

Breeding Triploid Plants: A Review

XILING WANG^{1,2}, ZONG-MING (MAX) CHENG², SHUANG ZHI¹ and FENGXIANG XU¹

¹College of Biotechnology, Southwest University, Chongqing, P.R. China;

²Department of Plant Sciences, University of Tennessee, Knoxville, USA

Abstract

Wang X., Cheng Z.-M., Zhi S., Xu F. (2016): Breeding triploid plants: a review. Czech J. Genet. Plant Breed., 52: 41–54.

Triploid plants have larger organs, greater biomass, and strong stress resistance by preserving relatively larger amounts of photosynthetic energy. The undesirable spread of non-native invasive crop and horticultural plants into natural areas can also be reduced or eliminated by the use of triploids, because they tend to be sterile and seedless. Triploid plants have great economic value and have been useful for developing new agronomic, horticultural, and forestry plant varieties. Because of rapid advances in DNA sequencing technology, triploids may become a focus of genomic research in the future, and will create unprecedented opportunities for discovering and monitoring genomic and transcriptomic changes in unbalanced genomes. One of the new trends in genomics research is to create synthetic triploid plants as materials for the study of first genomic responses that occur immediately after triploid formation. Here, we summarize recent progress in the use of triploid plants, approaches for obtaining triploid plants, including natural selection, artificial hybridization, and endosperm regeneration, the obstacles to obtain triploids, and possible ways to overcome these difficulties. This summary of the scientific progress on triploid plants will promote understanding of how they can be generated and assist plant breeders to design new strategies for triploid breeding.

Keywords: embryo rescue; endosperm; genomics; hybridization

Polyploidy occurs in many taxa, is particularly widespread in flowering plants, and is a prominent feature of the chromosome evolution of higher plants. At least half of the known angiosperm species have experienced polyploidy in their evolutionary history (HIETER & GRIFFITHS 1999; SHAKED *et al.* 2001; XING *et al.* 2010). Compared to their diploid counterparts, polyploid organisms often express specific characteristics such as larger cell and body sizes (SUGIYAMA 2005). MILLER *et al.* (2012) reported that ploidy level affects many morphological and fitness traits, including stomatal size, flower size, and seed weight in *Arabidopsis thaliana* (L.) Heynh. Triploid plants have three sets of chromosomes, and many desirable characteristics, including greater vigor; broad, thick, dark green leaves; and larger flowers or fruit, which result in higher yield or higher harvest index. For example, the Vertigo watermelon variety ($2n = 3x = 33$) has produced the highest watermelon yields (41 000 lb/acre) (CUSHMAN *et al.* 2003). Triploid cassava also has a high yield with outstanding culinary

and industrial qualities (HOSHINO *et al.* 2011). Triploid plants produce seedless fruits in different species like citrus, banana and watermelon. Only in citrus, international markets demand fruits without seeds and this characteristic is one of the most important for citrus and with special emphasis in mandarins. Sterile triploid crop and horticultural plants can reduce or eliminate the undesirable spread of nonnative invasive crop plants that produce numerous seeds into natural areas (LI *et al.* 2004). Thus, triploid plants will play an even more important role in agriculture, forestry, and ecology in the future.

One of the new trends in genomic research is to create synthetic polyploid plants to provide materials for studying initial genomic responses immediately after polyploid formation. Thus triploid plants have attracted more attention and there has recently been great progress in understanding the details of their formation after decades of investigation. In this review, we will summarize applications of triploid plants,

ways to generate triploid plants, possible obstacles to generating triploids, and some solutions to these obstacles.

Formation of triploid plants

Errors occur sometimes during meiosis in regular diploid plants and chromosomes fail to segregate properly to the daughter cells. Such an unreduced $2n$ gamete can unite with a normal, haploid gamete, resulting in a triploid zygote that may develop into a triploid plant. Triploid cells have three complete sets of chromosomes, and are designated $3n$. When meiosis occurs, the probability of obtaining $2n$ and n gamete is only $(1/2)^{x-1}$ (SU *et al.* 2012). For chromosome numbers $x > 8$, this probability is reduced by 1% (SU *et al.* 2012). Hybridization between one parent with unreduced gametes ($2n$ gametes) and another diploid parent is the typical way to triploid formation. Both $2n$ megagametophyte and $2n$ microgametophyte occur in both wild and cultivated hybrid and non-hybrid species. There are four mechanisms by which triploids form (Figure 1) in addition to somatic fusion. The female parent with unreduced gametes plays a particularly important role in triploid plant formation (RAMSEY & SCHEMSKE 1998). Two sets of chromosomes in the triploid plant in Figure 1A, B and D are derived from the female parent. Further, the triploid embryo needs nutrition provided by

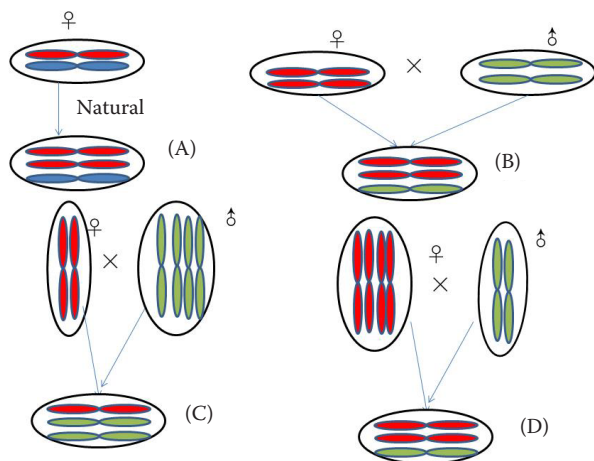


Figure 1. Triploid formation by hybridization: triploid endosperm recovered from a normal $2x \times 2x$ hybridization (A), sexual hybridization between diploid parents with one parent producing $2n$ gametes (female or male) (B), sexual hybridization between a diploid female parent and tetraploid male parent (C) and sexual hybridization between a tetraploid female parent and diploid male parent (D)

endosperm, which consists of two polar nuclei from female parent and sperm cell from the male parent. Successful hybridizations between different mating types showed that $2n$ female gamete is more efficient than the $2n$ pollen for the formation of triploid plants during hybridization. Embryo-endosperm balance number can determine the viability of seeds and the exit of cross direction (LU *et al.* 2013). These various triploid formation mechanisms result in different levels of offspring fertility and phenotypes.

Characteristics and application of triploid plants

Seedlessness of triploid plants and neutralizing the invasive plants

Prolonging flowering period. Although one of the chief characteristics of true triploids is partial or total sterility, this sterility can be horticulturally useful. Flowers of triploid plants are generally larger and more colorful than those of their diploid counterparts partly because the energy that is normally devoted to seed formation is used for flowers or other organs (MIYASHITA *et al.* 2009; TIKU *et al.* 2014). Triploid flowers often have longer shelf life and the triploid plants require little or no 'dead-heading' (the removal of faded or dead flowers from plants to maintain both a plant's appearance and to improve its overall flowering performance).

Neutralizing invasive plants. Weedy invasive plants have been a problem in the United States for years. The first comprehensive assessment of weedy invasive plants in the continental United States has found that nonnative plants are more widely distributed than are native plants (<http://news.sciencemag.org/biology/2015/01/invasive-plants-taking-over-us>). Gene flow mediated by pollen has also been demonstrated between commercial cultivars and weedy relatives (DENG *et al.* 2014). Thus, sterile triploid cultivars can be a vital strategy for reducing the invasiveness of crop plants. Many invasive plant species are considered noxious because they produce massive amounts of seeds, which can be dispersed by birds or other means and colonize surrounding areas of native flora (EVANS *et al.* 2005), resulting in major transformation of ecosystems such as forests, roadsides, parks, preserves, wildlife refuges, and urban areas. However, if this seed production can be blocked, these plants may behave well as crops or high-quality ornamentals without this invasive

doi: 10.17221/151/2015-CJGPB

tendency. One potential solution good for both the horticultural industry and for the environment is to create seedless versions of plants that have been shown to be, or that have potential to be invasive (LI *et al.* 2004). Thus, seedless triploid varieties can play an important role in neutralizing the invasiveness of introduced plants.

