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Thidiazuron: A potent cytokinin for efficient plant regeneration in Himalayan poplar (*Populus ciliata* Wall.) using leaf explants

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Abstract. *Populus* species are important resource for certain branches of industry and have special roles for scientific study on biological and agricultural systems. The present investigation was undertaken with an objective of enhancing the frequency of plant regeneration in Himalayan poplar (*Populus ciliata* Wall.). The effect of Thiadizuron (TDZ) alone and in combination with adenine and α-Naphthalene acetic acid (NAA) were studied on the regeneration potential of leaf explants. A high efficiency of shoot regeneration was observed in leaf (80.00%) explants on MS basal medium supplemented with 0.024 mg/l TDZ and 79.7 mg/l adenine. Elongation and multiplication of shoots were obtained on Murashige and Skoog (MS) basal medium, containing 0.5 mg/l 6. Benzyl aminopurine (BAP) + 0.2mg/l Indole 3-acetic acid (IAA) + 0.3 mg/l Gibberellic acid (GA3). High frequency root regeneration from in vitro developed shoots was observed on MS basal medium supplemented with 0.10 mg/l Indole 3-butyric acid (IBA). Maximum of the in vitro rooted plantlets were well accomplished to the mixture of sand: soil (1:1) and exhibited similar morphology with the field plants. A high efficiency plant regeneration protocol has been developed from leaf explants in Himalayan poplar (*Populus ciliata* Wall.).
Keywords regeneration, leaf explants, Thidiazuron, *Populus ciliata*.

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Introduction

Biotechnology holds promise for circumventing some of the major obstacles in the genetic improvement of woody perennials, namely the unwieldy size, the long life cycles and the lack of basic genetic and inheritance information. An efficient in vitro regeneration system

with cell and tissue culture is a pre-requisite for biotechnological application of the plant improvement programme (Confalonieri et al. 2003). The genus *Populus* L. (poplars, cottonwoods and aspens) comprises about 30 species (Park et al. 2004), widely distributed in the temperate climates of the northern hemisphere. *Populus ciliata* (Himalayan poplar) is one of the few forest species which are considered ideal for successful inter-cultivation with agricultural crops. It is a large sized, deciduous tree, with sexually differentiated male and female plants which occur in temperate and subtemperate regions of Himalayas, at altitudes of 1200-3500 m. Due to their exceptional qualities, such as high capacity for vegetative propagation and the fast growth rate, they have been extensively used for pulp and paper industry, reforestation of lowlands, and phytoremediation of contaminated soil (Balatinecz et al. 2001, Rishi et al. 2001). A growing interest has been shown in further improvement of the economically important traits in *Populus*, especially with modern methods of genetic engineering. However, *Populus ciliata* is severely affected by many biotic and abiotic stresses, leading to considerable yield loss. Also, high lignin content in the species makes the operational costs of delignification process in paper manufacturing quite expensive and therefore, making it an important species for in vitro genetic manipulation.

However, before taking this approach, it is important to know whether the somatic cells of this species are able to regenerate in such a way so as to give rise to whole plantlet and the conditions required for such plant regeneration. Efforts devoted to the use of explants of mature trees of proven worth for propagation through tissue culture technique have been well reported (Cheema 1989, Jafari et al. 1995, Shen et al. 1998, Dai et al. 2003, Thakur & Srivastava 2006, Thakur et al. 2008 & Thakur et al. 2012), but they are limited to very few genotypes and very few reports focused on shoot regeneration from leaf explants of Himalayan poplar (*Populus ciliata*)(Thakur & Srivastava 2006, Thakur et al. 2008).

Therefore, in the present study we established a high efficiency shoot regeneration system from leaf explants of *Populus ciliata,* which is the key step for genetic transformation of this tree species and the effect of Thiadizuron (TDZ) concentrations alone and along with adenine and α-Naphthalene acetic acid (NAA) was also investigated. We will further use this regeneration system for the transformation of Himalayan poplar by genetically engineered *Agrobacterium tumefaciens* strain.

