IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 15, Issue 6 Ser. I (June 2022), PP 24-36
www.iosrjournals.org

Factors affecting micropropagation of white poplar (*Populus alpa* L.)

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Abstract: *White Poplar (Populus. alpa L.) is a large, very fast-growing deciduous tree, has emerged as a model organism for forest biotechnology, and genetic modification is more advanced for this genus for another tree. It is tolerant to a wide range of soils and sites, including salty winds and windbreak tree.There is a need for applying non-conventional methods of propagation for sustainable utilization of the plant, therefore a study for creating an efficient micropropagation protocol was initiated, using stem nodal segments and shoot tips from mature trees as initial explants on Woody plant medium (WPM) and Murashige and Skoog (MS) medium. Obtained results showed that WPM supplemented with 1.0mg/l6-benzyl adenine (BA) + 0.1 mg/l α-naphthalene acetic-acid (NAA) was found to be highly suitable for shoot initiation from stem nodal segments during establishment stage. The multiplication rate was further enhanced by adding adenine sulphate (ADS) (20mg/l) and gave 8.63 shoots/explant with an average length of 11.3 cm. WPM medium containing 3mg/l indole–3-butyric acid (IBA) gave the highest mean number (4.7roots/shoot) and length of roots (6.9cm). Effect of phloroglucinol (PG) and arginine (Arg) at different concentrations in WPM medium containing IBA at 3mg/l on rooting improvement was evaluated. The highest number of roots (6.95roots/shoot) was obtained with 20mg/l PG. Also, this study indicates that PG or Arg increased chlorophyll a, chlorophyll b, carotenoids and carbohydrates content in the plant. A percentage of 90% of rooted plantlets were successfully acclimatized and grown normally in the greenhouse in a mixture of sand and peat moss at equal volumes.*

Keywords*: Stem nodal segments, shoot tips, plant growth regulators, micropropagation, additives*

Date of Submission: 03-06-2022 Date of Acceptance: 17-06-2022

I. Introduction

White Poplar (*Populus alba*L.)belong to the family Salicaceae. It is dioecious, with male and female flowers (catkins) occurring on separate trees. The diploid chromosome number in poplar is 2n = 38. The genus *Populus* comprises of five sections, consisting of more than 30 species widely distributed in the Northern Hemisphere (FAO, 1980). White Poplar (*P. alba* L.) is a widespread species found throughout the Mediterranean basin, Central Europe and the Middle East. Selected cultivars of White Poplar have been introduced and widely used in a commercial scale in a number of countries from Europe, North Africa, Near and Middle East during the second half of the twentieth century (Confalonieri et al., 2000). White Poplar should be grown in full sun and tolerates almost any soils, wet or dry (Christersson, 2010). Poplar trees are fast growing and have various uses on a large scale of climate zones for soils stabilization, minimizing wind dust and pollutants (Schnoor, 2000). The economical important of *Populus* species because of wood characters suitability for paper and timber industries (Rautio et al., 2001). This species represents a potentially large source for timber, veneer, plywood, matches, pulp, composites and paper/cellulose (Kishi and Fujita, 2008). Over 50% of the total wood obtained from exploitation is waste, in the form of branches, tops, or sawdust, which may be used as sustainable and renewable fuel (Liang et al., 2006; Verma et al., 2009; Verlinden et al., 2013). Generally, woody biomass is a renewable fuel because the amount of carbon dioxide resulting from burning is equal to that absorbed during tree growth. Some species including White Poplar are also used to reduce phreatic groundwater pollution. Beside its use for wood and biomass production, this species has a wide implementation in horticulture and landscaping, especially the genotypes with a pyramidal tree shape (Eggens et al., 1972; Kovacevic et al., 2010). The White Poplar is a pioneer species, found in humid areas, along rivers, in forest edges and in riparian forest lands. It is tolerant to salt and is used in coastal areas for windbreaks and stabilizing sand dunes. The tree requires plenty of light, good access to water and a well-textured soil. Poplar (*Populus* spp.) is one of the most important economical tree species in temperate regions of the world due to its desirable attributes in adaptability, growth rate, woody biomass, and versatility of its wood for industry (Confalonieri et al., 2003; Lin et al., 2006). Poplar seed has an extremely short period of viability and needs to be sown within a few days of ripening (Huxley,1992). Since the propagation of this species by stem cuttings is rather difficult and

is one of the main obstacles for wider growing of this species, there is a considerable interest in its propagation by tissue culture (Ahuja, 1984; Guzina et al., 1986).Thus, it is necessary to search for optimal culture conditions, in order to achieve satisfactory micropropagation rates and improve and optimize White Poplar tissue culture procedures (Confalonieri et al., 2000).