Triploid plants with larger organs and greater biomass

Triploid production increases the size of somatic cells and guard cells (JONES & REED 2007), and increases chloroplast number, which results in strengthening photosynthesis (PADOAN *et al.* 2013; TAPAN 2014). Therefore, many triploid plants are relatively more vigorous; have short internodes; broad, thick, dark green leaves, resulting in greater biomass or crop yield per plant. HOSHINO *et al.* (2011) found that triploids, including cassava (*Manihot esculenta* C.), watermelon, little gourd (*Coccinia grandis* (L.) J. Voigt), had higher yields and higher starch content. Today, over 80% of the watermelons produced in the US are seedless triploid (www.watermelon.org). The triploid seedless watermelon commands premium prices because of its high-quality flesh that is virtually free of seeds (ACTON 2013). The protein content of triploid mulberry leaves is 4.14% higher than that of diploid mulberry (YU *et al.* 2004). Therefore, the edibility and digestibility of triploid mulberry leaves is higher for silkworms. When fed triploid mulberry leaves, silkworms grow more rapidly, which reduces the length of their life cycle by about 2–3 days and increases whole cocoon weight, cocoon layer weight, and pupal weight over those fed with diploid leaves (YANG *et al.* 2000). Cocoon production is also increased by 14–16%, and fecundity improved by about 11%. As in these examples, the use of triploid plants can result in economic benefits in several kinds of farming systems (HOSHINO *et al.* 2011).

Use of triploid plants for studying genome evolution and genome plausibility

YANG *et al.* (2011) has reviewed the genomic aspects of research involving polyploid plants. Triploid plant species provide some unique characteristics for genome studies. After genome doubling, genomic characteristics at the individual or population level can be affected, including imbalances in gene dosage, genetic or epigenetic changes, genome size, genomic diversity and genomic rearrangements (SHAKED *et al.*

2001; EDGER & PIRES 2009). For example, some gene loss often occurs following polyploidization. TOWN *et al.* (2006) found that 35% of the genes inferred to be present when genome triplication occurred in the *Brassica* lineage have been lost in an interspersed pattern, most likely *via* a deletion mechanism. And they found genes encoding proteins involved in signal transduction or transcription were not preferentially retained in a triploidized *Brassica oleracea* genome. This result was not consistent with other studies which have indicated that dosage-sensitive genes involved in signal transduction and transcription may be preferentially retained after duplication. The DNA methylation status of newly formed species appears to be consistently affected following polyploidization. The genetically identical offspring of asexual triploid F₁ dandelion plants (a cross between a diploid sexual seed parent (TJX320) and a triploid apomict pollen donor (A681)) showed a modest level of methylation variation, indicating that *de novo* methylation was triggered by the formation of triploids (VERHOEVEN *et al.* 2010). Triploids, which carry three complete sets of chromosomes, in particular produce offspring with different chromosome numbers, including diploid and tetraploid progeny, as well as a swarm of aneuploid progeny. HENRY *et al.* (2009) reported that most of aneuploid gametes were viable and the chromosomal composition of swarms in *Arabidopsis thaliana* are strongly influenced by the dosage effect of the other gamete. There is thus strong selection against imbalance in gamete ploidy in crosses between triploids and diploids, even in the absence of aneuploidy (HENRY *et al.* 2009).

Ways to produce triploid plants

Triploid plants can also be produced by natural selection, sexual hybridization, endosperm culture *in vitro* and fusion of somatic diploid protoplasts with haploid microspore cells. There are few reports on protoplast fusion to produce triploid plants, so here we mainly talk about the other three ways to produce triploid plants.

Natural selection

Natural triploid poplar, especially *Populus tremula* L., is widely present in nature. In 1936, NILSSON discovered a natural triploid of *Populus tremula* L. in Sweden. It is named gigas form of *P. tremula* due to its huge leaves, rapid growth, and tall stature.

Compared with other trees of the same age, it has obvious advantages, therefore, it aroused the interest of scholars in various countries who carried out research on this variant. Subsequently, many triploid *P. tremula* L. variants were found in other countries (JOHNSON 1940; YABLOKOV 1941; SYLVEN 1943; SARVAS 1958; LI 2001). Triploid *P. alba* L. and *P. balsamifera* L. were also found by DILLEWIJN (1939) and GURREIRO (1944), respectively. These triploid poplars also had more desirable characteristics of volume growth, resistance, stem straightness, and fiber than did diploids of the same species. Triploid oak has also been found in a mixed stand of *Q. petraea* (Matt.) Liebl and *Q. robur* (Matt.) Liebl at a frequency of 0.48% (DZIALUK *et al.* 2007).

Unreduced gametes seem to occur more frequently when plants experience environmental stresses, such as frost, wounding, herbivory attack, and water or nutrient shortage (MASON *et al.* 2011). Noticeably, the frequency of unreduced gamete production occurs up to 50-fold more often in hybrids between divergent genomes than in non-hybrid systems (ZHANG *et al.* 2010). NISHIWAKI *et al.* (2011) found that new, naturally derived *Miscanthus* (*Poaceae*) triploid genotypes were identified more efficiently by flow-cytometry screening of seeds harvested from areas where tetraploid *M. sacchariflorus* F. plants grow sympatrically with diploid *M. sinensis* F. plants, than by random identification of triploids in the field. Triploid *Arachis pintoi* K. (*Leguminosae*) arose by autopolyploidy as evidenced by FISH and meiotic behaviour (LAVIA *et al.* 2011).

Artificial hybridization

Triploid plants can be recovered by $2x \times 2x$, $2x \times 4x$, $4x \times 2x$ or $2x \times 3x$ sexual hybridization. Most conventional methodology is from $2x \times 4x$ and $4x \times 2x$ hybridization (KESARA 2003; ALEZA *et al.* 2010, 2012; MORENO *et al.* 2010; ZHOU *et al.* 2012; JI *et al.* 2013). Since 1996, the Plant Protection and Biotechnology Center of the Instituto Valenciano de Investigaciones Agrarias (IVIA, Moncada, Spain) has developed an extensive citrus triploid breeding program based on interploid sexual hybridizations. A lot of citrus triploid hybrids have been successfully recovered from different $4x \times 2x$ and $2x \times 4x$ sexual hybridizations (ESEN & SOOST 1971; WILLIAMS & ROOSE 2004; VILORIA & GROSSER 2005; ALEZA *et al.* 2012). Most natural species and hybrids are diploid and spontaneous tetraploidy is extremely rare. So artificial

induction of tetraploid lines is necessary. Doubling of the diploid chromosome number may be achieved by the use of spindle inhibitors, mutation breeding (SMITH *et al.* 1993), protoplast fusion mediated by electricity or PEG (LIU *et al.* 2002, 2010; GUO *et al.* 2006; EECKHAUT *et al.* 2013). Mechanical damage such as top pinching over and over again can also achieve tetraploidy (TAO *et al.* 2007). Colchicine was one of the most commonly used spindle inhibitors and has been used to good effect in numerous plants either *ex vitro* or *in vitro* (VAINOLA 2000; BLAKESLEY *et al.* 2002; KADOTA & NIIMI 2002; SHAO *et al.* 2003). Oryzalin, and trifluralin were also used to disrupt spindle formation and preventing nuclear and cell division (ASCOUGH & VAN STADEN 2008; ALEZA *et al.* 2009). The effectiveness of these compounds depends strongly on the concentration applied, the duration of treatment, the type of explant, and the penetration of the compound (ALLUM *et al.* 2007). Colchicine has been used effectively in concentrations ranging from 0.25 mM (CHEN *et al.* 2006) to 38 mM (STANYS *et al.* 2006). Dimethyl sulfoxide (DMSO) can improve the permeability of drugs (TAO *et al.* 2007). But colchicine-induced tetraploids *ex vitro* were confronted with low mutation rate, high chimeric rate and reverse mutation, were difficult to select and vulnerable to environmental disturbance. Fusion of protoplasts together with colchicine-induction created homogenous tetraploid of *Citrus reticulata* Blanco (DUTT *et al.* 2010). But fusion of protoplasts is technically demanding (EECKHAUT *et al.* 2013).

