**Regular Article**
pISSN: 2288-9744, eISSN: 2288-9752
Journal of Forest and Environmental Science
Vol. 33, No. 3, pp. 197-201, August, 2017
https://doi.org/10.7747/JFES.2017.33.3.197

JFES
Journal of Forest and Environmental Science

# Interspecific Hybridization between *Populus caspica* L. × *P. deltoids* L 62/154 Using *in vitro* Embryo Development and Germination

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## Abstract

*Populus. caspica* L. is an Iranian indigenous poplar species which naturally distributed in the northern part of country. Unfortunately, overuse has removed many of the stems of better form, so that natural stands now usually appear small and crook. Therefore genetic variation for selection of new superior clone of this species is needed. Conventional hybridization system is currently used to induce genetic variation in poplar species but incompatibility barriers have been observed between them. *In vitro* ovule embryo culture was used to overcome incompatibility obstacle for interspecific hybridization between *Populus caspica* L. with *Populus deltoids* L.62/75. Female flowers of *Populus caspica* L. have artificially been pollinated with pollen grain of *P. deltoides* 62/75 in one direction using twig and pot crossing system. Ovaries at different ages (7, 14 and 21 days after pollination) were disinfected through 70% ethanol for 1 minute, 5% of sodium-hypochlorite solution for fifteen min followed by three time rising with sterile distil-water. Isolated ovaries were then transferred to MS hormone free medium containing 30 and 60 g/L sucrose for embryo development and germination. Collected data have been analyzed by two factorial experimental designs. The results indicated that there were significant differences between age of embryos for development and germination at $\alpha$=0.01%. Highest embryo germination (45%) was observed from 21 days old ovaries. No significant differences were observed between MS culture media containing 30 and 60 g/L for percentages of ovary-embryo germination and number of germinated embryo per ovary at $\alpha$=0.05%. Fourteen percentage of embryo germination obtained in MS medium supplemented with 60 g/L sucrose, while only 35% of isolated ovaries were able to germinate in MS containing 30 g/L sucrose. Induced plantlets in 4 cm height were transferred into pots containing soilless (1:1:1 peat, per lit and vermiculite) medium for acclimatization. After successful acclimatization, plants were delivered to nursery.

**Key Words:** ovary, embryo culture, *Populus deltoids* L., *Populus caspica* L., age of embryo

## Introduction

Poplar is a fast growing tree (growing up to 30 m) and belongs to the silcaseae family (Poplar and Willows). Among them *Populus caspica* L. is an Iranian indigenous poplar species which naturally distributed in the northern part of country (Golestan, Mazandaran, and Guilan provinces). This species is being used for reforestation program in degraded plain of Hyrcanian Forests (Jalilli 1999). Unfortunately, overuse has removed many of the stem

Received: February 15, 2017. Revised: April 4, 2017. Accepted: April 24, 2017.

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straightness individual from population (Jalilvand 1989). Therefore genetic variation for selection of new superior clone of this species is argent task for wood production program. Poplar plants are not usually multiplied by seed, but propagated by cutting (Jalilvand 1989). Vegetative propagated tree are identical in genetic point of view and no genetic variation is to be respected. For genetic variation, overcoming sexual reproduction barrier of this species need before any breeding method has to be done. Hybridization is currently used to induce genetic variation and to achieve hybrid vigor in many crops and trees (Tabaii Aghdaii and Jafari mofidabadi 2000). In poplars, many species are capable of hybridizing, but the hybridization rate depends on reflect of phyllogenetic relationships. Intra-sectional crosses showed more successful than inter-sectional crosses (Fernando et al. 2005).

In general, the *Populus caspica* L. are unable to hybridize with *Populus deltoids* L. species through conventional approaches. During the last fifteen years, a number of experiments have been made on pollen-stigma interactions in interspecific crosses of *Populus*, with emphasis on the sections Aigeiros, Leuce and tecamahaca (Willing and Pryor 1990; Rytter and Stener 2005; Lee et al. 2008). Many hybridization programs to improve the growth capacity of poplars have been carried out by several authors (Li et al. 1983; Li and Li 1985; Ahuja 1986; Raqiun and Trousard 1993; Jafari et al. 1999; Jafari Mofidabadi and Modir-Rahmati 2000; Calagari et al. 2003; Kevin 2004; Pyamnour et al. 2013). Hybrids obtained from the cross between *Populus euphratica Oliv*. with_*Populus alba* L. have exhibited outstanding characters, such as increased growth rate and stem straightness (Jafari Mofidabadi and Modr-Rahmati 2000). Valuable hybrid has been reported by interspecific hybridization between *Populus grandidentata* and *Populus alba* (Ahuja 1986).