Material and methods

Plant material and nutrient medium

Stem cuttings of *Populus ciliata* were procured from the fields of Tree Improvement and Genetic Resources, Shilly, Solan. These stem cuttings were then planted in the glass house conditions and young tender leaves of Himalayan poplar (*Populus ciliata* Wall.) were used as explants, which were obtained from glass house grown cuttings in department of Biotechnology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan. Leaf explants were thoroughly washed with teepol for half an hour under running tap water and then explants were surface sterilized with 0.2% Bavistin for 2-3 minutes and 0.1% $HgCl_2$ for 1-2 minutes. After, these explants were thoroughly washed with sterilized distilled water to remove the traces of $HgCl_2$ MS basal medium (Murashige & Skoog 1962) salts (Macro & Micro); vitamins supplemented with 100 mg/l mesoinositol, 3% sucrose and 0.8% agar-agar (used as gelling agent) was used as basal medium. Different concentrations of TDZ alone and in combination with adenine and NAA were used in the MS basal medium for plant regeneration studies. The pH of the medium was adjusted to 5.8 before adding agar-agar. The medium was autoclaved at 121°C and 15

pounds per square inch for 20 minutes. All the aseptic manipulations were carried out under vertical laminar air flow chamber.

Plant regeneration from leaf explants of Himalayan poplar

To optimize the culture medium for high frequency shoot regeneration leaf explants were excised from the stem cuttings, which were grown and maintained in the glass house. These explants were cut into approximately 0.5-1.0 cm size and cultured on MS basal medium supplemented with different concentrations of TDZ alone and in combinations with adenine and NAA (see Tables 1-3). For every combination, six flasks with five explants were inoculated. All the cultures were kept in the culture room under 16h photoperiod with the light intensity of 40 µmol m^{-2} s^{-1} provided by cool white fluorescent lamps, 70% - 80% humidity and at a temperature 26 ± 2° C. The regenerated shoots were separated and individual shoot was transferred to the MS basal medium containing various concentrations of auxin; Indole 3-butyric acid (IBA) for root induction to get complete plantlets (see Table 4).

Hardening of regenerated plantlets

After proper in vitro development, the plantlets were taken out of the tubes and flask in such a way so that no damage was caused to their root system. The roots were washed gently under running tap water, to remove the adhering medium and the plantlets were kept in running tap water for a few minutes, so that they do not wilt after transfer to pots. The survival and establishment of the plantlets were studied after transplanting the plantlets in pots containing a mixture of sterilized sand and soil (1:1). The plantlets were watered and covered with jam jars to maintain a high relative humidity. After 9-10 days, when the plantlets showed signs of establishment in pots with appearance of new leaves, they were uncovered for overnight and after 3-4 weeks the plantlets were fairly uncovered.

Statistical analysis

Each treatment consisted of at least 30 explants and each experiment was repeated three times. The data recorded for different parameters was subjected to Completely Randomize Design (Gomez & Gomez 1984). The statistical analysis based on mean values per treatment, was conducted using analysis of variance for Completely Randomize Design.

Results

Shoot regeneration from leaf explants

The leaf explants of Himalayan poplar were inoculated on MS basal medium (Fig. 1) supplemented with different concentrations of TDZ alone and in combinations with adenine and NAA for the shoot regeneration experiment. Colour of the leaf explants turned to light green from green after a few days of culturing. Swelling and expansion in the leaf explants was observed after one week of culturing and callus initiation was observed after 13-15 days of culturing from the cut edges of leaf explants. The complete callus formation was observed after 20 days of culturing, while shoot regeneration from callus was observed after 28 - 30 days of culturing (Fig. 1). No change in the colour of the medium was observed. All the media were able to induce indirect organogenesis from leaf explants of Himalayan poplar. For multiplication and elongation of shoots they were transferred to different medium (MS + 0.5 mg/l BAP + 0.2 mg/l IAA + 0.3 mg/l Gibberellic acid (GA_3).