Triploid production efficiency is determined by pollen viability, parents' compatibility and the frequency of unreduced gametes. The pollen germination rates were more dependent on genotypes than polyploidy (ALEZA *et al.* 2012). LURO *et al.* (2004), VILORIA and GROSSER (2005) and ALEZA *et al.* (2010) reported that the male parent and environmental conditions influence the production of citrus triploid plants. But ZHANG *et al.* (2001) thought that the female parent has a great influence on the success rate of hybridization. They found that $4x \times 2x$ crosses could produce 40.13% triploid seeds but $2x \times 4x$ crosses produce sterile seeds in False Pakchoi (*Brassica parachinensis* L.H.Bailey). YANG *et al.* (2000) found that both $4x \times 2x$ and $2x \times 4x$ crosses could generate a small number of triploid mulberry but the germination percentage of seeds from $4x \times 2x$ was small. ALEZA *et al.* (2012) reported 12/114 citrus seeds from $2x \times 4x$ were developed and 123/142 seeds from $4x \times 2x$ were developed. The efficiency

doi: 10.17221/151/2015-CJGPB

of triploid regenerated plants produced from $4x \times 2x$ was higher (114/116) than from $2x \times 4x$ (1/12) by *in vitro* embryo rescue. Environmental conditions, especially temperatures can affect pollen production, pollen size, pollen germination and pollen tube growth rate and the response to temperature during the reproductive phase is genotype-dependent (YOUNG & STANTON 1990; HEDHLY *et al.* 2005). ALEZA *et al.* (2010) demonstrated that the frequency of unreduced gametes is an intrinsic characteristic of each genotype. It suggested that the recovery efficiency in triploid plants was more dependent on the genotype and combination.

In $4x \times 2x$ or $4x \times 2x$ hybridisations, three seed types are obtained: undeveloped seeds, developed seeds (normal seeds) and developed small seeds. Only the developed normal seed can germinate. Seeds tend to abort due to endosperm degeneration during early embryogenesis (ESEN & SOOST 1973; SANFORD 1983). ESEN and SOOST (1973) proposed that the 3/4 ploidy ratio of embryo and endosperm caused the induction of seed abortion and endosperm degeneration. ALEZA *et al.* (2012) confirmed those triploid plants could be originated as a consequence of unreduced megagametophyte with haploid pollen grain with a most suitable endosperm/embryo ploidy ratio (3/5) or maternal/paternal contribution. So embryo rescue is an indispensable technique for triploid breeding programs that are based on interploid hybridizations (HIRAMATSU *et al.* 2003; GUO 2011; ALEZA *et al.* 2012). Embryo rescue was utilized by plant breeders to rescue inherently weak, immature and/or abortive embryos, breeding seedless crosses and triploid plants, and distant hybridization between different species (SHARMA 1995). Thus far, embryo rescue was extensively applied in rescuing many fruit crops, including apple (DANTAS *et al.* 2006), banana (UMA *et al.* 2011), citrus (VILORIA & GROSSER 2005; ALEZA *et al.* 2012), grape (SUN *et al.* 2011), etc. LI *et al.* (2015) provided an overview of the factors that affect its efficiency, including genotype, the time point of removing ovules, medium, culture method and condition, plant growth regulators, etc. According to the results of citrus embryo rescue, the undeveloped seeds were 49–75% smaller than normal seeds and the undeveloped seeds had either one (monoembryonic) or multiple embryos (polyembryonic) which is difficult to individualize or isolate. All normal seeds contained only one well-formed embryo. But the efficiency of triploid plants recovered from small seeds was higher than from normal seeds (ALEZA

et al. 2012). These results provided great help for breeding of triploid plants. In the future, maker-assisted selection technique together with embryo rescue technique will continuously play an important role in the efficient evaluation and selection of the triploid hybrids.

Endosperm culture in vitro

As endosperm is a triploid tissue, it would be reasonable to assume that natural triploids could be successfully regenerated plants from endosperm tissues. The first attempts at endosperm culture *in vitro* took place in the 1930s (LAMPE & MILLS 1936). Endosperm culture has now been attempted for triploid plant regeneration in nearly 64 species, but successful initiation of buds or shoots from endosperm explants has been reported in only 32 species, 30 of which are listed in Table 1. Triploid plantlets have been regenerated only from 15 of these species. Thus, regeneration from endosperm tissues is often technically challenging.

Genotype, sampling times, and culture media are important aspects of endosperm culture systems. First, the efficiency of endosperm response has been found to be genotype-dependent in many species (reviewed by POPIELARSKA-KONIECZNA *et al.* 2013).

Second, because either immature and mature endosperms have been used for successful endosperm culture (18 out of 30 species from mature endosperm and 14 out of 30 species have successfully used immature endosperm), it is not clear how critical endosperm developmental stage is for regeneration (Figure 2). But BAJAJ *et al.* (1980) found striking differences in the growth responses of immature or mature rice endosperm of various cultivars cultured on different media. The immature endosperm underwent two modes of differentiation, *i.e.*, direct regeneration of plants without callus phase, and indirect regeneration after the differentiation of callus. The mature endosperm, however, first proliferated to form callus, and the plants differentiated 4–6 weeks later. THAMMINA *et al.* (2011) compared the efficiency of regeneration from immature or mature endosperm in *Euonymus alatus*. They found that the mature endosperm formed callus at a lower rate than did immature endosperm tissues, but that after being transferred to bud induction medium, mature endosperm-derived calli initiated buds more easily. The stage of immature endosperm, usually calculated as days after pollination (DAP), varies from plant

Table 1. Response of endosperm culture in vitro on various basic culture media supplemented with different concentrations of plant growth regulators

Species	Status of endosperm	Basic media	The best combination of plant growth regulator	Regeneration	Reference
<i>Zea mays</i>	mature	White	YE	no calli	STRAUS and LA RUE (1954)
<i>Exocarpus cupressiformis</i>	mature	White	9.04 μM 2,4-D + 23.2 μM KT + 2.5 g/l CH	calli	JOHRI and BHOJWANI (1965)
<i>Dendrophthoe falcate</i>	mature	White	24.6 μM IBA 23.2 μM KT or 148 μM Ad	callus shoot 80%	JOHRI and NAG (1968)
<i>Taxillus vestillus</i>	mature	White	50 μM KT	shoot	JOHRI and NAG (1970)
<i>Ricinus communis</i>	mature	WB	9.04 μM 2,4-D + 23.2KT + 2.5 g/l YE	calli	SRIVASTAVA (1971)
<i>Croton bonplandianum</i>	mature	White	9.04 μM 2,4-D + 23.2 μM KT + 2.5 g/l YE 0.1 μM IBA	callus root	BHOJWANI and RAZDAN (1971)
<i>Lolium multiflorum</i>	mature	White	5.7 μM IAA + 117 mM Sucrose + 5 g/l YE	calli	SMITH and STONE (1973)
<i>Codiaeum varigatum</i>	mature	White	4.5 μM 2,4-D + 4.65 μM KT + 0.5 g/l CH + 10% CM 4.5 μM 2,4-D + 0.5 g/l CH + 10% CM	calli root; shoot	CHIKKANNAIAH and GAYATRI(1974)
<i>Oryza sativa</i>	immature	White	2,4-D 10^{-5}M + YE IAA + YE or YE + KT YE + 2,4-D	calli shoot root	NAKANO <i>et al.</i> (1975)
<i>Oryza sativa</i>	mature and immature	MS	9.04 μM 2,4-D 9.29 μM KT + 21.4 μM IAA No growth regulator	calli shoot root; plantlet	BAJAJ <i>et al.</i> (1980)
<i>Santalum album</i>	mature	MS White	4.5–9.0 μM 2,4-D or 2.22–8.88 μM BA + 5.37 μM NAA 1.33 μM BA + 5.34 μM IAA or 1.39 μM KT + 2.89 μM GA 2.67 μM IAA	calli shoot root; plantlet	LAKSHMI SITA <i>et al.</i> (1979)
<i>Hordeum vulgare</i>	immature	MS	4.5 μM 2,4-D + 0.5 g/l CH 2.32 μM KT + 1.07 μM NAA	calli shoot	SUN and ZHU (1981)
<i>Emblica officinale</i>	mature	MS	1 mg/l BAP + 1 mg/l IAA or 1mg/l 2,4-D + 1 mg/l KT 0.2 mg/l BAP + 0.1 mg/l IAA 0.002 mg/l NAA	calli shoot root	SEHGAL and KHURANA (1985)
<i>Annona squamosa</i> Linn	mature	White NT	0.46 μM KT + 0.89 μM BA + 5.37 μM NAA + 2.89 μM GA ₃ 2.69 μM NAA + 8.88 μM BA	callus shoot	NAIR <i>et al.</i> (1986)
<i>Actinidia</i>	mature	LS	13.7 μM zeatin + 0.54 μM NAA + 0.4 g/l CH 4.6 μM zeatin + 0.4 g/l CH + 3% sucrose 490 μM IBA	calli shoot root; plantlet	KIN <i>et al.</i> (1990)
<i>Citrus</i>	immature	MT 2 MT	22.2 μM BA + 9.04 μM KT + 23.2 μM 2,4-D + 1 g/l CH + 0.5 g/l ME 5.5 μM BA + 14.8 μM Ad + 5.77 μM GA ₃ + 0.5 g/l CH	calli shoot; plantlet	GMITTER <i>et al.</i> (1990)
<i>Lycopersicon esculentum</i> Mill	mature		0.44 μM BA + 4.5 μM 2,4-D + 28.9 μM GA ₃	callus	KAGAN-ZUR <i>et al.</i> (1990)
<i>Acacia nilotica</i>	immature	MS	25 μM BAP + 10 μM 2,4-D + 1 g/l CH	calli	GARG <i>et al.</i> (1996)

