucrose (semi-solid) Regeneration: BA (2 μM) Growth and development of shoot primordia: GA_3 (29 μM) + CH (1000 $mg \cdot l^{-1}$) Shoot elongation: MS (free of plant growth regulators) Rooting: NAA (5 μM)	–	<Regeneration of adventitious shoots> 85% <Growth and development of shoot primordia> 100% <Shoot elongation> All shoots developed normally.	Mohamed et al. (1996)
Petroselinum hortense	Induction of embryogenic callus: MS + 3% sucrose + 0.6% agar	–	–	Masuda et al. (1977)
Santalum album	Callus induction: MS + BAP (0.5–2 $mg \cdot l^{-1}$) + NAA (1 $mg \cdot l^{-1}$) Embryoid differentiation: MS＋GA (1–2 $mg \cdot l^{-1}$) or MS + BAP (0.3 $mg \cdot l^{-1}$) + IAA (1 $mg \cdot l^{-1}$) or MS + GA (1 $mg \cdot l^{-1}$) + kinetin (0.3 $mg \cdot l^{-1}$) Shoot and root development: White's medium or White's medium + IAA (0.5 $mg \cdot l^{-1}$)	Endosperm from green fruits (0.6–0.8 cm in diameter)	–	Lakshmi Sita et al. (1980)

MS: Murashige and Skoog medium (Murashige and Skoog, 1962), BAP (BA): benzylaminopurine, CH: casein hydrolysate, CM: coconut milk, 2,4-D: 2,4-dichlorophenoxyacetic acid, GA_3: gibberellic acid, IAA: indole-3-acetic acid, IBA: indole-3-butyric acid, NAA: 1-naphthaleneacetic acid, YE: yeast extract.
White's medium (White, 1963)
Nitsch's medium (Nitsch, 1969)
–: No information

Figure legend

Figure 1

Schematic model for the production of different polyploid plants using endosperm culture. By using endosperm culture, these polyploid plants can be produced efficiently.

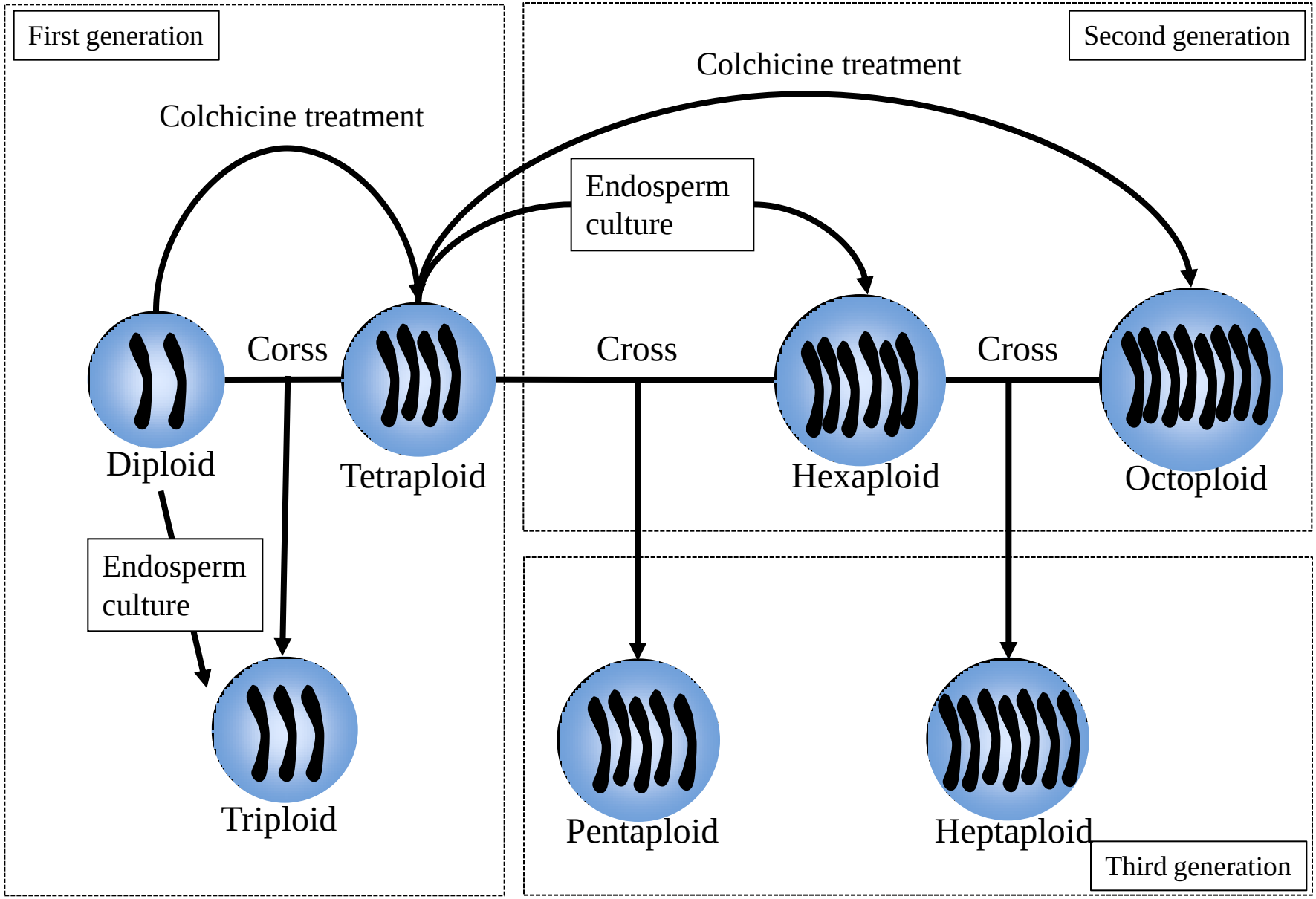
First generation
Second generation
Colchicine treatment
Colchicine treatment
Endosperm culture
Corss
Cross
Cross
Diploid
Tetraploid
Hexaploid
Octoploid
Endosperm culture
Triploid
Pentaploid
Heptaploid
Third generation

Figure 1