Effect of different concentrations

Effect of different concentrations of TDZ. A maximum percentage of shoot regen-

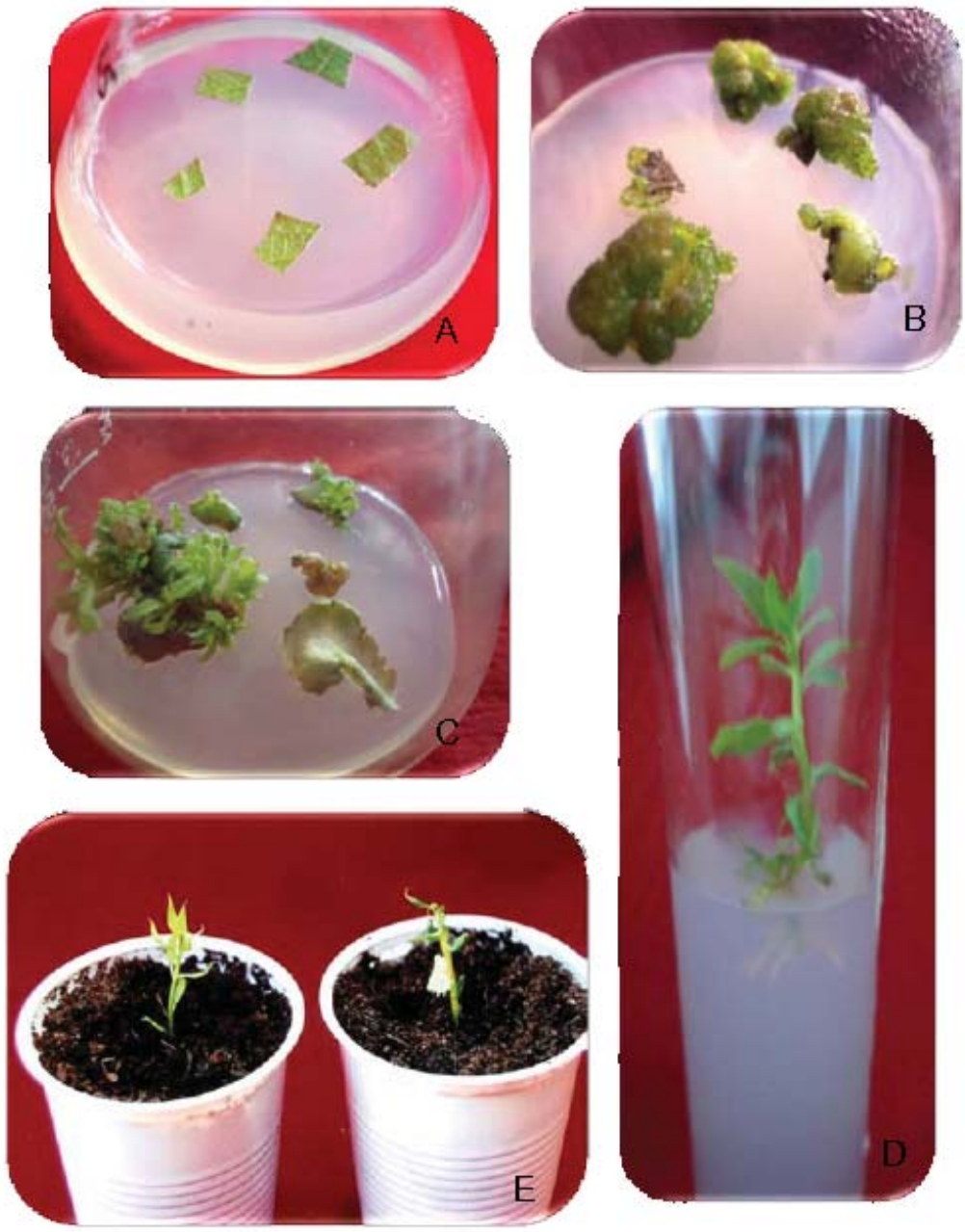

Figure 1 Plant regeneration studies in poplar (*Populus ciliata* Wall.) from leaf explants