doi: 10.17221/151/2015-CJGPB

Table 1 to be continued

Species	Status of endosperm	Basic media	The best combination of plant growth regulator	Regeneration	Reference
<i>Passiflora foetida</i>	mature	MS	25 µM BA 5 µM NAA	shoot root	MOHAMED <i>et al.</i> (1996)
<i>Diospyros kaki</i>	immature	MS	10 µm zeatin + 10 µm IAA + 500 mg/l CH 10 µm zeatin + 0.1 µm IAA	calli shoot	TAO <i>et al.</i> (1997)
		1/2MS	1.5 mM IBA 5 µM 2,4-D	root calli	
<i>Morus alba</i>	immature	MS	1 µM TDZ or 5 µM BA + 15 µM NAA 1/2 MS + IBA	shoot plantlet	THOMAS <i>et al.</i> (2000)
<i>Azadirachta indica</i> A. Juss	immature	MS	2 µM BAP + 5 µM NAA + 0.5 g/l CH 5 µM BAP	callus shoot	CHATURVEDI <i>et al.</i> (2003)
		1/2 MS	2.46 µM IBA	root; plantlet	
<i>Actinidia deliciosa</i> cv. Hayward	mature	MS	23.2 µM KT + 9.04 µM 2,4-D 2.27 µM TDZ 2.7 µM NAA and 2.22 µM BA	calli shoot root	GORALSKI <i>et al.</i> (2005)
<i>Carthamus tinctorius</i>	immature	MS	4.44 µM BAP or 2.32 µM KT or 4.5 µM 2,4-D; 2.27 µM TDZ	calli; shoot	WALIA <i>et al.</i> (2007)
<i>Eucalyptus</i>	immature	MS	6.66 µM BA + 5.34 µM IAA 4.44 µM BA + 5.37 µM NAA	calli shoot	LI <i>et al.</i> (2008)
		1/2 MS	2.46 µM IBA	root; plantlet	
<i>Lonicera caerulea</i> var. <i>emphylocalyx</i>	immature	MS	4.44 µM BA + 4.92 µM IBA 0.44 µM BA and 2.89 µM GA ₃	calli shoot	MIYASHITA <i>et al.</i> (2009)
		1/2 MS	no regulator	root; plantlet	
<i>Euonymus alatus</i>	mature or immature	MS	2.22 µM BA + 2.7 µM NAA 4.4 µM BA + 0.5 µM IBA	calli shoot	THAMMINA <i>et al.</i> (2011)
		WPM	4.9 µM IBA	root; plantlet	
<i>Jatropha curcas</i>	mature	MS	1.82 µM TDZ + 9.04 µM 2,4-D + 1.44 µM GA ₃ + 1 g/l YE 6.8 µM ZT + 1.33 µM IAA	calli shoot	ZHU (2011)
		1/2 MS	0.49 µM IBA	root; plantlet	
<i>Carica papaya</i>	immature	MS	6.0 µM 2,4-D + 2.5 µM NAA + 4.0 µM KT 3.0 µM TDZ + 1.5 µM NAA or 1.5 µM BA + 3.0 µM IAA	calli shoot 93.8%	SUN <i>et al.</i> (2011)
		1/2MS	2.0 µM IBA	root 90%; plantlet	
<i>Sapium sebiferum</i>	mature	MS	4.44 µM BA + 5.37 µM NAA 8.88 µM BA + 1.07 µM NAA	calli 80% shoot 40–66.7%	TIAN <i>et al.</i> (2012)
			9.84 µM IBA + activated charcoal	root 80%; plantlet	
<i>Phlox drummondii</i>	immature	MS	5 µM BAP + 10 µM NAA 10 µM BAP + 2.5 µM IAA	calli shoot; plantlet	TIKU <i>et al.</i> (2014)

MS – MURASHIGE and SKOOG (1962); White – WHITE (1954); WB – modified White's semi-solid (agar 0.8%) medium containing 2% sucrose; NT – NITSCH and NITSCH (1969); MT – MURASHIGE and TUCKER (1969); WPM – woody plant medium; LS – LINSMAIER and SKOOG (1965); CH – casein hydrolysate; 2,4-D – 2,4-dichlorophenoxyacetic acid; GA₃ – gibberellic acid; IAA – indole acetic acid; IBA – indole butyric acid; 2iP – 2 isopentenyl adenine; NAA – naphthalene acetic acid; ZT – zeatin; BAP (BA) – benzylaminopurine; TDZ – thidiazuron; PAA – phenyl acetic acid; PEG – polyethylene glycol

to plant, as reviewed by THOMAS and CHATURVEDI (2008). MIYASHITA *et al.* (2009) investigated the histology of endosperm development and explained different stages of development in haskap (*Lonicera caerulea*). They confirmed the endosperm was at best stage for callus formation (88.9%) when developing embryos had reached the globular to early torpedo-stage.

Third, culture media composition can be the decisive factor that determines the success of triploid plant development. Most experiments on endosperm culture attempt to determine the most efficient media for the given species. The following three points can be concluded from Figure 1B: MS (MURASHIGE & SKOOG 1962), White (WHITE 1954), LS (LINSMAIER & SKOOG 1965) and MT (MURASHIGE & TUCKER 1969) were used as basic medium and (1) MS is the most commonly used basal medium. (2) Plant growth regulators are essential for regeneration from immature endosperm in most species. About 70% of the reports we have identified have found that 2,4-D is the most effective plant growth regulator to induce callus from endosperm. And BAP appears to be the most popular cytokinin in endosperm culture (Table 1). Successful callus induction without cytokinin has been reported, but there have been no reports of successful callus induction without auxin. The concentrations and ratios of cytokinins (BA, KT) and auxins (2,4-D, NAA) are key factors for differ-

ent genotypes or endosperm stage. THAMMINA *et al.* (2011) reported that callus was not induced with *Euonymus alatus* endosperm on hormone-free MS medium. SUN *et al.* (2011) also reported that all papaya (*Carica papaya*) explants turned brown and died without any sign of growth on basal medium. TIAN *et al.* (2012) reported that high callus formation rates (approx. 80%) were obtained on media containing both BA and NAA. BA is the most popular cytokinin and IAA and NAA are the most popular auxins used for shoot induction. Auxin is not indispensable, but cytokinin is almost always present in shoot induction media (Figure 1B). TIAN *et al.* (2012) also found that increasing BAP concentration from 4.44 to 22.2 μM did not significantly affect callus induction rates, but that higher concentrations were more effective for shoot induction. Stimulation of endosperm metabolism (increasing alpha-amylase activity and hydrolysis) by the addition of exogenous gibberellic acid (GA) has been reported by many workers. MOHAMED *et al.* (1996) found that a medium supplemented only with GA₃ and casein hydrolysate could increase the development of shoot primordia in *Passiflora foetida* L. from 2 shoots per explant on all other treatments to more than 20 shoots per explant. KIN *et al.* (1990) found that the presence of GA₃ in the medium was apparently not necessary and did not enhance callus production for *Actinidia*.

