

Vahideh PAYAMNOUR,
Samereh HASHEMI, Ali Jafari MOFIDABADI and Akram AHMADI¹

**INTER-SPECIFIC AND INTER-GENERIC HYBRIDIZATION IN
SALICACEAE (*POPULUS NIGRA* × *P. DELTOIDES* AND *P. NIGRA* ×
SALIX ALBA) USING AN EMBRYO RESCUE TECHNIQUE**

SUMMARY

Tree breeding has been developed new horizon in producing new hybrids which play basic role in supplying wood and help to prevent forest reduction. The main aim of the current project was to investigate inter-specific and inter-generic hybridization in *Salicaceae* with the target of increasing genetic diversity and to achieve new hybrids suitable for producing woody biomass. This is the successful attempt to perform Inter-specific crossing between *Populus nigra* × *P. deltoides* and inter-generic crossing between *P. nigra* × *salix alba*. An embryo rescue technique as an efficient method was used on ovaries isolated from unwanted pollens. After pollination, 10 and 14-days-old embryos were transferred to MS and 1/2 MS media containing 30 gr/lit sucrose. In view of the results, inter-specific and inter-generic hybridization in the willow family was possible with these species. The high rate of germination was occurred 14 days after pollination and in the MS medium. This hybridization is accomplished through an *in vitro* system. These results defined a successful protocol for *in vitro* breeding of *P. nigra* × *P. deltoides* and *P. nigra* × *salix alba*.

Keywords: Hybridization, Breeding, Embryo rescue, Inter-specific, Inter-generic.

INTRODUCTION

Hybridization and gene flow can have a profound effect on persistence and evolution of wild populations and it is an important evolutionary process for many groups of species (Vanden et al., 2005). It was an interesting fact, that increasing genetic diversity by developing new pre-breeding material is a continuous challenge for breeders. Plant breeders achieve this goal by making inter-specific and inter-generic crosses; however, possibilities are limited by the occurrence of various incongruity and incompatibility barriers. A solution can be reached in some cases using various techniques such as *in vitro* embryo rescue and poly-ploidization. Somatic hybridization circumvents sexual crossing barriers between plant species. It is necessary to note, that hybridization is

¹ Vahideh Payamnour, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Samereh Hashemi, Akram Ahmadi (corresponding author: ahmadi.1870@gmail.com) Gorgan University of Agricultural Sciences and Natural Resources, Iran, Ali Jafari Mofidabadi Cotton Research Institute of Iran (CRII), Gorgan, Iran

currently used to combine desirable traits and to achieve hybrid vigor in many crops and trees (Stettler, 1980).

Tree species of poplar and willow are coming rapidly to be the forefront of research due to interest in their use as biomass crops in temperate regions of the world. Willow and poplar are excellent candidates for short-rotation coppice cultures, which rely on species that are characterized by fast growth and dynamic production of biomass. Moreover, biomass crops can be improved genetically by a variety of experimental breeding, selection and inter-specific hybridization programs (Agnieszka et al., 2010).

Taxonomy and classification in poplar and willow, as the most plentiful species of the Salicaceae family, are made complex because of either natural or artificial hybridization that can be derived from them (Dickman and Stuart, 1993).

The poplar genus has much variety with between 22 to about 85 species, distributed throughout the northern hemisphere in both temperate and subtropical zones. Poplar species are predominantly deciduous and thus suitable for cross fertilization (FAO, 1972). Poplar wood has many uses comprised of application in the pulp and paper industry, as timber plywood, pallets, soft board and hardboard (Vanden et al., 2005). There has been a keen and sustained interest in hybridized poplars because of the benefit of heterosis and its ability to combine desirable traits to improve quality and amounts of wood production (Stettler, 1980). *P. deltoides* and *P. nigra* are the most important species used for breeding poplar in the world. Most of the commercial poplar plantations throughout Europe have derived from inter-specific crosses between *P. deltoides* and *P. nigra* and their backcrosses (Vanden et al., 2005). The hybrid obtained from these species is called *P. euramericana* and it is important due to its high growth rate.

