

Intraspecific Hybridization of *Populus euphratica* Oliv. Using *in vitro* Technique

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Abstract

In order to investigate the effect of different geographic origins of pollen grain in intraspecific hybridization and to achieve hybrid vigor, artificial hybridization has been carried out between different local genotype of *Populus euphratica* Oliv. using conventional and embryo-rescue techniques. Because of long period requirement for *Populus euphratica* Oliv. embryo development, as a maternal plant, 45 day old pollinated catkins were used for ovary and ovule culture. For embryo germination, isolated ovaries and ovules were transferred to different kinds of media (MS, Half-MS and DKW). The mean effect of pollinated *Populus euphratica* Oliv. ovaries was 34.03% to produce plantlets, whereas the value for pollinated ovules was 47.46%. There was a significant difference between ovaries and ovules for embryo germination at 0.05% level. In spite of no significant difference between media for embryo germination, the highest percentage of embryo germination was observed in MS medium (43.22%). Due to existing of high number of ovule in each ovary (15-30), the highest number of plantlet was observed in ovary culture. No significant differences were observed between pollen grains with different origin (geographic origin) in percentage of fertilization and embryo germination. Morphological variation was observed among regenerated plants. One hundred thirty seven plantlets were successfully acclimatized in a green house and transferred to the field.

Keywords: *Populus euphratica*; Intraspecific hybridization; Ovary and ovule culture

Introduction

The variation in stem straightens that occur within individuals in different regions, are relatively compared with the differences between species that habitually occupy different types of habitat [11]. However, even

within species there are often genetic differences in response to geographic conditions between populations from different locations [12]. There are striking cases where the intraspecific hybridization led to obtain superior offspring in some crop species. There has been a long interest in the hybridization of poplars largely

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because of the benefits derived from capturing heterosis and combining desirable traits to improve the quality and amount of wood production [10].

The importance of *Populus euphratica* Oliv has been recognized; populations through its wide range in the sub-tropics, from China to Morocco, show varying degrees of tolerance to the salinity, periodic water-logging, cold and arid conditions. Unfortunately, overuse has removed many of the stems of better form, so that natural stands now usually appear small and crooked. Numerous attempts have been done to improve this highly vegetative propagated tree, using genetic manipulation process (interspecific hybridization between *Populus euphratica* Oliv. and *Populus alba* L. [4,5]; Gametoclonal variation, [6]). With the aim to understand the role of different geographical source of pollen grain in intraspecific hybridization of *Populus euphratica* Oliv. to obtain superior genotype, different crosses were carried out between several local genotypes (ecotype) of this very wide distributed species. Due to possible embryo abortion, and efficient plantlet induction, embryo rescue technique was used.

Materials and Methods

A ten year old grown *Populus euphratica* Oliv. tree in research center of Albourz in Karaj (with origin of Gotvand-Karoon river in Khozistan province) was chosen as a maternal parents for artificial intraspecific hybridization. The female inflorescence buds were covered with transparent papers before anthesis to avoid contamination. Artificial pollination was made on the surface of *Populus euphratica* Oliv stigma with pollen grain of different ecotypes of this species [Sarakhs (Tajan river), Doroud (Khorram Abad river), Tehran (Jajroud river) and Gotvand (Karron river)]. In order to provide pollen grain, branches of different ecotype of *Populus euphratica* Oliv. were collected and forced to anthesis in green-house. Pollination was carried out on covered female inflorescence by high dusting pollen grain. Forty five day old pollinated catkins were then collected from branches. Closed capsules, still attached to the axis of the catkin, were then surface disinfected for 12 min in a solution of calcium hypochlorite followed by three 5-min rinses in sterile deionized water. To rescue fertilized embryos, pollinated ovaries and ovules were isolated and transferred to the hormone free MS [6], half-MS and DKW [1] agar medium in small test tube. Cultures were incubated at 24°C under 16 h photoperiod with light provided by 40 watt cool white fluorescent lamps (4000 to 5000 lux).

No growth regulators were added to the medium but two concentrations of sucrose (20 and 30 mg/L) were

used. The medium was autoclaved for 20 min at 120°C and then dispensed in small test tube (5×2.5 cm) and jars with 5 ml, 20 ml, respectively. Plantlets 1 to 2 cm in height were transferred to jars containing the same medium and kept for two months before acclimatization.