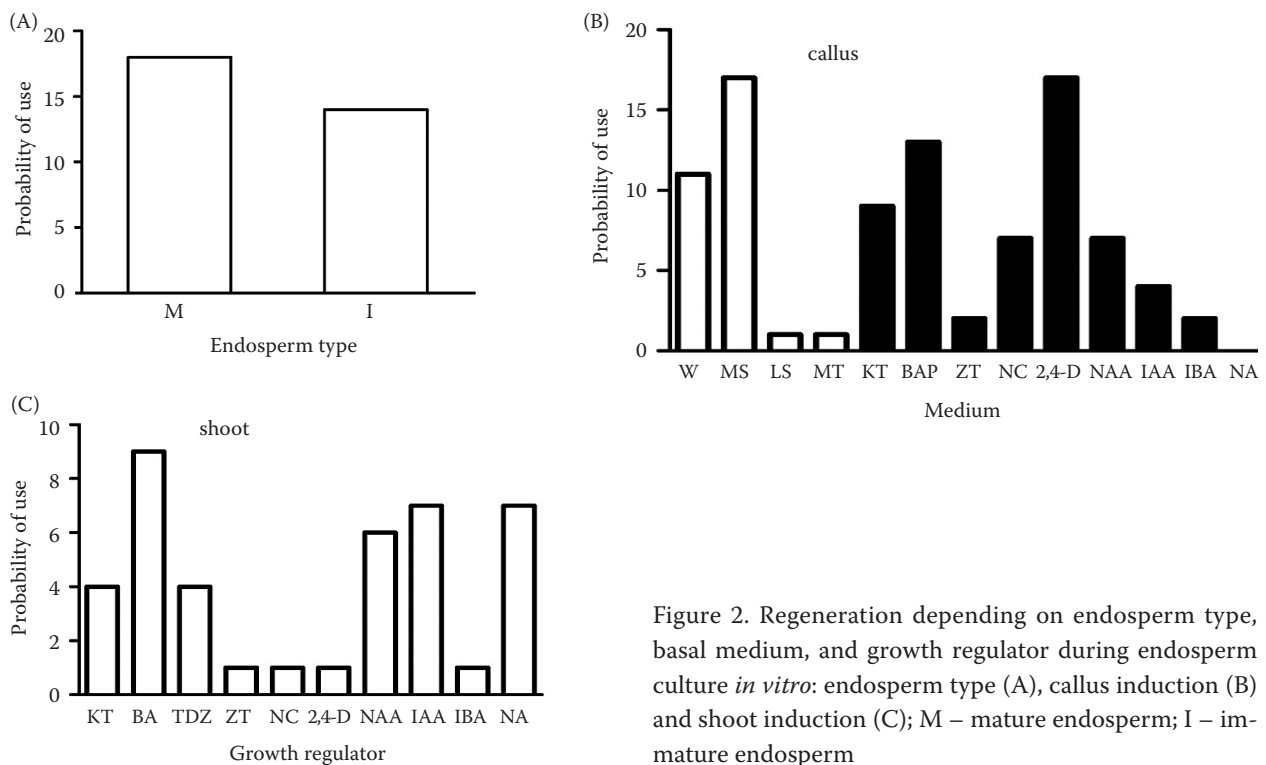


Figure 2. Regeneration depending on endosperm type, basal medium, and growth regulator during endosperm culture *in vitro*: endosperm type (A), callus induction (B) and shoot induction (C); M – mature endosperm; I – immature endosperm

doi: 10.17221/151/2015-CJGPB

Many obstacles can decrease the probability of obtaining viable triploid plants in endosperm culture. But despite its challenges, endosperm culture is still a common method for producing triploid plants, but a limited number of species are amenable to endosperm culture.

Albinism. Albinism often occurs in progeny of interspecific crosses and in endosperm culture regenerants. Albino seedlings typically do not survive because they lack chlorophyll pigments and chloroplast membranes (KITA *et al.* 2005; EECKHAUT *et al.* 2007; KUMARI *et al.* 2009). KUMARI *et al.* (2009) suggested that the important post-zygotic barrier to successful *in vitro* embryo or ovule cultures could be due to any one or a combination of factors including genotype, environment, meiotic abnormalities, hormonal imbalance, nuclear-plastid genome incompatibility, deletions in plastid DNA, mutations in genes responsible for chlorophyll biogenesis, or a metabolic block in pathways leading to chlorophyll biosynthesis. Genetic factors are one of the most important causes of albinism in plants. Also, the degree of genetic relatedness between parents of a cross can also affect the chances of recovering albino seedlings (SHARMA 1995).

Mixoploid chimeras. Mixoploid chimeras are one of the common outcomes of interploidy crosses or endosperm culture. The majority of cells in endosperm culture have several ploidy levels, and aneuploidy is frequently seen (TIKU *et al.* 2014). For example, plants generated from apple ($2n = 2x = 34$) endosperm had chromosome numbers ranging from 29 to 56, most of which ranged from 37 to 56, while only 2% to 3% of cells were true triploids (WU 1978). Ploidy levels, however, have been found to be relatively stable in some endosperm-generated plantlets and these kinds of endosperm cells often can maintain a long-term ability to differentiate organs. SUN *et al.* (2011) reported that over 75% of endosperm-derived papaya plants were triploid with chromosome number $2n = 3x = 27$. TIKU *et al.* (2014) analyzed the chromosome number of 80 *Phlox drummondii* plants derived from endosperm cultures. Of these plants, 70% were triploids ($2n = 3x = 21$) and 5% were diploids ($2n = 2x = 14$), whereas the remaining 25% were aneuploids. HUANG *et al.* (1982) reported that kiwi fruit could generate triploid and diploid plants from endosperm from the same plant. When 0.5 mg/l NAA was added to the medium, the plantlets were diploid ($2n = 2x = 58$); whereas when 1 mg/l 2,4-D was substituted in the medium, the plantlets were triploid ($2n = 3x = 87$). The authors suggested that

the internal structure of the endosperm together with appropriate hormone components in the culture medium could affect the proliferation and differentiation of certain cell types, and affect the likelihood of recovering plantlets with particular ploidy levels.

Conclusions and perspectives

Triploid plants are rare in nature because of their inviable seeds and resulting lack of progeny, so it is challenging to detect naturally occurring triploid plants. However, due to their faster growth and seedlessness, they will be useful for improving biomass, fruit and flower traits, and other qualities of economically important food, medicinal, bioenergy, and ornamental plants, reducing or eliminating the invasiveness of many crop and horticultural plants. So scientists have intentionally bred triploids through traditional and modern technologies. Natural selection, interploid sexual hybridization, endosperm culture, protoplast fusion were used for production of triploids. A lot of plant species produced triploid plants and popular application. There are more talks about interploid sexual hybridization and endosperm culture. As endosperm is a triploid tissue, it is thought that endosperm culture is the most direct and efficient method for production of triploid plants. Although endosperm culture is not yet entirely routine, many successful protocols have been developed over the last 15 years. In suitable media, 82% shoot and 80% root regeneration can be achieved from endosperm cultures of *Phlox drummondii* (TIKU *et al.* 2014). This paper reviewed effect of many factors on the endosperm culture, which may help further study. Protoplast fusion technology has been utilized in many crops to generate allotetraploid somatic hybrids and sometimes triploids can be produced (FU *et al.* 2003; CAI *et al.* 2009; GROSSER & GMITTER 2011). It is important to combine the traditional methods with modern methods to promote development of breeding triploid. In the future, marker-assistant selection technique, which has already been used in grape breeding (AKKURT *et al.* 2012), together with embryo rescue technique will continuously play an important role in the breeding triploid plants.

Other new strategies might be developed to induce triploid plants. With the rapid development of genomics research and advanced biology technologies, perhaps new methods to induce formation of triploids and new avenues of research into and using triploid plants will become possible.

Acknowledgments. This research is supported by the Tennessee Agricultural Experiment Station to Z.-M. CHENG and supported by Fundamental Research Funds for the Central Universities (XDJK2014C065)