Note: (A) In vitro cultured leaf explants on shoot regeneration medium [MS (basal medium) + 0.024 mg/l TDZ + 79.1 mg/l Adenine]; (B) Callus formation was observed from leaf explants on shoot regeneration medium [MS (basal medium) + 0.024 mg/l TDZ + 79.1 mg/l Adenine]; (C) Shoot regeneration from callus derived cultures of leaf explants on shoot regeneration medium [MS (basal medium) + 0.024 mg/l TDZ + 79.1 mg/l Adenine]; (D) Root regeneration on medium [MS (basal medium) + 0.10 mg/l IBA]; (E) Successful hardened plants on sterilized cocopeat mixture

Table 1 Effect of various concentrations of TDZ (in MS basal medium) on shoot regeneration from leaf explant of Himalayan poplar (*Populus ciliata* Wall.)

Sr. No.	Medium composition	Average number of shoots per explant	Percent shoot regeneration
1	MS basal medium + 0.022 mg/l TDZ	2.60	52.00 (46.15)
2	MS basal medium + 0.044 mg/l TDZ	7.40	72.00 (58.06)
3	MS basal medium + 0.110 mg/l TDZ	2.80	60.00 (50.77)
4	MS basal medium + 0.154 mg/l TDZ	3.60	56.00 (48.45)
5	MS basal medium + 0.220 mg/l TDZ	3.20	52.00 (46.15)
$CD_{0.05}$		0.30	3.04
SE±		0.14	1.37

Table 2 Effect of various concentrations and combinations of TDZ and adenine (in MS basal medium) on shoot regeneration from leaf explants of Himalayan poplar (*Populus ciliata* Wall.)

Sr. No.	Medium composition	Average number of shoots explants	Percent shoot regeneration
1	MS basal medium + 0.004 mg/l TDZ + 79.7 mg/l adenine	2.89	57.14 (49.11)
2	MS basal medium + 0.024 mg/l TDZ + 79.7 mg/l adenine	6.63	80.00 (63.45)
3	MS basal medium + 0.123 mg/l TDZ + 79.7 mg/l adenine	2.91	76.00 (60.67)
4	MS basal medium + 0.616 mg/l TDZ + 79.7 mg/l adenine	1.66	68.00 (55.56)
$CD_{0.05}$		0.30	3.98
SE±		0.13	1.29

Table 3 Effect of various concentrations and combinations of TDZ and NAA (in MS basal medium) on shoot regeneration from leaf explants of Himalayan poplar (*Populus ciliata* Wall.)

Sr. No.	Medium composition	Average number of shoots regenerated per leaf explant	Percent shoot regeneration in leaf explants
1	MS basal medium + 0.1 mg/l TDZ + 0.02 mg/l NAA	2.30	53.28 (46.88)
2	MS basal medium + 0.3 mg/l TDZ + 0.02 mg/l NAA	4.70	64.35 (53.34)
3	MS basal medium + 0.5 mg/l TDZ + 0.02 mg/l NAA	3.40	57.71 (49.44)
4	MS basal medium + 1.0 mg/l TDZ + 0.02 mg/l NAA	2.90	54.17 (47.39)
$CD_{0.05}$		0.30	2.12
SE±		0.13	0.92

eration (72.00%) was observed on MS basal medium supplemented with 0.044 mg/l TDZ with average number of shoots (7.40) per explants (Table 1).

Effect of different concentration of TDZ in combination with adenine. Out of the various concentrations of TDZ with adenine in MS basal medium, the 0.024 mg/l TDZ + 79.7 mg/l adenine was proved to be best. Maximum percentage of shoot regeneration (80.00%) was observed on this medium with average number of shoots (6.63) per explant (Table 2).

Effect of different concentration of TDZ in combination with NAA. MS basal medium supplemented with 0.3 mg/l TDZ and 0.02 mg/l NAA gave rise to maximum percentage of shoot regeneration (64.35%) with

average number of shoots (4.7) per explant (Table 3).