II. Materials and Methods

1. Plant material and explant sources

White Poplar explants were collected from a mature plant grown in AlqanaterAlkharyrya Research Station, HorticulturalResearch Institute, Agricultural Research Center, Egypt. Explants were moistened and wrapped in wet papers until used.

2. Disinfection of explants

Explants were washed under running tap water for 30 min. Surface sterilization of explants was carried out in a transfer hood. Stem nodal segments were cut into 3-4 cm and then were soaked in 50% (v/v) Clorox bleach solution (2.5% sodium hypochlorite) for 30 min, and shoot tips were soaked in 20% (v/v) Clorox (1.0% sodium hypochlorite) for 15 min, providing them gentile agitation, followed by five sequential rinses in sterilized distilled water. Subsequently, explants were treated with 1% (w/v) mercuric chloride ($HgCl_2$) for one min, followed by washing with sterilized distilled water for six times to remove the traces of $HgCl_2$ solution.

3. Culture medium and conditions

The basal media used in this study were Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) and woody plant medium (WPM; Lloyd and McCown 1980) (Duchefa, Haarlem, the Netherlands) supplemented with 100mg/l myo-inositol and 30g/l sucrose. The pH of the medium was adjusted to 5.7-5.8 before being solidified with 2.8g/l phytagel (Duchefa, Haarlem, the Netherlands). Media were in dispensed in large jars capped with autoclavable polypropylene lids, then autoclaved at a pressure of 1.06 kg/cm^2 and 121°C for 20min. The cultures were incubated at approximately 25°C±2°C with a 16-h photoperiod under cool white florescent tubes (F140t9d/38, Toshiba).

4. Shoot initiation

Two basal media were used and compared to detect suitable medium for shoot initiation. MS and WPM supplemented with different concentrations of the cytokinin; 6-benzyl adenine (BA), at concentrations of 0.0, 0.25, 0.5, 0.75 and 1.0mg/l in combination with α-naphthalene acetic-acid (NAA) at0,0 and 0.1mg/l, in addition to plant growth regulators (PGRs)-free media as control treatment. After six weeks of culturing, shoot initiation %, mean no. of shoots (per explant) and mean length shoots(cm) were recorded.

5. Shoot multiplication

Initiated shoots were used for shoot multiplication, WPM medium was supplemented with BA, Thidiazuron (TDZ) and Kinetin (Kin), individually at concentrations of 1.0, 1.5, 2.0 and 3.0 mg/l, for each, with indole-3-acetic-acid (IAA) at the concentration of 0.5 mg/l. Mean number of shoots (per explant) and mean length of shoot(cm) were recorded after eight weeks.

6. Effect of additives on shoot multiplication

For increase shoot multiplication, rate adenine sulphate (ADS), casein hydrolysate (CH) and glutamine (GLU) were added individually to the multiplication medium at various concentrations of 10.0, 20.0, 40.0 mg/l with the best concentration of cytokinins in order to determine their individual effects on shoot multiplication. Mean number of shoots (per explant) and mean length of shoot(cm) were recorded after six weeks.

7. Rooting stage

For root induction, WPM medium was supplemented with indole–3-butyric acid (IBA), IAA and NAA, individually at different concentrations of 1.0, 1.5, 2.0 and 3.0 mg/l. An experiment for rooting improvement was carried out using phloroglucinol (PG) and arginine (Arg), individually at concentrations of 10, 20 and 40 mg/l for each on the best rooting induction medium. Root formation %, mean number of roots (per shoot) and root length(cm) were recorded after six weeks.

8. Acclimatization stage

The plantlets were removed from the rooting media, washed in water to remove phytagel and soaked in a fungicide solution (Benlate) and then transferred to plastic pots containing equal volume of sand and peat moss. The transplanted plants were covered with plastic bags to maintain humidity, placed in the greenhouse;

the plastic bags were opened gradually. The survival percentage of the *in vitro* propagated plants was evaluated after ten weeks.