References

- Acton Q.A. (ed) (2013): Researchers submit patent application “Inbred tetraploid watermelon line 4xasss4” for approval. In: Cell Nucleus Structures – Advances in Research and Application, Atlanta, Scholarly Editions: 150–151.
- Akkurt M., Çakir A., Shidfar M., Çelikkol B.P., Söylemezoğlu G. (2012): Using SCC8, SCF27 and VMC7f2 markers in grapevine breeding for seedlessness via marker assisted selection. *Genetics and Molecular Research*, 11: 2288–2294.
- Aleza P., Juárez J., Ollitrault P., Navarro L. (2009): Production of tetraploid plants of non apomictic citrus genotypes. *Plant Cell Reports*, 28: 1837–1846.
- Aleza P., Juárez J., Cuenca J., Ollitrault P. (2010): Recovery of citrus triploid hybrids by embryo rescue and flow cytometry from $2x \times 2x$ sexual hybridizations and its application to extensive breeding programs. *Plant Cell Reports*, 29: 1023–1034.
- Aleza P., Juárez J., Cuenca J., Ollitrault P., Navarro L. (2012): Extensive citrus triploid hybrid production by $2x \times 4x$ sexual hybridizations and parent-effect on the length of the juvenile phase. *Plant Cell Reports*, 31: 1723–1735.
- Allum J.F., Bringloe D.H., Roberts A.V. (2007): Chromosome doubling in a *Rosa rugosa* Thunb. hybrid by exposure of *in vitro* nodes to oryzalin: The effects of node length, oryzalin concentration and exposure time. *Plant Cell Reports*, 26: 1977–1984.
- Ascough G.D., van Staden J. (2008): Effectiveness of colchicine and oryzalin at inducing polyploidy in *Watsonia lepida* N.E. Brown. *HortScience*, 43: 2248–2251.
- Bajaj Y.P.S., Saini S.S., Bidani M. (1980): Production of triploid plants from the immature and mature endosperm cultures of rice. *Theoretical and Applied Genetics*, 58: 17–18.
- Bhojwani S.S., Razdan M.K. (1971): *Plant Tissue Culture: Theory and Practice. A Revised Edition.* Amsterdam, Elsevier.
- Blakesley D., Allen A., Pellny T.K., Roberts A.V. (2002): Natural and induced polyploidy in *Acacia dealbata* Link. and *Acacia mangium* Willd. *Annals of Botany*, 90: 391–398.
- Cai X.D., Fu J., Chen C.L., Guo W.W. (2009): Cybrid/hybrid plants regenerated from somatic fusions between male sterile Satsuma mandarin and seedy tangelos. *Scientia Horticulturae*, 122: 323–327.
- Chaturvedi R., Razdan M.K., Bhojwani S.S. (2003): An efficient protocol for the production of triploid plants from endosperm callus of neem, *Azadirachta indica* A. Juss. *Journal of Plant Physiology*, 160: 557–564.
- Chen L.P., Wang Y.J., Zhao M. (2006): *In vitro* induction and characterization of tetraploid *Lychnis senno* Siebold et Zucc. *HortScience*, 41: 759–761.
- Chikkannaiah P.S., Gayatri M.C. (1974): Organogenesis in endosperm tissue culture of *Codiaeum variegatum* Blume. *Current Science*, 43: 23–24.
- Cushman K.E., Horgan T.E., Snyder R.G., Hudson P.M., Coker C.H., Ely M. (2003): Evaluation of elongated and oval triploid (seedless) watermelon genotypes. Annual Report 2002 of the North Mississippi Research & Extension Center. Mississippi Agriculture & Forestry Experiment Station Information Bulletin, 398: 339–345.
- Dantas A.C.M., Boneti J.I., Nodari R.O., Guerra M.P. (2006): Embryo rescue from interspecific crosses in apple rootstocks. *Pesquisa Agropecuária Brasileira*, 41: 969–973.
- Deng J., Duan H., Deng Z.N., Zhao D.G., Yi G.J., McAvoy R., Li Y. (2014): Molecular strategies for addressing gene flow problems and their potential applications in abiotic stress tolerant transgenic plants. *Critical Review in Plant Sciences*, 33: 190–204.
- Dillewijn C. (1939): Cytology and breeding of *Populus*. *Nederland Boschbouw Tijdschr*, 12: 470–481.
- Dutt M., Vasconcellos M., Song K.J., Gmitter F.G., Grosser J.W. (2010): *In vitro* production of autotetraploid Ponkan mandarin (*Citrus reticulata* Blanco) using cell suspension cultures. *Euphytica*, 173: 235–242.
- Dzialuk A., Chybicki I., Welc M., Sliwiska E., Burczyk J. (2007): Presence of triploids among oak species. *Annals of Botany*, 99: 959–964.
- Edger P.P., Pires J.C. (2009): Gene and genome duplications: the impact of dosage-sensitivity on the fate of nuclear genes. *Chromosome Research*, 17: 699–717.
- Eeckhaut T., De Keyser E., Van Huylenbroeck J., De Riek J., Van Bockstaele K. (2007): Application of embryo rescue after interspecific crosses in the genus *Rhododendron*. *Plant Cell, Tissue and Organ Culture*, 89: 29–35.
- Eeckhaut T., Lakshmanan P.S., Deryckere D., Van Bockstaele E., Van Huylenbroeck J. (2013): Progress in plant protoplast research. *Planta*, 238: 991–1003.
- Esen A., Soost R.K. (1971): Unexpected triploids in citrus: their origin, identification and possible use. *Journal of Heredity*, 62: 329–333.
- Esen A., Soost R.K. (1973): Seed development in Citrus with special reference to $2x \times 4x$ crosses. *American Journal of Botany*, 60: 448–462.
- Evans C.W., Barger C.T., Moorhead D.J., Douce G.K. (2005): *Invasive weeds in Georgia.* Athens, University of Georgia: 1–2.
- Fu C.H., Guo W.W., Liu J.H., Deng X.X. (2003): Regeneration of *Citrus sinensis* (+) *Clausena lansium* intergeneric triploid and tetraploid somatic hybrids and their identification by molecular markers. *In Vitro Cellular & Developmental Biology – Plant*, 39: 360–364.

doi: 10.17221/151/2015-CJGPB

- Garg L., Bhandari N.N., Rani V., Bhojwani S.S. (1996): Somatic embryogenesis and regeneration of triploid plants in endosperm cultures of *Acacia nilotica*. *Plant Cell Reports*, 15: 855–858.
- Gmitter E.G.J., Ling X.B., Deng X.X. (1990): Induction of triploid *Citrus* plants from endosperm calli *in vitro*. *Theoretical and Applied Genetics*, 80: 785–790.
- Goralski G., Popielarska M., Slesak H., Siwinska D., Batycka M. (2005): Organogenesis endosperm of *Actinidia deliciosa* cv. 'Hayward' cultured *in vitro*. *Acta Biologica Cracoviensia, Series Botanica*, 47: 121–128.
- Grosser J.W., Gmitter F.G.J. (2011): Protoplast fusion for production of tetraploids and triploids: applications for scion and rootstock breeding in citrus. *Plant Cell, Tissue and Organ Culture*, 104: 343–357.
- Guo Y., Zhao Y., Li K., Liu Z., Lin H., Guo X., Li C. (2011): Embryo rescue of crosses between diploid and tetraploid grape cultivars and production of triploid plants. *Africal Journal of Biotechnology*, 10: 19005–19010.
- Guo W.W., Cheng Y.J., Chen C.L., Deng X.X. (2006): Molecular analysis revealed autotetraploid, diploid and tetraploid cybrid plants regenerated from an interspecific somatic fusion in Citrus. *Scientia Horticulturae*, 108: 162–166.
- Gurreiro M. (1944): The silvicultural improvement of *Populus*. *Publ Serv Flor Aquic Portugal*, 11: 53–117.
- Hedhly A., Hormaza J.L., Herrero M. (2005a): Influence of genotype-temperature interaction on pollen performance. *Journal of Evolutionary Biology*, 18: 1494–1502.
- Henry I.M., Dilkes B.P., Tyagi A.P., Lin H.Y., Comai L. (2009): Dosage and parent-of-origin effects shaping aneuploid swarms in *Arabidopsis thaliana*. *Heredity*, 103: 458–468.
- Hieter P., Griffiths T. (1999): Polyploidy – More is more or less. *Science*, 285: 210–211.
- Hiramatsu M., Wakana A., Park S.M., Fukudome I. (2003): Production of triploid plants from crosses between diploid and tetraploid grapes (*Vitis* complex) through immature seed culture and subsequent embryo culture. *Journal of Faculty Agriculture Kyushu University*, 48: 51–57.
- Hoshino Y., Miyashita T., Thomas T.D. (2011): *In vitro* culture of endosperm and its application in plant breeding: approaches to polyploidy breeding. *Scientia Horticulturae*, 130: 1–8.
- Huang Z.X., Huanpu Y.L., Xu L.Y. (1982): Production of triploid plants from endosperm cultures of *Actinidia chinensis* Planchon. *Chinese Science Bulletin*, 27: 247–250.
- Ji W., Li Z.Q., Zhou Q., Yao W.K., Wang Y.J. (2013): Breeding new seedless grape by means of *in vitro* embryo rescue. *Genetics and Molecular Research*, 12: 859–869.
- Johnsson H. (1940): Cytological studies of diploid and triploid *Populus tremula* and crosses between them. *Hereditas Lund*, 26: 321–352.
- Johri B.M., Bhojwani S.S. (1965): Growth responses of mature endosperm in culture. *Nature*, 208: 1345–1347.
- Johri B.M., Nag K.K. (1968): Experimental induction of triploid shoot *in vitro* from endosperm of *Dendrophthoe falcata* Ettings. *Current Science*, 21: 606–607.
- Johri B.M., Nag K.K. (1970): Endosperm of *Taxillus vestitus* (Wall.). A system to study the effect of cytokinins *in vitro* in shoot bud formation. *Current Science*, 39: 177–179.
- Jones K.D., Reed M. (2007): Analysis of ploidy level and its effects on guard cell length, pollen diameter, and fertility in hydrangea macrophylla. *HortScience*, 42: 483–488.
- Kadota M., Niimi Y. (2002): *In vitro* induction of tetraploid plants from a diploid Japanese pear cultivar (*Pyrus pyrifolia* N.cv. Hosui). *Plant Cell Reports*, 21: 282–286.
- Kagan-Zur V., Mills D., Mizrahi Y. (1990): Callus formation from tomato endosperm. *Acta Horticulturae*, 280: 139–143.
- Kesara A.J. (2003): Preparation of chromosomes from plant leaf meristems for karyotype analysis and *in situ* hybridization. *Methods in Cell Science*, 25: 91–95.
- Kin M.S., Fraser L.G., Harvey C.F. (1990): Initiation of callus and regeneration of plantlets from endosperm of *Actinidia* interspecific hybrids. *Scientia Horticulturae*, 44: 107–117.
- Kita K., Kurashige Y., Yukawa T., Nishimura S., Handa T. (2005): Plastid inheritance and plastome-genome incompatibility of intergeneric hybrids between *Menziesia* and *Rhododendron*. *Journal of the Japanese Society for Horticultural Science*, 74: 318–323.
- Kumari M., Heather J.C., Ian S., Kadambot H.M.S. (2009): Albinism in plants: A major bottleneck in wide hybridization, androgenesis and doubled haploid culture. *Critical Review Plant Science*, 28: 393–409.
- Lakshmi Sita G., Raghava Ram N.V., Vaidyanathan C.S. (1979): Differentiation of embryos and plantlets from shoot callus of sandalwood. *Plant Science Letters*, 14: 265–270.
- Lampe L., Mills C.O. (1936): Growth and development of isolated endosperm and embryo of maize. *Bulletin Torrey Botany Club*, 63: 365–382.
- Lavia G.I., Ortiz A.M., Robledo G. (2011): Origin of triploid *Arachis pintoii* (Leguminosae) by autopolyploidy evidenced by FISH and meiotic behavior. *Annals of Botany*, 108: 103–111.
- Li J., Wang X.H., Wang X.P., Wang Y.J. (2015): Embryo rescue technique and its applications for seedless breeding in grape. *Plant Cell, Tissue and Organ Culture*, 120: 861–880.
- Li S.L., Zhuang N.S., Wang Y., Lin F., Qiu H.Y. (2008): Callus induction and plant regeneration from endosperm culture of *Eucalyptus* 12ABL. *Journal of Northwest Forest University*, 23: 101–104.
- Li Y. (2001): Advances in studies of triploid poplar breeding. *Chinese Bulletin Botany*, 18: 451–458.
- Li Y., Cheng Z.M., Smith W.A. (2004): Invasive ornamental plants: problems, challenges, and molecular tools to neu-