Out of the 13 different concentrations and combinations of TDZ (Table 1, 2 and 3) tried, MS basal medium supplemented with 0.024 mg/l TDZ + 79.7 mg/l adenine was proved to be best one with maximum percentage of shoot regeneration (80.00%) and average number of shoots (6.63) per explant (Table 2). Multiplication and elongation of shoots were observed on the MS basal medium containing 0.5 mg/l BAP + 0.2 mg/l IAA + 0.3 mg/l GA_3.

Root regeneration in adventitious shoots and hardening of plantlets

Elongated shoots (about 2-3 cm in height) obtained from the leaf explants were excised and cultured on MS basal medium supplemented with different concentrations of IBA for root induction (Table 4). Complete root formation was observed after 24 days of transfer to the root regeneration medium. Maximum percentage of (76%) root regeneration was observed with MS basal medium supplemented with 0.10 mg/l IBA (Fig. 1). After complete development of roots, the plantlets were taken out from the culture tubes and transferred to the plastic pots containing a mixture of sterilized sand and soil (1:1) (Fig. 1). The plantlets were watered and covered with glass jar to maintain high humidity. Himalayan poplar plantlets were able to regenerate within 2-3 months.

Discussion

Plant regeneration studies in *Populus* species have also been carried out by various studies in order to achieve an efficient and reliable regeneration system (Charenon & Taris 1960, Mathes 1964, Winton 1968, Venverloo 1973, Walter et al. 1988, Costache et al. 1995, Gangoo & Khurana 2002, Thakur & Srivastava 2006, Thakur et al. 2008). During the present investigation concentrations of plant growth regulators and age of the explants proved to be important factors affecting the frequency of shoot regeneration. The young and tender leaf explants were more effective for an efficient shoot regeneration in *P. ciliata,* in agreement with the previously reported results in *P. ciliata* by Thakur & Srivastava 2006, Thakur et al. (2008). Mehra & Cheema (1980) were successful to induce multiple shoots on young and tender leaf disc of Himalayan poplar. But, the mature leaf disc of Himalayan poplar under identical conditions did not respond.

In the present study, TDZ was used in the MS medium for shoot regeneration studies. TDZ is a synthetic phenyl urea cytokinin like compound that has been proven to be highly effective regulator of shoot morphogenesis (Huetteman & Preece 1993, Murthy et al. 1998, Hui-mei et al. 2008). It is also effective in terms of shoot regeneration in many recalcitrant species (Pelah et al. 2002, Schweenand Schwenkel 2002, Liu et al. 2003, Mithila et al. 2003). The effect of various concentrations of TDZ alone and in combination with adenine and NAA were studied for enhancing the shoot regeneration frequency from the leaf explants in *Populus ciliata*. Highest shoot regeneration frequency was obtained on a MS basal medium supplemented with TDZ in combination with adenine. Many published protocols for poplar

Table 4 Composition of root regeneration media having different concentration of IBA

Sr. No.	Medium composition	Percent root regeneration
1	MS basal medium + 0.05 mg/l IBA	50 (55.67)
2	MS basal medium + 0.10 mg/l IBA	75 (68.50)
3	MS basal medium + 0.20 mg/l IBA	45 (53.56)
$CD_{0.05}$		1.42
SE±		0.50

regeneration whether they include transformation step or not are based on TDZ (De Block 1990, Leple et al. 1992, Noel et al. 2002, Ma et al. 2004, Cseke et al. 2007, Tsvetkov et al. 2007, Hui-mei et al. 2008, Yevtushenko & Misra 2010) reported the TDZ based media found to be very efficient for enhancing the frequency of shoot regeneration in *Populus* species. We also found that the concentration of TDZ affect shoot regeneration greatly. The concentration of TDZ up to a certain extent favoured shoot regeneration; further increase in concentration of TDZ would lead to decrease in shoot regeneration frequency, which is in agreement with the similar results reported by Ledbetter & Preece (2004). Malik & Saxena (1992) reported that higher concentration (5-10 μM) and continous exposure to TDZ may cause loss of regeneration in vitro culture. Hence, a precaution is necessary while using TDZ in in vitro cultures. In Himalayan poplar, TDZ in combination with adenine (in MS medium) and an exposure for 2 weeks after regeneration was found to be optimum for maximum in vitro shoot regeneration from leaf explants.