9. Determination of photosynthetic pigments

Chlorophyll a and b and carotenoids were extracted from the shoots and estimated according to Fadeel's method (1962) and Von Wettstein (1957). The testing procedures were carried out at low light to protect the pigments from breakdown. Fresh leaf samples (0.5 g) were homogenized in mortar with 85% acetone according to Fadeel's method (1962). The optical densities were measured spectrophotometrically using Pharamisia LKB Novasspec at 622, 644 and 440 nm. The pigment concentrations were calculated using Wettstain's formula (Von Wettstein, 1957) as following:

Chlorophyll a (mg/l) = 9.784 x E662– 0.99 x E644

Chlorophyll b (mg/l) = 21.426 x E644 – 4.65 x E662

Carotenoids (mg/l) = 4.695 X E440 – 0.268 (Chlorophyll a+b)

Where, E = Optical density at the wavelength indicated. The concentration of pigments was then expressed in mg/g fresh weight of leaves according to the following formula:

mg/g = (mg/l x dilution) / (sample weight x 1000)

10. Determination of total carbohydrates

For determination of total carbohydrates content, a known weight of powdered samples was extracted from the dried terminal shoots in1 N HCl solution for six h at 100˚C and filtered. The filtrate was raised to known volume. Total carbohydrates content was determined photometrically at 485 nm according to the phenol sulphuric acid method described by Dubois et al. (1956). Phenol reagent was prepared by adding 10 ml of H_2O to 90 ml of the 5% phenol solution. The resultant solution should be colorless, but may develop a pale-yellow color with time. Standard glucose solution was prepared by adding 50 mg of glucose dissolved in water and raised to a final volume of 100 ml. A standard curve was prepared with a range of 20-150 µl of glucose standard solution. A blank of distilled water was used. Five ml of phenol reagent were added to each tube and mixed thoroughly, then rapidly 5 ml of H_2SO_4 were added to each tube. Samples were allowed to stand at room temperature for 30 min and the absorbance at 485 nm was recorded. The concentrations of glucose were plotted against the corresponding absorbance to generate a standard curve. The concentration of the unknown carbohydrates is determined graphically.

11. Experimental design and statistical analysis of data

The experiments were subjected to a completely randomized design. Analysis of variance (ANOVA) and Duncan' s multiple range test (Duncan,1955), as modified by Snedecor and Cochron (1998) were used to analyze the obtained data. Each treatment of PG and Arg had three replicated and each replicate consisted of nine jars. The experiments were repeated twice. The differences between the averages of the recorded parameters for all treatments were tested for significance at the 5% level. The averages followed by the same letter are not significantly different at $p<0.05$.

III. Results and Discussion

1. Shoot initiation stage

The desertification processes existing in Egypt include urban encroachment on expenses of arable land, wind erosion, water erosion, salinization and water logging (UNECA, 2007). Desertification reversal is accompanied by increases in the content of soil organic carbon, improvements in the physical and chemical properties of soil, stabilization of shifting sand to produce more typical zonal soil, increases in land productivity and biodiversity, and restoration of the ecological balance (Cheng et al., 2004).

The establishment of aseptic culture is the first critical step in *in vitro* propagation process. There are many reports on successful application of sodium hypochlorite for surface sterilization of initial explants (Norton and Norton, 1986; Grant and Hammatt 1999; Debnath 2004). Stem nodal segments and shoot tips were inoculated on basal MS and WPM medium and were further tested using BA and NAA in the present study. The effect of type of medium, explant type and PGRs on shoot initiation%, mean no. of shoots and mean length of shoot(cm) was evaluated. As presented in Table 1 and Figure 1, WPM medium was significantly superior to MS medium which gave the highest shoot initiation percentage and mean no. of shoots and mean length of shoot for both explants. The percentage of shoot initiation reached 100% for stem nodal segments and 83.33% for shoot tips,the mean no. of shoots reached 2.80 and 2.30, respectively andmean length of shoots reached 5.7and 2.8cm, respectively.

Table 1. Effect of media (MS and WPM) and explant type on shoot initiation of White Poplar cultured *in vitro* after six weeks.