Results and Discussion

Germination of embryo in all crosses was observed 3 and 10 days from cultured ovule and ovary respectively on the surface of agar medium.

In ovary culture, an average of 34.03% of cultured capsules produced plantlets, while the value was 47.46% for ovule culture. Due to induction of more than 15 plantlets in each cultured ovary, high numbers (yield) of induced plantlets were observed in ovary culture, while only one tiny shoot per ovule culture each. These phenomena were observed for ovary culture of *P. euphratica*, which was pollinated with *P. alba* L pollen grain [5]. There was a significant difference between ovary and ovule embryo germination at 0.05% level using chi-square test (Tables 1 and 2). An average of 7.8% cultured ovaries on all applied media, became necrotic and discarded. Malformed ovules with abnormal plantlets were observed in 10 days cultured and died later. The same results were observed in ovary, ovule and embryo culture of many species such as poplar [4,5], cherry [3] and apricot [2]. Analysis on the effect of different geographical sources of pollen grain (Fig. 1) on combining abilities and embryo-germination showed no significant differences between them, but better results of embryo germination were obtained on combination of *P. euphratica* Karaj (Gotvand) x *P. euphratica* Tehran (Khojir). High degree of morphological variation was observed among regenerated plants (Fig. 2). No significant difference was observed between culture media on embryo germination and hybrid release in all crosses, but the highest percentage of embryo germination was observed in MS medium (43.22%). Due to relatively long term

Table 1. Analysis of independent variables on embryo germination using Chi-square test

Independent variables	Estimated Chi-square value	Df	Significance
Explants	3.735	1	0.053 *
Media	0.261	2	0.877 **
Origin of pollen grain	5.699	3	0.127 **

* Significant difference at 0.05 level

** No Significant difference

Table 2. Mean effect of independent variables on embryo germination in intraspecific hybridization of *Populus euphratica* Oliv.

Variables	Embryo germination and plantlet production
<i>Explants</i>	% of embryo germination *
Ovule	47.46
Ovary	34.93
<i>Media</i>	% of embryo germination **
MS	43.22
1/2 MS	41.87
DKW	39.69
<i>Source of pollen grain (Province)</i>	% of embryo germination **
Tehran	69.09
Doroud	53
Sarakhs	59.45
Gotvand	62.91

* Significant at the 0.05 level

** no significant difference

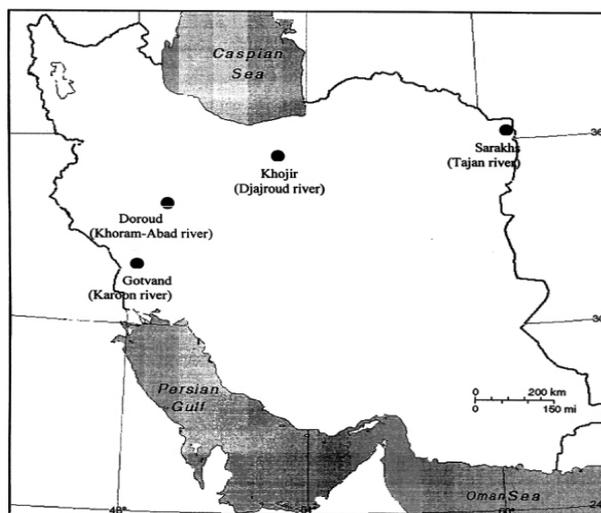


Figure 1. Location of *Populus euphratica* Oliv. Genotypes used as male parents.



Figure 2. Morphological variation in intraspecific hybrid of *Populus euphratica* (left), and control pollination (right).



Figure 3. Forty five day old embryo germination of *Populus euphratica* Gotvand x *P. euphratica* Sarakhs intraspecific hybrid.

P. euphratica embryo development, at least 45 day old embryo (the day after pollination) was necessary for *in vitro* embryo germination (Fig. 3). The same observation has been seen in *P. euphratica* x *P. alba* L. interspecific hybridization when *P. euphratica* was chosen as maternal plant [5]. In *Populus euphratica* x *P.*

simonii hybridization due to abnormal behavior of pollen tubes and immaturation of pollen tube and embryo sac, no fertilization occurred [7]. In contrast, when *Populus simonii* were considered as a maternal donor plant in cross with *P. euphratica*, a few hybrid seeds were preformed [7].

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