During the present investigation, TDZ was found superior over the various cytokinin in promoting shoot regeneration from leaf explants in *Populus ciliata.* We have obtained the maximum percent (80%) of shoot regenetaion from leaf explants on the MS medium supplemented with TDZ and adenine. Whereas, Thakur & Sriviastava (2006) were used BAP and Kinetin in promoting shoot regeneration from leaf explants in Himalayan poplar and they have reported as maximum as 72% shoot regeneration from leaf explants. The TDZ was highly efficient in inducing shoot regeneration from poplar plants, thus confirming its higher biological activity and the promotive abilities demonstrated in previous tissue culture research (Huttermann & Preece 1993, Mok et al. 1987). The shoot regeneration was obtained through callus formation on TDZ supplemented MS medium. But Thakur & Srivastava (2006) obtained the direct shoot regeneration from the leaf explants of Himalayan poplar on MS basal medium supplemented with different concentrations and combinations of cytokinin and auxins. In contrast, various studies reported plant regeneration from callus cultures derived from leaf discs of *Populus* species and hybrids (Mehra & Cheema 1980, Park & Son 1988, Son & Hall 1990, Xiao et al. 1996, Shen et al. 1998, Liu et al. 2004, Phan et al. 2004 & Kang et al. 2006), which is in agreement with our results obtained during the present studies.

After the present investigation, that a protocol for high frequency plant regeneration from leaf explants in Himalayan poplar (*Populus ciliata* Wall.) has been developed. This protocol allows the regeneration and acclimatization of male plants of Himalayan poplar within 2-3 months. It can be successfully used to carry out transformation studies to transfer various biotic and abiotic stress resistance genes. Thus, the present analysis will provide a platform for in vitro genetic manipulations and mass multiplication of improved genotypes of Himalayan poplar.

References

Balatinecz J.J., Kretschmann D.E., Leclercq A., 2001. Acheivements in the utilization of poplar wood-guideposts for the future. Forestry Chronicle 77: 265-269.

Charenon J., Taris B., 1960. Remarques sur la structure des tissus neoformes et l'apparition d'organes specializes chez quatre cultivars de *Populus* et chez *Salix alba* cultives in vitro. CR Academic Science 251: 2070-2071.

Cheema G.S., 1989. Somatic embryogenesis and plant regeneration from cell suspension and tissue cultures of mature Himalayan poplar (*Populus ciliata*). Plant Cell Reports 8(2): 124-27.

Confalonieri M., Balestrazzi J., Bisoffi S., Carbonera D., 2003. In vitro culture and genetic engineering of *Populus* spp. synergy for forest tree improvement. Plant Cell Tissue and Organ Culture 72(2): 109-138.

Costache I.M., Lowe K.C., Davey M.R., Power J.B., 1995. Improved micropropagation of *Populus* spp. by Pluronic F-68. Plant Growth Regulation 17(3): 233-239.

Cseke L.J., Cseke S.B., Podila G.K., 2007. High efficien-

cy poplar transformation. Plant Cell Reports 26: 1529-1538.

Dai W.H., Cheng Z.M., Sargent W., 2003. Plant regeneration and *Agrobacterium*-mediated transformation of two elite aspen hybrid clones from *in vitro* leaf tissue. In vitro Cellular and Developmental Biology-Plant 39(1): 6-11.

De Block M., 1990. Factors influencing the tissue culture and *Agrobacterium tumefaciens*-mediated transformation of hybrid aspen and poplar clones. Plant Physiology 93: 1110-1116.

Gangoo S.K., Khurana D.K., 2002. In vitro multiplication of poplars: effects of growth regulators and genotypes. Applied Biology Research 4(1/2): 11-16.