Although pollination between different poplar species can be done but due to post-zygotic barriers which are expressed in the endosperm, embryo abortion has been observed (Zenkteler 1990; Sharma et al. 1996; Jafari Mofidabadi et al. 1999). Attempts have been done to interbreed the two species producing populations with intermediate characteristics between the two species but there are some reproductive barriers that may prevent successful hybridization by conventional approaches. *In vitro* immature and mature embryo culture has been suggested to overcome post zygotic barriers and developing interspecific hybrids in poplar trees.

## Materials and Methods

Artificial pollination was made between female flowers of *Populus caspica* L. as a maternal plant with pollen grain of *P. deltoids* L. (62/154) using twig and pot system (tap water culture) in one direction. Branches containing female flowers of *Populus caspica* L. collected from north forest of Golestan Province in Iran and put them in a pot containing tap water. In order to provide pollen grains, the male flowers of *Populus deltoids* L. (62/154) were forced to anthesis in green-house. The female inflorescence buds were covered with transparent papers before anthesis to avoid contamination. Pollinations were then carried out on female inflorescence of *Populus caspica* L. stigma by high dusting of *Populus deltoids* L.62/154 pollen grain in isolated greenhouse. Catkins were then collected 7, 14 and 21 days after pollination (DAP). Closed capsules, still attached to the axis of the catkin, were disinfected in 70% (v/v) ethanol for 1 min followed by sodium hypochlorite solution (0.5 % active chlorine in water) for 15 min and finally rinsed three times in sterile distilled water. Isolated ovaries were aseptically transferred to surface of agar MS (Murashige and Skoog 1962) hormone free media supplemented with two different sucrose concentrations (30 and 60 g/L) for *in vitro* embryo development and germination. Media were autoclaved for 20 min at 120°C and then dispensed 40 ml in each sterile jar. PH was adjusted to 5.7 before autoclaving. Cultures were incubated at 24°C under a 16 h photoperiod with light provided by cool white 40-watt fluorescent lamps (4000-5000 lux). Collected data (percentage of germinated ovary and number of plant produced per isolated ovary) were analyzed using factorial experimental design with two factors (age of embryo and sucrose concentration) and 10 replications. Plantlets at 1-2 cm in height were transferred to jars containing the same medium and kept for two months under the same conditions before being acclimatized. The plantlets were subsequently transferred to the green-house in pot containing peat, perlit and vermiculite in the ratio of 1:1:1 for hardening phase.

## Results and Discussion

### *Effect of ovaries ages on embryo development and germination*

All developed embryos from isolated capsules of *P. caspica* L. pollinated with *P. deltoides* L. 62/154 pollen grain started to germinate after one week on the surface of hormone free MS medium supplemented with both 30 and 60 g/L sucrose (Fig. 1). Analysis of collected data based on two factorial experimental design, indicated that there are highly significant differences between age (number of days after pollination) of isolated ovaries (7, 14 and 21 DAP) for mean percentage of ovary-embryo germination and mean number of germinated embryo per ovary at $\alpha=0.01$ level (Table 1). Highest mean percentages of embryos germination (45%) and highest number of plantlets formation per ovary (2.31 averages) were observed from 21 days old embryo (Table 2 and 3). Due to formation of multi embryos of *p. caspica* L. capsule (ovary), often more than one plants were produced on one isolated ovary (Table 3). Twenty one days old *in vitro* embryo development and germination have been previously reported for *P. alba* L. (Jafari Mofidabadi et al. 1999), *P. nigra* L. and *P. caspica* L. (Jafari Mofidabadi 2015).

In contrast to the report of (Li et al. 1983; Li and Li 1985), which white- cream, loose and friable callus were induced from three days old embryos (days after pollination), we were able to obtain plantlets from at least 7 days old isolated ovaries. Eight days old capsule of *Salix viminalis* L. was necessary for *in vitro* ovule embryo germination in intergeneric crosses with *P. alba* L (Bangniewska et al. 2011) and for Salix aegyptica ovary culture when pollinated with *Populus caspica* L. pollen grain (Ahmedi et al. 2008).

Germination of *P. euprahtica* Oliv. ovary pollinated with *P. alba* L. was observed within 45 days after culture initiation (Jafari Mofidabadi and Modir-rhmati 2000; Calagari et al. 2003). This long term (45 days after pollination) embryo development and germination of *P. euprahtica* Oliv., has been caused that the crossing has to be only done on mature tree instead of twig and pot breeding system (Jafari and Modir-rahmeti 2000).