- tralize their invasiveness. *Critical Review Plant Science*, 23: 381–389.
- Linsmaier E.M., Skoog F. (1965): Organic growth factor requirements of tobacco tissue cultures. *Physiology of Plant*, 18: 100–127.
- Liu J.F., Liu J.H., Cheng Y.Q., Zhong X., Chen Z.W. (2010): Acquiring homozygous tetraploid germplasm by PEG-mediated protoplast fusion of *Rhodiola sachalinensis*. *China Journal of Chinese Material Medica*, 35: 1781–1786.
- Liu J.H., Hu C.G., Deng X.X. (2002): Production of citrus intergeneric tetraploid somatic hybrids plants from electricity-mediated protoplast fusion. *Acta Horticulturae Sinica*, 29: 372–374.
- Lu M., Zhang P.D., Kang X.Y. (2013): Induction of $2n$ female gametes in *Populus adenopoda* Maxim. by high temperature exposure during female gametophyte development. *Breeding Science*, 63: 96–103.
- Luro F., Maddy F., Jacquemond C., Froelicher Y., Morillon R., Rist D., Ollitrault P. (2004): Identification and evaluation of diplogyny in clementine (*Citrus clementina*) for use in breeding. In: 11th Eucarpia Symposium on Fruit Breeding and Genetics. *Acta Horticulturae*, 663: 841–847.
- Mason A., Nelson M., Yan G., Cowling W. (2011): Production of viable male unreduced gametes in *Brassica* interspecific hybrids is genotype specific and stimulated by cold temperatures. *BMC Plant Biology*, 11: 103.
- Miller M., Zhang C.Q., Chen Z.J. (2012): Ploidy and hybridity effects on growth vigor and gene expression in *Arabidopsis thaliana* hybrids and their parents. *Genetics*, 2: 505–514.
- Miyashita T., Ohashi T., Shibata F., Araki H., Hoshino Y. (2009): Plant regeneration with maintenance of the endosperm ploidy level by endosperm culture in *Lonicera caerulea* var. *emphylocalyx*. *Plant Cell, Tissue and Organ Culture*, 98: 291–301.
- Mohamed M.E., Hicks R.G.T., Blakesley D. (1996): Shoot regeneration from mature endosperm of *Passiflora foetida*. *Plant Cell, Tissue and Organ Culture*, 46: 161–164.
- Moreno R., Espejo J.A., Gi J. (2010): Development of triploid hybrids in asparagus breeding employing a tetraploid landrace. *Euphytica*, 173: 369–375.
- Murashige T., Skoog F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiology of Plant*, 15: 473–497.
- Murashige T., Tucker D.P.H. (1969): Growth factor requirements of citrus tissue culture. In: Chapman H.D. (ed.): *Proc. 1st Int. Citrus Symposium*, Riverside, Mar 16–26, 1968, Vol. 3: 1155–1161.
- Nair S., Shirgurkar M.V., Mascarenhas A.F. (1986): Studies on endosperm culture of *Annona squamosa* Linn. *Plant Cell Reports*, 5: 132–135.
- Nakano H., Tashiro T., Maeda E. (1975): Plant differentiation in callus tissue, induced from immature endosperm of *Oryza sativa* L. *Zeitschrift für Pflanzenphysiologie*, 76: 444–449.
- Nilsson E.H. (1936): Über eine in der Natur gefundene Gigasform von *Populus tremula*. *Hereditas*, 21: 379–393.
- Nishiwaki A., Mizuguti A., Kuwabara S., Toma Y., Ishigaki G., Miyashita T., Yamada T., Matuura H., Yamaguchi S., Rayburn A.L., Akashi R., Stewart J.R. (2011): Discovery of natural *Miscanthus* (Poaceae) triploid plants in sympatric population of *Miscanthus sacchariflorus* and *Miscanthus sinensis* in southern Japan. *American Journal of Botany*, 98: 154–159.
- Nitsch J.P., Nitsch C.S. (1969): Haploid plants from pollen grains. *Science*, 163: 85–87.
- Padoan D., Mossad A., Chiancone B., Germana M.A., Khan P.S.S.V. (2013): Ploidy levels in Citrus clementine affects leaf morphology, stomatal density and water content. *Theoretical and Experimental Plant Physiology*, 25: 283–290.
- Popielarska-Konieczna M., Koziaradzka-Kiszkurno M., Tuleja M., Ślesak H., Paweł K., Marcińska I., Bohdanowicz J. (2013): Genotype-dependent efficiency of endosperm development in culture of selected cereals: histological and ultrastructural studies. *Protoplasma*, 250: 361–369.
- Ramsey J., Schemske D.W. (1998): Pathways, mechanisms, and rates of polyploid formation in flowering plants. *Annual Review of Ecology Systematics*, 29: 467–501.
- Sanford J.C. (1983): *Methods in Fruit Breeding*. West Lafayette, Purdue University Press: 100–123.
- Sarvas R. (1958): Two triploid aspen and two triploid birches. *Communicationes Instituti Forestalis Fenniae*, 49: 25.
- Sehgal C.B., Khurana S. (1985): Morphogenesis and plant regeneration from cultured endosperm of *Embllica officinalis* Gaertn. *Plant Cell Reports*, 4: 263–266.
- Shaked H., Kashkush K., Ozkan H., Feldman M., Levy A.A. (2001): Sequence elimination and cytosine methylation are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. *The Plant Cell*, 13: 1749–1759.
- Shao J., Chen C., Deng X. (2003): In vitro induction of tetraploid in pomegranate (*Punica granatum*). *Plant Cell, Tissue and Organ Culture*, 75: 241–246.
- Sharma H. (1995): How wide can a wide cross be? *Euphytica*, 82: 43–64.
- Smith M.M., Stone B.A. (1973): Study on *Lolium multiflorum* endosperm in tissue culture. *Australian Journal of Biology Science*, 26: 123–133.
- Smith M.K., Hamill S.D., Langdon P.W. (1993): *Mutation Breeding*. Newsletter, 40: 4–5.
- Srivastava P.S. (1971): In vitro growth requirements of mature endosperm of *Ricinus communis*. *Current Science*, 13: 337–339.