Gomez K.A., Gomez A.A., 1984. Statistical procedures for agricultural research. John Wiley and Sons, New York.

Hui-mei Wang, Hong-mei Liu, Wen-Jie Wang, Yuan-gang Zu., 2008. Effects of Thidiazuron, basal medium and light quality on adventitious shoot regeneration from *in vitro* culture stem of *Populus alba* x *P. berolinensis*. Journal of Forestry Research 19(3): 257-259.

Huttenman C.A., Preece J.E., 1993. Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tissue and Organ Culture 33: 105-119.

Jafari M.A., Kiss J., Gergacz J., Heszky L.E., 1995. High efficiency callus induction and plant regeneration in petiole culture of four poplar genotypes. Acta Biologica Hungarica 46(1): 51-59.

Kang W., Zheung J., Liu K.Y, Peng J.X., Hong H.ua Z., 2006. Study on plant regeneration of excised leaf from *Populus deltoides* (I-63 x I-69). Journal of Wuhan Botanical Research 24(1): 83-86.

Ledbetter D.I., Preece J.E., 2004. Thidiazuron stimulates adventitious shoot production from *Hydrangea quercifolia* Bartr. Leaf explants. Scientia Horticulturae 101: 121-126.

Leple J.C., Brasileiro A.C.M., Michel M.F., Delmotte F., Jouanin L., 1992. Transgenic poplars: expression of chimaeric genes using four different constructs. Plant Cell Reports 11: 137-141.

Liu C.L., Shen H., Chang Li.L., HaiLong S., Zhang Y., Huang J., 2004. Tissue culture techniques of *Populus alba* L. var. pyramidalis Bge. Journal of Northeast Forestry University 32(1): 5-7.

Liu C.Z., Murch S.J., Demerdash M.E.L., Saxena P.K., 2003. Regeneration of the Egyptian medicinal plant *Artemisia judaica* L. Plant Cell Report 21: 525-530.

Ma C., Strauss S.H., Meilan R., 2004. *Agrobacterium*-mediated transformation of the genome sequenced poplar clone, Nisqually-1 (*Populus trichocarpa*). Plant Molecular Biology Reporter 22: 1-9.

Malik, K.A., Saxena. P.K., 1992. Thidiazuron induces high frequency shoots regeneration in intact seedlings of pea (*Pisum sativum*), Chickpea (*Cicer arietinum*) and lentil (*Lens culinaris*). Australian Journal of Plant Physiolgy 19: 731-740.

Mathes M.C., 1964. Culture of isolated triploid aspen tissue. Forest Science 10: 35-38.

Mehra P.N., Cheema G.S., 1980. Clonal multiplication in vitro of Himalayan poplar (*Populus ciliata*). Phytomorphology 30: 336-343.

Mithila J., Hall J.C., Victor J.M.R., Saxena P.K., 2003. Thidiazuron induces shoot organogenesis at low concentration and somatic embryogenesis at high concentration on leaf and petiole explants of African violet (*Saintpaulia ionantha* WEndl.). Plant Cell Report 21: 408-414.

Mok M.C., Mok D.W.C., Turner J.E., Mujer C.V., 1987. Biological and biochemical effects of cytokinin active phenylurea derivatives in tissue culture systems. Horticultural Science 22(6): 1194-1196.

Murashige T., Skoog F., 1962. Revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Planta 15: 211-218.

Murthy B.N.S., Murch S.J., Saxena P.K., 1998. Thidiazuron: a potent regulator of in vitro plant morphogenesis. In Vitro Cellular and Developmental Biology 34: 267-275.

Noel N., Leple J.C., Pilate G., 2002. Optimization of in vitro micropropagation and regeneration for *Populus* x *interamericana* and *Populus* x *euramericana* hybrids (*P. deltoides, P. trichocarpa*, and *P. nigra*). Plant Cell Reports 20(12): 1150-1155.

Park S., Oh S., Han K.H., 2004. Large scale computational analysis of poplar ESTs reveals the repertoire and unique features of expressed genes in the poplar genome. Molecular Breeding 14: 429-440.