The willow genus has expanded more naturally than poplar and it is distributed throughout the northern and southern hemispheres and often in regions along riverbanks (FAO, 1978). Willows are rich in species and gene resources and they are easy to cross and to propagate. They are a typical short-rotation species due to early fast growth. Willow wood is white and even in its structure. Willow also plays an important role in natural restoration of riverbank ecosystems and for biomass production (Ahmadi et al., 2010). It has high tolerance to flood and drought, and moderate tolerance to salt. Many programs exist to breed willow for biomass. They are based on the use of inter-specific hybrids (Zsuffa, 1990; Lindegaard and Barker, 1997) with contrasting characteristics that are important for breeders, such as growth capacity, survival and resistance to rust fungi (Ronnberg-Wastljung and Gullberg, 1999).

The principal genera of the Salicaceae family, *Salix* and *Populus*, do not hybridize under natural conditions because of a variety of crossing barriers. However, many inter-specific hybridizations have been readily achieved under experimental conditions within the genera *Populus* (Willing and Pryor, 1976, Stettler, 1980, Smulders et al., 2008) and *Salix* (Argus, 1974).

The detailed review of earlier studies reveals that there are some published data on Inter-specific and inter-generic hybridization in poplar genus through *in vitro* conditions by Spies and Barnes (1982), Jafari Mofidabadi and Modirrahmati (1998), Jafari Mofidabadi and Modirrahmati (2000), Calagari et al. (2004) and Vanden et al. (2005). Several investigation reported that hybridization in willow genus achieved a hybrid with vigor thus increasing genetic diversity (Orien et al., 2000, Argus, 1986, Ahmadi et al., 2010).

Therefore, the experiment was undertaken considering the objectives: (i) to perform inter-specific hybridization between *P. nigra* × *P. deltoides* creating Euroamerica hybrid to avoid loss of genetic diversity in Iran because of its asexual propagation, (ii) to find out inter-generic hybridization possibilities between *P. nigra* × *S. alba* for new hybrids in salicaceae as woody biomass resources.

MATERIAL AND METHODS

This research was carried out using biotechnology methods in order to investigate possibility of inter-specific and inter-generic hybridization for producing new hybrids in Salicaceae. *P. nigra* stigma collected from Chamestan in Noor, which were pollinated with *P. deltoids* and *s. alba* pollens collected from Chahar bagh and Kordkoy in Golestan (Iran).

After opening of buds and when the flowers were ready, pollination was performed with a brush dipped in pollen in an isolated environment. The embryo rescue method was used for germinating 10 and 14-days-old hybrid embryos by transferring to medium. Before transferring ovaries to media, they were surface-sterilized aseptically in 70% ethanol for 1 min and then 1% NaOCl for 5 minutes and then, they were washed three times with sterile distilled water carefully during 10 minutes. Ovaries were transferred to the media culture, MS and half-MS media containing 30 gr/l sucrose and 6.8 gr/l agar (Murashige and Skoog, 1962). The cultured ovaries were put in a 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 inter-generic (*Populus nigra* × *Salix alba*) and also, inter-specific (*P.nigra* × *P.deltoides*) crosses 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 Ahmadi et al. (2010).

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

Hybrid	Source of variance (S.O.V)	Degree of freedom (df)	X ²
<i>P. nigra</i> × <i>P. deltoides</i>	Age (A)	1	5.714*
	Media (M)	1	8.803**
	A×M	3	30.000**
<i>P. nigra</i> × <i>S. alba</i>	Age (A)	1	5.500*
	Media (M)	1	8.909**
	A×M	3	29.00**

**significant at the 0.01 level.

* significant at the 0.05 level.