Explant type								
Type of medium	**PGRs**		**Stem nodal segment**			**Shoot tip**		
	BA	**NAA**	**Shoot initiation %**	**Mean no of shoots/explant**	**Mean length of roots (cm)**	**Shoot initiation %**	**Mean no of shoots/explant**	**Mean length of shoots (cm)**
MS	0.00	0.1	33.33	1.00 ^{c}	2.10 ^{b}	26.66	1.00 ^{b}	0.80 ^{b}
	0.25	0.1	43.33	1.00 ^{c}	2.10 ^{b}	26.66	2.00 ^{b}	0.80 ^{b}
	0.50	0.1	53.33	1.46 ^{bc}	2.90 ^{b}	36.66	3.00 ^{b}	1.13 ^{b}
	0.75	0.1	70.00	1.93 ^{abc}	3.46 ^{b}	53.33	4.00 ^{b}	1.30 ^{b}
	1.00	0.1	93.33	2.10 ^{abc}	3.80 ^{ab}	63.33	5.00 ^{b}	1.76 ^{b}
WPM	0.00	0.1	33.33	1.00 ^{c}	2.10 ^{b}	26.66	6.00 ^{b}	0.80 ^{b}
	0.25	0.1	63.33	1.80 ^{abc}	2.90 ^{b}	40.00	7.00 ^{b}	0.96 ^{b}
	0.50	0.1	73.33	1.96 ^{abc}	4.41 ^{ab}	56.66	1.46 ^{b}	1.80 ^{b}
	0.75	0.1	86.66	2.56 ^{ab}	4.46 ^{ab}	66.66	1.96 ^{a}	1.90 ^{b}
	1.00	0.1	100.00	2.86 ^{a}	5.76 ^{a}	83.33	2.30 ^{a}	2.80 ^{a}

Mean followed by the same letter within a column are not significantly different at $p > 0.05$

The early finding reported by several investigators on pomegranate (Kantharajah et al., 1998, Naik et al., 2000, Murkute et al., 2004) are in harmonywith the results obtained in the present study. The results prove that WPM medium containing BA was the most suitable medium for the shoots initiation, also Ankita et al. (2021) reported the same results for production of multiple shoots on *Ficus carica*var. Black Jack. For woody plants, usually the more diluted media are used such as WPM with lower concentrations of nitrogen and potassium salts (Lloyd and McCown, 1980). These experiments proved that the WPM composition is suitable for shoot regeneration in *Daphne mezereum* 'Alba', especially in the long-term cultures. Shoots were intensively green, without any leaf discolorations and both stems and leaves were well formed (Karolina et al.,2019). The explants successfully produced several new shoots over six weeks, which indicates that the best media was WPM. The highest average number of shoots regenerated from original explants of*Juniperus procera*was obtained on WPM (Abdalrhamanet al.,2021). WPM was also the best medium for stimulating shoot length and the development of adventitious buds of goji (Arleta et al.,2021). A nutrient medium containing a reduced salt level has been employed by various workers in *in vitro* culture of *Salix* species (Chalupa,1983, Bergman et al.,1984). WPM medium has also been used for micropropagation of *Lonicera periclyme*num (Boonnour et al.,1988). Shoot bud induction of*Santalum album* was initiated in WPM medium supplemented with different combinations and concentrations of BA and NAA andshoots became greenish, nodular and more organized (Singh, et al.,2016). Regarding the variation of different concentrations of BA and NAA in the culture media (MS and WPM), shoot initiation percentage, mean no. of shoots per explant and mean length of shoot(cm) increased with increasing concentration of BA. The maximum shoot bud sprouting (100%), shoot number (2.80) and shoot length (5.76 cm) occurred in stem nodal segments and proved better than shoot tip explant. The differential responses of the two explant types are likely due to differences in endogenous hormone levels. Faye et al. (2015) found that the responses of organogenesis of *Manihot esculenta* from the assessed variables changed based on the type and concentration of PGRs, regarding the effect type of explant, nodal explants were responsive to all treatments tested with PGR indicating the high regenerative potential of nodal explants.Nodal explants were superior to shoot tip explants in terms of multiple shoot induction and proliferation of *Pimpinella anisum* (Ahmed and Hnaa,2019). The superiority of nodal over shoot tip explants for multiple shoot proliferation has been demonstrated in other medicinal plants including *Menthapiperit*a (Sujana et al., 2011), *Withaniasomnifera* (Fatima and Anis, 2012), *Agastachefoeniculum* (Moharami et al., 2014) and *Teucrium scorodonia* (Makowczyńska et al., 2016).The production of nodal segments is a relevant factor for micropropagation, since it reflects the generation of new plants at each sub-cultivation (Flores et al., 2009). Ribeiro et al. (2014)said that the larger number of leaves implies a higher multiplication rate due to the increased formation of nodal segments of *Calla lily*. The nodal segments of*Ficus religiosa*induced shoot buds on WPM supplemented with 1.0 mg/l BAP and 0.5 mg/l IAA (the medium yielding maximum number of healthy-looking shoot buds) (Priyanka and Anita,2011). For woody plants, using a cotyledonary node as an explant is relatively easy to promote adventitious shoots (San-Jose et al., 2001; Huang et al., 2014; Li et al., 2016).