Necroses ovaries (5.6%) and malformed plantlets (12.4%) were observed in ovary culture of *p. caspica* L. after two sub culture. The same phenomena were reported by Jafari Mofidabadi et al. (1998) for *p. alba* L. when used as a maternal parent in *Populus alba* L. × *P. euphratica* Oliv. hybridization.

**Fig. 1.** Fourteen days old hybrid embryo (*Populus caspica* L. × *P. deltoides* 62/154) germinated on in MS medium containing 30 g/L sucrose.

**Table 1.** Effect of age of ovary and MS medium supplemented with 30 and 60 g/L sucrose on % of germinated embryo and number of plantlets per ovary

| Source of variation | Df | MS | |
|---|---|---|---|
| | | % of germinated ovary germination | No of plantlets/ovary |
| Age | 2 | 3.9856** | 70.4755** |
| Medium | 1 | 0.1530 ns | 7.2871 ns |
| Age * medium | 2 | 0.0515 | 0.4182 |
| Error | 45 | 0.1195 | 6.5137 |
| Total | 59 | | |

**Table 2.** Effect of age (DAP) ovaries on percentage of germinated embryo

| Duncan grouping | Mean | N | Age |
|---|---|---|---|
| A | 45 | 20 | 21 |
| A | 37.7 | 20 | 14 |
| B | 5 | 20 | 7 |

**Table 3.** Effect of age (DAP) ovaries on the mean number of germinated embryo per isolated ovary

| Duncan grouping | Mean | N | Age |
|---|---|---|---|
| A | 2.31 | 20 | 21 |
| A | 2.06 | 20 | 14 |
| B | 1.10 | 20 | 7 |

### *Effect of sucrose concentration embryo development and germination*

Analysis of collected data showed that there are no significant differences between MS medium containing 30 or 60 g/L sucrose concentration for mean percentage of germinated ovary and for average number of plantlets formation per ovary (Table 1). In spite of no significant differences between sucrose concentrations, highest mean percentage of germinated ovary (40%) and mean number of plantlets per isolated ovary (2.008) were observed in MS medium containing 60 g/L sucrose (Table 4 and 5). MS hormone free medium supplemented with 30 g/L sucrose were used for *in vitro* embryo nutrition in intra specific and inter specific hybridization of poplar (Jafari Mofidabadi et al. 1999; Jafari Mofidabadi and Modir-Rahmeti 2000; Calagari et al. 2003; Kevin 2004; Ahmedi et al. 2008) and got nearly the same results. MS medium supplemented with different growth regulators hormone were used by several authors for intra and interspecific poplar tree hybridization (Li et al. 1983; Li and Li 1985; Bangniewska et al. 2011). Half-MS supplemented with 3o g/L (3%) sucrose was used for immature embryo development and germination of *Salix viminalis* L. as maternal species in crosses with *P. alba* L. (Bangniewska et al. 2011). In contrast with Bangniewska et al. 2011 who transferred germinated seedling into the MS medium containing 0.2 mg/L naphtaleneacetic acid for plantlet growth, we used hormone free MS medium for further subculture.

Three percentage of sucrose was also used for *in vitro* embryo development and germination of intergeneric (*Salix viminalis* L. × *P. alba* L.) by Ahmedi et al. 2008., for interspecific (*Populus euphratica* Oliv × *P. alba* L.) by Jafari Mofidabadi et al. 2000., for interspecific (*Populus euphratica* Oliv × *P. euphratica* oliv.) hybrid production of different ecotypes by Calagari et al. 2003. Thirty one plantlets with morphological variation were successfully acclimatized in green house and transferred to the nursery. Due to highly vegetative propagation system in poplar trees, selected of any superior hybrid clones could be easily proliferated. The results of this article showed that conventional breeding and successful *in vitro* embryo development and germination of poplar made it possible to obtain new superior clones through sexual reproduction, intra and interspecific hybridization.

**Table 4.** Effect on MS medium supplemented 30 and 60 g/L on percentage of germinated embryo

| Duncan grouping | Mean | N | Medium + |
|---|---|---|---|
| A | 40 | 20 | 60 g/L sucrose concentration |
| A | 35 | 20 | 30 g/L sucrose concentration |

**Table 5.** Effect o MS medium supplemented 30 and 60 g/L on the number of germinated embryo per ovary

| Duncan grouping | Mean | N | Medium + |
|---|---|---|---|
| A | 2.0086 | 20 | 60 g/L sucrose concentration |
| A | 1.9076 | 20 | 30 g/L sucrose concentration |

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