Variance analysis of data (Table 1) demonstrated that there was significant difference at 5 % level in culture embryos of different ages and at 1% level in different media. Embryo age (number of days after pollination) affected the ability of an embryo to respond to culture media (Jafari Mofidabadi and Modirrahmati, 2000). In this research the highest embryo germination rate was obtained in ovaries collected 14 days after pollination so that germination percentages in 14 day-old ovaries in *P.nigra* × *P. deltoids* cross and *P. nigra* × *S. alba* cross was 45% and 55%, respectively. Ahmadi et al. (2010) and Jafari Mofidabadi et al. (2008) also observed the highest germination in 14 day-old ovaries. Moreover, the analysis of embryogenesis in this inter-generic and inter-specific hybridization indicated that the lowest germination percentage in ovary culture between 10 and 14 days after pollination occurred at 10 days embryos (25% and 33%, respectively). This decreasing in germination is because of incomplete growth of ovaries which revealed this time is not suitable for embryo rescue due to early development of ovaries.

The effect of two media components on the germination percentage of ovaries showed significant difference between media components. The highest germination rate was observed on the MS medium (containing the full concentrations of micro and macro nutrients) supplemented with 30 gr/lit sucrose (48% germination in *P. nigra* × *P. deltoids* cross and 58% germination in *P. nigra* × *S. alba*). Analogous investigations were carried out by Jafari Mofidabadi and Modirrahmati (2000), Ahmadi et al. (2010), Jafari Mofidabadi (1998) and Calagari et al. (2004).

The results revealed that, the half-MS medium showed the minimum germination in both crosses in compared to MS medium (20% germination in *P. nigra* × *P. deltoids* cross and 30% germination in *P. nigra* × *S. alba*). In addition,

there was significant difference on interaction effects between different embryo developmental stages and media.

In light of the above results it appears that inter-specific and inter-generic hybridization in salicaceae with this species through ovary culture is possible. Additionally, as a result of advances in this study, embryo rescue method may be an effective technique for develop aforementioned hybrids which can be useful for breeding, improvement and genetic studies. Indeed, more efforts are necessary on its heterosis plants derived from these hybrids and commercial characteristics of these inter-genetic and inter-specific hybrids.



Figure 1: a. male catkin of *P.deltoides*, b. male catkin of *S.alba*, c. female flowers of *P.nigra* for pollination

ACKNOWLEDGEMENTS

This project is supported by Gorgan university of agricultural sciences and natural resources and all authors here express their deep appreciations.

REFERENCES

- Bagniewska-Zadworna, A. Wojciechowicz, M. K. Zenkteler, M. Jezowski, S. & Zenkteler, E. (2010): Cytological analysis of hybrid embryos of inter-generic crosses between *Salix viminalis* and *Populus* species. *Australian Journal of Botany.*, 58: 42–48.
- Ahmadi, A. Azadfar, D. & J-mofidabadi, A. (2010): Study of inter-generic hybridization possibility between *Salix aegyptica* and *Populus caspica* to achieve new hybrids. *Iranian Journal of Plant Production.*, 4(2): 143-147.
- Argus, G. W. (1974): Experimental study of hybridization and pollination in *Salix* (willow). *Canadian Journal of Botany.*, 52: 1613–1619.
- Argus, G. W. (1986): The genus *Salix* (Salicaceae) in the Southeastern United States. *Systematic Botany Monographs.*, 9:1-70.
- Calagari, M. J-mofidabadi, A. tabari, M. & Hosseini, S. M. (2004): Intra-specific Hybridization of *Populus euphratica* Olive. Using *in vitro* Technique. *Journal of Science Islamic Republic of Iran.*, 15(2): 109-112.
- Dickmann, D. I. & Stuart, K. W. (1993): The culture of Poplars in eastern North America. East Lansing: Michigan state university. Department of forestry. pp. 168.