2. Multiplication stage

The probable attribute for this synergism is cross-talk mechanism of auxin and cytokinins, which induce new bud primordia, subsequent bud formation and shoot regeneration in cultures (Lodha et al., 2014; Zhang et al., 2015). Multiplication of primary explants is highly dependent on plant species and the type of cytokinins used (Sandal et al., 2001).

Shoot multiplication was evaluated by varying concentrations of BA, TDZ, Kin, IAAadded to WPM medium to study their effect on the multiplication rate. The number of shoots per explant was significantly affected by the type and level of cytokinin. BA was the most efficient cytokinin in the proliferation of axillary buds (Table 2). The highest mean number of shoots was recorded on BA at 3.0 mg/l +NAA at 0.5 mg/l, compared to the media containing different concentrations of TDZ or Kin, they reached 5.86 shoots per explant. Superior results in response to BA for shoot induction compared to other cytokinins have been reported in different species, Zimmerman and Scorza (1994) reported that BA at a 2.3 mg/l concentration resulted in a greater shoot multiplication rate for three peach genotypes. Moreover, BA has been well documented as an efficient inducer for shoot multiplication in many woody plants, including *Eucalyptus impensa*(Bunn, 2005) and *Sapindus trifoliatus* (Asthana et al., 2011). The number and frequency of shoot induction were dependent mainly on the concentration of cytokinin used in the culture medium (Sharma, 2017). Auxin has been proved to be efficient for woody plant shoot proliferation (Mathur et al., 2002; Siwach and Gill, 2011; Li et al., 2016). The response of shoot regeneration to the combination of cytokinins (BA,Kin) and auxins (NAA) was better compared to the effects of cytokinins only(Van et al.,2018). BA was reported in literature as the most effective cytokinin for shoot induction and multiplication in cucurbits (Ananthakrishnan et al., 2003),*Lagenariasiceraria*(Saha et al., 2007) and *Citrullus lanatus*(GanasanandHuyop, 2010). Furthermore, supply of auxin (NAA) at low concentration to the media containing cytokinin enhanced shoot proliferation (Reuveni et al.,1990).

However, maximum shoot length(6.8 cm) was observed on Kin at 3.0 mg/l + NAA at 0.5 mg/l, the shoot length increased with increasing concentration of Kin. With BA, TDZ shoot length decreased on increasing concentration (1.0-3.0mg/l). Dantu and Bhojwani (1987) and Prasad and Chaturvedi (1992) also found that higher levels of BA reduce the shoot elongation of *in vitro* plantlets of *Gladiolus* and *Amaryllis*, respectively. Kin is strongly recommended than using Zeatin, TDZ and BA to obtain the highest shoot length for nodal explants of cucumber (Saeid et al., 2015). Romanov et al. (2000) and Sota et al. (2020) reported that Kin induced cell elongation.The best shoot length (11.7 mm) of *Matthiolaincana*was obtained when 2 mg/l Kin was used (Afshin et al., 2011).However, Zhe et al. (2021) found that Kinhad a slightly better effect on shoot elongation of*Hylocereuspolyrhizus* than BA as Kinproduced shoots with longer length than BA.

Table 2. Effect of different concentrations of BA, TDZ, KIN and IAA in WPM medium on shoot multiplication of White Poplar after six weeks.

Conc. (mg/l)				Mean no. of shoots/explant	Mean length of shoots (cm)
BA	TDZ	KIN	IAA		
1	0.0	0.0	0.5	2.53 ^{b}	4.56 ^{bc}
2	0.0	0.0	0.5	2.56 ^{b}	4.56 ^{bc}
2	0.0	0.0	0.5	4.60^{ab}	4.56 ^{bc}
3	0.0	0.0	0.5	5.86 ^{a}	2.86 ^{cd}
0	1.0	0.0	0.5	2.53 ^{b}	3.46 ^{cd}
0	1.5	0.0	0.5	2.53 ^{b}	2.76 ^{cd}
0	2.0	0.0	0.5	3.10 ^{b}	1.86 ^{d}
0	3.0	0.0	0.5	3.23 ^{b}	2.96 ^{cd}
0	0.0	1.0	0.5	2.90 ^{b}	3.36 ^{cd}
0	0.0	1.5	0.5	3.80 ^{b}	4.20 ^{bc}
0	0.0	2.0	0.5	3.80 ^{b}	5.80 ^{ab}
0	0.0	3.0	0.5	4.20 ^{ab}	6.80 ^{a}