Park Y.G., Son S.H., 1988. In vitro organogenesis and somatic embryogenesis from punctured leaf of *Populus nigra* x *P. maximowiczii*. Plant Cell Tissue Organ Culture 15(2): 95-105.

Pelah D., Kaushik R.A., Mizrahi Y., Sitrit Y., 2002. Organogenesis in the vine cactus *Selenicereus megalanthus* using Thidiazuron. Plant Cell Tissue and Organ Culture 71: 81-84.

Phan C.T., Jorgensen J., Jouve L., Hausman J.F., Polle A., Teichmann T., 2004. Micropropagation of *Populus euphratica* Olivier. Belgian Journal of Botany 137(2): 175-180.

Rishi A.S., Nelson N.D., Goyal A., 2001. Genetic modification for improvement of *Populus.* Pysiology **7**: 7-21.

Schween G., Schwenkel H.G., 2002. In vitro regeneration in *Primula* Sp. via organogenesis. Plant Cell Report 20: 1006-1010.

Shen H., Watanabe S., Ide Y.HaiLong S., Watanabe S., Ide Y., 1998. Establishment of a callus culture system of *Populus euphratica*, *Populus alba* cv. Pyramidalis and *Populus maximowiczii* x *Populus plantierensis*. Bulletin of the Tokyo University Forests 99: 19-26.

Shen H.L., Wantanabe S., Ide Y., 1998. Establishment of callus culture system of *Populus euphratica*, *Populus alba* cv. Pyramidalis and *Populus maximowiczii x Populus plantierensis*. Bulletin of the Tokyo University Forests 99: 19-26.

Son S.H., Hall R.B., 1990. Plant regeneration capacity of callus derived from leaf, stem and root segments of

Populus alba x *Populus grandidentata* Michx. Plant Cell Reports 9(6): 344-347.

Thakur A., Sharma S., Srivastava D.K., 2008. Direct organogenesis and plant regeneration from petiole explant of male Himalayan poplar (*Populus ciliata* Wall.). Phytomorphology 58(1/2): 49-55.

Thakur A.K., Saraswat A., Srivastava D.K., 2012. In vitro plant regeneration through direct organogenesis and in *Populus deltoids* clone G48 from petiole explants. Journal of Plant Biochemistry & Biotechnology 21(1): 23-29.

Thakur A.K., Srivastava D.K., 2006. High efficiency plant regeneration from leaf explants of male Himalayan poplar (*Populus ciliata* Wall.). In vitro Cellular and Developmental Biology Plant 42(2): 144-147.

Tsvetkov I., Hausman J.F., Jouve L., 2007. Thidiazuron induced regeneration in root segment of white poplar (*P. alba* L.). Bulgarian Journal of Agriculture Science 13: 623-626.

Venverloo C.J., 1973. Formation of adventitious organs in cytokinin induced formation of leaves and shoots in callus cultures of *Populus nigra* L. Acta Botanica Netherland 22: 390-398.

Walter M.W., Grima-pettenati J., Grand C., Boudet A.M., Lamb C., 1988. Cinnamyl alcohol dehydrogenase, a molecular marker specific for lignin synthesis: cDNA cloning and mRNA induction by fungal elicitor. Proceedings of National Academy of Sciences, USA 85: 5546-5550.

Winton L.L., 1968. Plantlets from aspen tissue culture. Science 160: 1234-1235.

Xiao D.S., Xin N.Z., Shi J.Y., Xu L., Sheng Q.Z., Dong Shen-X., Ning Zhang-X., Yuan-ShiJ., Liu X., Qing Zhang-S., 1996. Effects of growth regulators on leaf callus induction and plantlet regeneration in *Populus tomentosa*. Ningxia Journal of Agricultural and Forestry Science and Technology (6): 18-20.

Yevtushenko P.D., Misra S., 2010. Efficient Agrobacterium-mediated transformation of commercial hybrid poplar *Populus nigra* x *Populus maximowiczii* A. Henry. Plant Cell Reports 29: 211-221.