- FAO. (1972): Poplars and Willows. published under the auspices of the international Poplar Commission. pp. 328.
- J-Mofidabadi, A. & M-Rahmati, A. (1998): Application of Ovary & Olive culture in *Populus alba* L. × *P. euphratica* Olive. Hybridization. *Silvae Genetic.*, 47: 5-6.
- J-Mofidabadi, A. & M-Rahmati, A. (2000): Production of *Populus euphratica* Olive. × *P. alba* L. hybrid poplars through ovary and olive culture. *Plant Genetic Resources Newsletter.*, 122: 13-15.
- J-Mofidabadi, A. Zarrinbal, A. Etamad, A. & Shariatzadeh, S. H. (2008): Production of *Populus Caspica*. × *P. alba* L. hybrid Poplar through ovary cultures. *Iranian journal of Agricultural Science and Natural Resources.*, 13(2): 36-13.
- Lindegaard, K. & Barker, J. (1997): Breeding willows for biomass. *Aspects of Applied Biology.*, 49: 155–162.
- Murashing, T. & Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiological Plant.*, 15:473-497.
- Orians, C. M. Griffiths, M. E. Roche, L. B. & Fritz, R. S. (2000): Phenolic glycosides and Condensed tannins in *Salix Sericea*, *S. eriophala* and their F1 hybrids: not all hybrids are created equal. *Biochemical Systematics and Ecology.*, 28:619-632.
- Ronnberg-Wastljung, A. C., & Gullberg, U. (1999): Genetics of breeding characters with possible effects on biomass production in *Salix viminalis* L. *Theoretical and Applied Genetics.*, 98: 531–540
- Smulders, M. J. M. Beringen, R. Volosyanchuk, R. Broeck, A. V. Vander Schoot, J. Arens, P. & Vosman, B. (2008): Natural hybridisation between *Populus nigra* L. and *P. canadensis* Moench. Hybrid offspring competes for niches along the Rhine river in the Netherlands. *Tree Genetics and Genomes.*, 4: 663–675.
- Stettler, R. F. (1980): Inter-specific crossability studies in Poplars. *Theoretical and Applied Genetics.*, 58: 273-282.
- Spies, T. A. & Barnes, B. V. (1982): Natural hybridization between *Populus alba* L and Native aspens in Southeastern Michigan. *Canadian Journal of Forest Research.*, 12(3): 653-660.
- Vanden, B. Marc, V. Erik, V. & Jos, V. (2005): Natural hybridization between cultivated poplar and their wild relatives: evidence and consequences for native poplar population. *American Journal of Botany.*, 62: 601-613.
- Willing, R. R. & Pryor, L. D. (1976): Inter-specific hybridization in Poplar. *Theoretical and Applied Genetics.*, 47: 141–151.
- Zsuffa, L. (1990): Genetic improvement of willows for energy plantations biomass. *Biomass.*, 22: 35–47.

Vahideh PAYAMNOUR,
Samereh HASHEMI, Ali Jafari MOFIDABADI, Akram AHMADI

**MEĐUVRSNA I INTERGENERIČNA HIBRIDIZACIJA SALICACEAE
(*POPULUS NIGRA* × *P. DELTOIDES* AND *P. NIGRA* × *SALIX ALBA*)
POMOĆU TEHNIKE SPAŠAVANJA EMBRIONA**

SAŽETAK

Oplemenjivanje drvnih vrsta je otvorilo nove poglede u proizvodnji novih hibrida koji imaju suštinsku ulogu u eksploataciji drveta, te pomažu u sprečavanju smanjenja šuma. Osnovni cilj ovog projekta je da se ispita međuvrsna i intergenerična hibridizacija Salicaceae, sa ciljem povećanja genetskog diverziteta i stvaranja novih hibrida koji su pogodni za proizvodnju drvene biomase. Ovo je uspješan pokušaj vršenja međuvrsnog ukrštanja između *Populus nigra* × *P. deltoides* i intergeneričkog ukrštanja između *P. nigra* × *Salix alba*. Na plodnicama neželjenog polena je kao efikasan metod korišćena tehnika spašavanja embriona. Nakon oprašivanja se 10 i 14 dana stari embrioni prenose na MS i 1/2 MS medijum koji sadrži 30 gr/lit saharoze. Prema rezultatima, međuvrsna i intergenerička hibridizacija u porodici vrbe je moguća sa ovim vrstama. Visoka stopa klijanja je postignuta 14 dana nakon oprašivanja i na MS medijumu. Ovakva hibridizacija je postignuta pomoću in vitro sistema. Ovakvi rezultati su definisali protokol za in vitro oplemenjivanje *P. nigra* × *P. deltoides* i *P. nigra* × *Salix alba*.

Ključne riječi: Hibridizacija, Oplemenjivanje, Spašavanje embriona, Međuvrsna, Intergenerična.