Means followed by the same letter within a column are not significantly different at $p > 0.05$

3. Influence of additives on shoot multiplication

A shoot multiplication protocol could have a large impact on our ability to rapidly multiply *in vitro* desirable fruit rootstocks and ensure plant availability throughout the year accordingly (Vujović et al.,2012). Influence of additives was studied after the optimum cytokinin to improve the quality of shoots (Table 3). Results showed that all additives supplemented individually, positively affected the shoot multiplication. The highest number of shoots formed was obtained on medium supplemented with 40 mg/l ADS with an average of 8.63 shoots per explant. Also,it gave the highest mean length of shoot (11.3 cm), comparing to the different concentrations of additives.

Table 3. Effect of adenine sulphate, casein hydrolysate and glutamine supplemented in different concentrations to WPM medium having 3.0 mg/l BA on shoot multiplication of White poplar after six weeks.

ADS(mg/l)	CH (mg/l)	GLU (mg/l)	Mean no.of shoots/explant	Mean length of shoots(cm)
10	0	0	6.46 [ab]	8.96 [ab]
20	0	0	6.93 [ab]	8.76 [ab]
40	0	0	8.63 [a]	11.30[a]
80	0	0	7.20[ab]	9.40[ab]
0	10	0	5.60[ab]	7.90[ab]
0	20	0	5.96 [ab]	8.46 [ab]
0	40	0	6.26 [ab]	9.26 [ab]
0	80	0	7.63 [ab]	9.53 [ab]
0	0	10	6.20[ab]	5.43 [b]
0	0	20	6.96 [ab]	6.60[b]
0	0	40	4.86 [ab]	5.86 [b]
0	0	80	4.26 [b]	5.80[b]

Mean followed by the same letter within a column are not significantly different at $p > 0.05$

This observation was similarly reported by Nandagopal and Ranjitha (2006) andMedzaMve et al. (2010), who demonstrated that adenine has the same activity as cytokinin and adding it to the culture medium will improve growth. ADS Followed by CH at 80 mg/l which gave 7.63 shoots per explant. The addition of GLU (20 mg/l) was shown to promote the formation of multiple shoots of *Hibiscus moscheutos*and could increase the mean number of multiple shoots. GLU was found to be superior to other PGRs for shoot growth of *Hibiscus moscheutos* when produced using tissue culture practices (Zhitong and John,2018). Data also revealed that multiplication rate was gradually decreased with increasing additives concentration. In an agreement with these results,optimization of the amino acid content can bring about different morphogenetic responses however; higher concentrations of amino acids have been shown to be general growth inhibitors in *Cicer arietinum* (John and Mukherjee, 1997).

4. Rooting stage

The nutritional quality of the shoot is related to rooting, and the presence of roots likely favors an adequate plant metabolism, resulting in the production of more vigorous shoots (Brondani et al., 2012). WPM medium was supplemented with three auxins tested for rooting of White Poplar, presented in Table 4. The highest rooting percentage (100%) was obtained by using IBA and IAA. The highest mean number (4.73 roots/shoots) and length (6.93 cm) of roots was observed on WPM medium with 3.0 mg/l of IBA, which was found to be the most suitable for *in vitro* rooting comparing to other auxins tested. IBA plays a cardinal role in rooting, where its utilization in culture media presents the highest percentage of roots, and also contributes to a better induction of lateral roots compared to IAA as reported by De Klerk(2002) andChannuntapipat et al.(2003). Here the result showed consistency with other studies where the addition of IBA promoted the induction of roots in several systems including *Citrus reticulata*, *Citrus limon* (Singh et al., 1994), *Ocimumbasilicum* (Sahoo et al., 1997), *Salvia sclarea* (Liu et al., 2000),*Artemisia judaica* (Liu et al., 2003), *Dioscoreazingiberensis* (Chen et al., 2003), *Bixa orellana* (Neto et al., 2004), *Woodfordia fruticose* (Islam et al., 2009) and *Ophiorrhizaeriantha* (Jaimsha et al., 2010). In addition to IBA concentration, the period of pulse treatment has been shown to have significant effects on the average number of roots produced per shoot on *Catharanthus roseus* (Rupesh et al., 2013). Exogenous IBA also had a significant effect on root induction rates and the number of roots (Zhiming and Jun,2016). IBA was found to induce number of roots when compared to NAA (Ayyadurai and Ramar, 2019).