doi: 10.17221/151/2015-CJGPB

- Stanys V., Weckman A., Staniene G., Duchovskis P. (2006): *In vitro* induction of polyploidy in Japanese quince (*Chaenomeles japonica*). Plant Cell, Tissue and Organ Culture, 84: 263–268.
- Straus J., La Rue C.D. (1954): Maize endosperm tissue grown *in vitro* I. Culture requirements. American Journal of Botany, 41: 687–694.
- Su J., Wang X.D., Wang J., Guo Q.G. (2012): Research progress of triploid plant breeding. South China Agriculture, 6: 78–80.
- Sugiyama S.I. (2005): Polyploidy and cellular mechanisms changing leaf size: Comparison of diploid and autotetraploid populations in two species of *Lolium*. Annals of Botany, 96: 931–938.
- Sun D.Q., Lu X.H., Liang G.L., Guo Q.G., Mo Y.W., Xie J.H. (2011): Production of triploid plants of papaya by endosperm culture. Plant Cell, Tissue and Organ Culture, 104: 23–29.
- Sun J.S., Zhu Z.Q. (1981): The induction of endosperm plantlets and their ploidy of barley *in vitro*. Acta Botanica Sinica, 23: 262–267.
- Sylvén N. (1943): Annual report on the work of the Association for Forest Tree Breeding during the year. Svensk Papptidn, 47: 38.
- Tao D.-H., Liu M.-Y., Xiao J.-Z., Deng J.-P. (2007): Advances in the research on induction means of biopolyploid. Life Science Research, 11: 6–13. (in Chinese)
- Tao R., Ozawa K., Tamura M., Sugiura A. (1997): Dodecaploid plant regeneration from endosperm culture of persimmon (*Diospyros kaki* L.). Acta Horticulturae, 436: 119–128.
- Tapan K.M. (2014): Polyploid Breeding. Breeding and Biotechnology of Tea and its Wild Species. Delhi, Springer India: 9–34.
- Thammina C., He M.Y., Lu L.T., Cao K.S., Yu H. (2011): *In vitro* regeneration of triploid plants of *Euonymus alatus* ‘Compactus’ (Burning Bush) from endosperm tissues. Hortscience, 46: 1141–1147.
- Thomas T.D., Chaturvedi R. (2008): Endosperm culture: a novel method for triploid plant production. Plant Cell, Tissue and Organ Culture, 93: 1–14.
- Thomas T.D., Bhatnagar A.K., Bhojwani S.S. (2000): Production of triploid plants of mulberry (*Morus alba* L.) by endosperm culture. Plant Cell Reports, 19: 395–399.
- Tian L.T., Ke Y.N., Gan S.R., Chen Y.Q., Chen Y., Yang Z.F., Wang X.G. (2012): Triploid plant regeneration from mature endosperms of *Sapium sebiferum*. Plant Growth Regulator, 68: 319–324.
- Tiku A.R., Razdan M.K., Raina S.N. (2014): Production of triploid plants from endosperm cultures of *Phlox drummondii*. Biology of Plant, 58: 153–158.
- Town C.D., Cheung F., Maiti R., Crabtree J., Haas B.J., Wortman J.R., Hine E.E., Althoff R., Arbogast T.S., Tallon L.J., Vigouroux M., Trick M., and Bancroft I. (2006): Comparative genomics of *Brassica oleracea* and *Arabidopsis thaliana* reveal gene loss, fragmentation, and dispersal after polyploidy. Plant Cell, 18: 1348–1359.
- Uma S., Lakshmi S., Saraswathi M.S., Akbar A., Mustaffa M.M. (2011): Embryo rescue and plant regeneration in banana (*Musa* spp.). Plant Cell, Tissue and Organ Culture, 105: 105–111.
- Vainola A. (2000): Polyploidization and early screening of *Rhododendron* hybrids. Euphytica, 112: 239–244.
- Verhoeven K.J.F., Van Dijk P.J., Biere A. (2010): Changes in genomic methylation patterns during the formation of triploid asexual dandelion lineages. Molecular Ecology, 19: 315–324.
- Viloria Z., Grosser J.W. (2005): Acid citrus fruit improvement via interploid hybridization using allotetraploid somatic hybrid and autotetraploid breeding parents. Journal of American Society Horticulture Science, 130: 392–402.
- Walia N., Kaur A., Babbar S.B. (2007): Proliferation and differentiation from endosperms of *Carthamus tinctorius*. Biologia Plantarum, 51: 749–753.
- White P.R. (1954): The Cultivation of Animal and Plant Cells. New York, Ronald Press.
- Williams T.E., Roose M.L. (2004): ‘TDE2’ Mandarin hybrid (Shasta Gold Mandarin), ‘TDE3’ Mandarin hybrid (Tahoe Gold Mandarin) and ‘TDE4’ Mandarin hybrid (Yosemite Gold Mandarin): three new mid and late-season triploid seedless Mandarin hybrids from California. In: Proc. 10th Int. Society of Citriculture, Agadir, Feb 2004: 394–398.
- Wu X.J., Liu S.Q., Zhou Y.K., Qian N.F., Zhang P., Xie H.Q., Zhang F.S., Yan Z.L. (1978): Induction of triploid apple plants from endosperm calli *in vitro*. Chinese Science, 355–360.
- Xing S.C., Cai Y.H., Zhou K.D. (2010): A new approach for obtaining rapid uniformity in rice (*Oryza sativa* L.) via a $3x \times 2x$ cross. Genetics and Molecular Biology, 33: 325–327.
- Yablokov A.S. (1941): A giant form of *Populus tremula* in the forests of U.S.S.R. Transactions of the All-Union Forest Research Institute, No. 23: 52.
- Yang X.H., Yang J.H., Luo C.J. (2000): Review and prospect of mulberry polyploidy breeding. Agriculture Science of Zhejiang, 6: 304–306.
- Yang X.H., Ye C.Y., Cheng Z.M., Timothy J.T., Stan D.W., Yin W.L., Xia X.L., Gerald A.T. (2011): Genomic aspects of research involving polyploid plants. Plant Cell, Tissue and Organ Culture, 104: 387–397.
- Young H.J., Stanton M.L. (1990): Influence of environmental quality on pollen competitive ability in wild radish. Science, 248: 1631–1633.

- Yu M.D., Jing C.J., Wu C.R., Lu C. (2004): Breeding of new artificial triploid mulberry variety Jialing No. 20. *Science of Sericulture*, 3: 225–229.
- Zhang C.H., Zhang S.L., Shen S.X., Wang M., Wang Y.H. (2001): Observation on obtaining the triploid by $4x \times 2x$ and its cytoembryology in false pakchoi. *Acta Horticulture Sinica*, 28: 317–322.
- Zhang L.Q., Liu D.C., Zheng Y.L., Yan Z.H., Dai S.F., Yan Z.H., Dai S.F., Li Y.F., Jiang Q., Ye Y.Q., Yen Y. (2010): Frequent occurrence of unreduced gametes in *Triticum turgidum*-*Aegilops tauschii* hybrids. *Euphytica*, 172: 285–294.
- Zhou S.J., LI K.H., Zhou G.X. (2012): Analysis of endosperm development of allotriploid \times diploid/tetraploid crosses in *Lilium*. *Euphytica*, 184: 401–412.
- Zhu X.H., Liu J., Zheng X.J., Xu Y., Chen F. (2011): Regeneration of plantlets from mature endosperm of *Jatropha curcas* L. and analysis of their stomata. *Chinese Journal of Applied & Environmental Biology*, 17: 353–358.

Received for publication October 20, 2015

Accepted after corrections April 20, 2016

Corresponding author:

Dr. ZONG-MING (MAX) CHENG, University of Tennessee, Department of Plant Sciences, Knoxville, USA;
e-mail: zcheng@utk.edu
