

Study of inter-generic hybridization possibility between *Salix aegyptica* and *Populus caspica* to achieve new hybrids

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Abstract

Hybrids as various resources have valuable importance in tree breeding. Today, improvement programs by using poplar and willows and their hybrids play basic role in supplying wood and decreasing pressure on natural forests. This research was carried out in order to study inter-generic hybridization possibility between *Salix aegyptica* and *Populus caspica* species from Salicaceae to achieve new hybrids. Embryo rescue technique was used in order to produce new inter-generic hybrids in salicaceae. The experiment was performed in factorial completely randomized design with 3 replication in embryo developmental stages and different media treatments. The analysis of data showed that inter-generic cross ability between *Salix aegyptica* and *Populus caspica* was possible and 14 day after pollination and MS medium containing 3 percent sucrose were the best time and medium for obtaining high amount of germinated hybrids.

Keywords: Inter-generic hybridization; *Populus caspica*; *Salix aegyptica*; Tree breeding.

Introduction

The poplars, genus *Populus* and the willows, genus *Salix* belong to the salicaceae family (FAO, 1972). Willows are rich in species and gene resources and they are easy to cross and vegetative to propagate. They are typical short-rotation species because of early fast growth. Their wood is white and has even structure (Mingiian et al., 2000). Also, willows play important role in natural communities, restoration of rivers edge and biomass production. Willows have main features as high growth speed, good performing in lowland areas and having positive effects such as medical consumption and prevention from erosion (Drusilla and Bertholdsson, 2000; Bergner, 2001).

Poplars are fast growth, ease of vegetative propagation and versatility of end uses determined the special role of the genus *Populus* in agricultural systems. Today, poplars represent an important growing stock of poplar is in natural forests (Confalonieri et al., 2003). Poplars are often considered as part of agro-forestry system that are used for

producing wood, fuel and windbreaker (Dickmann, 2006). However, long generation time of trees, the presence of seasonal dormancy and the prolonged period required for evaluation of mature traits are strong limitations for classical breeding and selection. The development of methods for *in vitro* culture and genetic engineering has increased the possibility of producing poplar genotypes improved in insect pest resistance, herbicide tolerance, growth rate and wood quality or reduction in undesirable traits (Confalonieri et al., 2003).

Inter-specific and intra-specific hybridization in Poplar genus (Sout et al., 1927; Mofidabadii, 1998; Jafari mofidi and Modir Rahmati, 2000; Asadi et al., 2001; Kalagari et al., 2003) and willow (Argus, 1974 and 1986; Mosseler and Papadopol, 1989; Orians and Fritz, 1995; Hardig et al., 2000; Orians et al., 2000; Fritz, 2006) have been done by many researchers that they lead to increase genetic resources to achieve heterosis. Inter-generic hybridization between willow and poplar genera have been reported by Zenkteler et al. (2005) that they examined the inter-generic cross ability of *salix vernalis* and four populus species.

Hybridization between forest trees is one of method to increase genetic diversity and to obtain genetic improvement. These methods result in phenotype and genetic variation which increase basis materials for improvement works (Heszky et al., 1992). This article was performed in order to study inter-generic hybridization possibility between *salix aegyptica* and *populus caspica* to achieve new hybrids in salicaceae as woody biomass resources.

Materials and Methods

Pollination was performed with Twig and Pots system (Jafari mofidi and Modir Rahmati, 2000) and *Salix aegyptica* stigma were pollinated with *Populus caspica* pollens. The explants were cultured at 10 days after pollination and were prolonged in 14 and 21 days. Before transferring of ovaries to media, the ovaries were surface-sterilized aseptically.

The ovaries were cultured on MS and Half-MS medium (Murashige and Skoog, 1962) containing 20 and 30 g/l sucrose and 8 g/l agar. The Cultured ovaries were put in growth cabinet in 16 hours light conditions and 4500-5000 lux in temperatures varying from 15 °C at night to 20-25 °C during the day. The number of germinated ovules was recorded daily for six months. The specimens were sub-cultured every 15 days. Variance analysis of germination parameter was carried out in factorial experiment completely randomized design at 3 replication with embryo developmental stage (10, 14, 21 day-old after pollination) and medium treatment (MS and Half-MS without plant growth regulators). Then the mean differences among media and time and their intercept effects were compared by using Duncan multiple comparisons.

Results and Discussion

The results of this research indicated that the initiation time of ovaries germination in *Salix aegyptica* × *Populus caspica* embryos occurred at 7-10 days after transferring of

ovaries to media that This is in accordance with the results obtained by Mofidabadi et al. (1998) and Kalagari et al. (2003).

Variance analysis of data was demonstrated that there was significant differences at 1% level in germination of inter-generic hybrid ovaries (Table 1) between embryo developmental stages (10, 14 and 21 days). The highest and lowest ovule germination was obtained in ovaries collected at 14 and 21 day-old after pollination, respectively. Jafari Mofidabadi (2005, 1998) and Raquin and Troussard (1993) also observed the highest germination in 14 day-old embryos. Moreover, the analysis of embryogenesis in this inter-generic hybridization indicated that the lowest germination percentage in ovary culture was observed in 21 day-old embryos that it is similar to Jafari Mofidi et al. (2006, 1998) results. This decreasing in germination is because of white cottons that surround ovules and exist at late development of ovaries.

Table 1. Germination variance analysis in embryo developmental stages and medium type.

| Source | Degree of freedom (df) | Mean Square (ms) |
|-----------------|------------------------|------------------|
| Corrected Model | 11 | 199/818** |
| Intercept | 1 | 464/815** |
| media | 3 | 349/750** |
| age | 2 | 17/343** |
| media * age | 6 | 6/917* |
| Error | 24 | |
| Total | 35 | |

**significant at the 0.01 level.

*significant at the 0.05 level.

Also, the effect of several media components on the germination percentage of ovules in this inter-generic cross by using ovary culture showed significant difference among media components. Maximum percentage of germination was observed on media containing the full concentrations of micro-and macro-nutrients of the MS medium supplemented with 30 g/l sucrose and minimum percentage of germination was resulted in media containing the half concentrations MS supplemented with 20 g/l sucrose.

In addition, there was significant difference on interaction effects between different embryo developmental stages and media. Furthermore, based on Duncan multiple comparisons in simultaneous consideration of media and embryo developmental stages on ovaries, the highest and lowest germination have been obtained from ovaries which were collected at 14 and 21 days after pollination and transferred to MS medium supplemented with 30 and 20 g/l sucrose, respectively (Figure 1).

In general, this research may allow breeders to develop inter-generic hybrids (*Salix aegyptica* × *Populus caspica*) through hybridization using new tissue culture methods. More research is need for improving genetic variability of inter-generic hybrids in salicaseae. Also, more efforts are necessary on its heterosis plants and commercial characteristics of this new inter-genetic hybrid.



Figure 1. Inter-generic hybrid obtained from ovary at 14 days after pollination in MS medium supplemented with 30 g/l sucrose.

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