Table 4. Effect of different concentrations of auxins (IBA, IAA and NAA) in WPM medium on *in vitro* rooting of White Poplar after six weeks.

Auxins conc. (mg/l)			Root formation %	Mean no.of roots/shoot	Mean root length(cm)
IBA	IAA	NAA			
1.0	0.0	0.0	100.00	2.83 ^{bc}	4.76 ^{ab}
1.5	0.0	0.0	100.00	3.10 ^{bc}	4.76 ^{ab}
2.0	0.0	0.0	100.00	3.83 ^{b}	4.76 ^{ab}
3.0	0.0	0.0	100.00	4.73 ^{a}	6.93 ^{a}
0.0	1.0	0.0	88.88	2.86 ^{bc}	3.86 ^{b}
0.0	1.5	0.0	93.33	2.93 ^{bc}	4.76 ^{ab}
0.0	2.0	0.0	100.00	3.10 ^{bc}	4.9 ^{ab}
0.0	3.0	0.0	100.00	3.63 ^{b}	5.6 ^{ab}
0.0	0.0	1.0	46.80	2.20^{c}	3.96 ^{b}
0.0	0.0	1.5	67.70	2.66 ^{bc}	4.86 ^{ab}
0.0	0.0	2.0	80.80	2.93 ^{bc}	4.96 ^{ab}
0.0	0.0	3.0	80.80	3.20 ^{bc}	5.16 ^{ab}

Mean followed by the same letter within a column are not significantly different at $p > 0.05$

5. Effect of Different additives on rooting

Root quality is an important index of *in vitro* plantlets quality and a critical factor for transplanting, adventitious rooting. Shoots formed roots on WPM medium containing 3.0 mg/l IBA and different concentrations of PG and Arg separately (Table5). The highest roots number and length was achieved on 20 and 40 mg/l PG, which gave 6.96 and 6.0 roots/shoot, respectively (Figure5). Also,they gave 7.3 and 10.5 cm length, respectively. Followed by 20 mg/l Arg that gave 5.82 roots/shoot. In agreement with these results, Rafael et al. (2021) reported that PG acted synergistically with auxin, whichstimulated the rooting of *in vitro* shoots of sugarcane cultivar C90-469. PG strongly promotes adventitious root inductionin WPM medium. Rooting efficiency is a critical parameter for the success of commercial micropropagation technology (De Klerk et al., 2011; Daud et al., 2013;Norhayatiet al., 2013).

Table 5. Effect of PG and Arg in WPM medium with 3.0mg/l IBA on rooting formation of White Poplarafter six weeks from culturing.

Additives conc. (mg/l)		Mean no. of roots/shoot	Mean length of roots (cm)
PG	Arg		
10	0	5.44 ^{ab}	7.30^{ab}
20	0	6.96 ^{a}	7.36 ^{ab}
40	0	6.00^{ab}	10.50^{a}
0	10	4.90^{b}	7.20^{ab}
0	20	5.82 ^{ab}	8.20^{ab}
0	40	5.10^{ab}	6.43 ^{b}

Means followed by the same letter within a column are not significantly different at $p > 0.05$

The results of this study show a synergistic effect of PG with IBA, an analog of auxin which can be degraded by decarboxylation. PG and its precursors (such as phloridzin) or its products of metabolism (such as phloretic acid) have the potential to influence a wide range of plant growth processes and development, although it is expected, as for most other phenolic compounds and PGRs, that excessively high concentrations would have an inhibitory effect (Jaime et al., 2013). PG combined with IBA stimulated root elongation, formation of new roots, and increased percent rooting as compared to the treatment without PG with zeolite (Laisyn et al., 2016). Copper-containing enzymes catalyze the oxidation and decarboxylation of IAA and may be responsible for the presumed acceleration of IBA breakdown in WPMmedium. By adding PG, these enzymes would be inhibited and allow a more sustained presence of IBA in WPM medium, which is favorable for the cultivation of

woody plant species due to a reduced nitrogen content. The increase in root number per rooted explant in the CAB-6P apple rootstock could be ascribed to the efficient use of Arg as an N source. Furthermore, protein synthesis from Arg is more efficient and needs less energy consumption in comparison to inorganic N. It is evident that the increased needs for N due to promotion of metabolism, when auxin was added, could be easily covered in the culture medium by the supplied Arg (Orlikowska, 1992). The role of Arg in rooting may be also considered as a source of components for auxin conjugates, which can affect rooting (Zelena and Fuksova, 1991) or a source of polyamine precursors (Evans and Malmberg, 1989). Finally, a special role for Arg in the stimulation of apple rooting can be connected with the fact that it is a main nitrogen storage compound in dormant apple trees (Tromp andOvaa, 1973). L-arginine promotes the positive effect of IBA on rooting with regard to both root number and root length in both cherry rootstocks (Virginia et al., 2014). The enhancement of the rooting capacity due to Arg could also be ascribed to Arg absorption from the culture medium and its metabolism, producing proteins rich in Arg during DNA transcription. Assimilation of Arg from the nutrient medium was reported in grape (*Vitis vinifera*) protoplasts (Theodoropoulos and Roubelakis-Angelakis, 1989). It is worth mentioning that the amino acid Arg has a direct effect on *in vitro* rooting of the CAB-6P and Gisela 6 apple explants.Couée et al. (2004) indicated that the stimulation of root growth and development by polyamines and consequently by Arg may be related to increased ethylene synthesis.

Chlorophyll pigment content

Chlorophyll contents are the key indicator of the photosynthetic activity, and the carotenoids are involved in the defense mechanism against oxidative stress (Shah et al., 2017). The effect of PG and Arg on chlorophyll a, chlorophyll b, carotenoids and carbohydrates content were evaluated (Table6). It was clear that leaf chlorophyll a and b, carotenoids and carbohydrates content were increased in treated plantlets at the different treatments compared with the control. The highest amount of chlorophyll a was recorded on WPM medium supplemented with 20mg/l PG, it reached 0.47. Chlorophyll b reached the maximum of 0.21 mg/g at 40 mg/l PG. While the maximum carotenoids and chlorophyll content was obtained with 10 mg/l Arg (0.21 and 2.25 mg/g, respectivle). 0n the other hand, the control had the lowest amount of chlorophyll a and b, carotenoids and carbohydrates contents.

Table 6. Effect of different concentration of PG and Arg on the contents of chlorophyll a, and b, carotenoids and carbohydrates of White Poplar.

Conc. (mg/l)		Chl. a (mg/g)	Chl. b (mg/g)	Carotenoids (mg/g)	Carbohydrates (mg/g)
PG	**Arg**				
0	0	0.01^{e}	0.04^{d}	0.08^{d}	0.03^{d}
10	0	0.05^{d}	0.16^{b}	0.13^{c}	1.35^{b}
20	0	0.47^{b}	0.20^{a}	0.15^{b}	1.28^{b}
40	0	0.10^{c}	0.21^{a}	0.08^{d}	2.12^{a}
0	10	0.04^{d}	0.10^{c}	0.21^{a}	2.25^{a}
0	20	0.05^{d}	0.15^{b}	0.07^{d}	1.32^{b}
0	40	0.77^{a}	0.21^{a}	0.10^{d}	0.83^{c}

Mean followed by the same letter within a column are not significantly different at $p > 0.05$

The foliar chlorophyll contents are the key indicator of the photosynthetic activity, and the carotenoids are involved in the defense mechanism against oxidative stress (Shah et al., 2017). In *Caricapapaya*, the best results obtained with 158 µMPG, which produced higher contents of chlorophyll a and b in the leaves of plants as compared with the control (Laisyn et al., 2016). Also, Pérez et al. (2016) found that incorporation of 79 µM PG in the medium improved the rate of photosynthesis in *Carica papaya*. The carotenoids were increased to protect the photosynthetic apparatus against the stress impact of increased PG in micropropagated*Coccolobauvifera*(Manokari et al., 2021). Aremu et al. (2015) reported that PG improved morphology, bioactive compounds, and enhanced bulblets formation in *Ecklonia maxima*, which indicates the positive role of PG in the development of storage organs in plants. Regarding the effect of Arg, the results indicated that, the levels had significant positive effect on chlorophyll a and b, carotenoids and carbohydrates compared with control medium. On the other hand, the leaf chlorophyll content significantly increased withthe addition of IBA, regardless of its concentration, withoutArg, as compared to the control (Virginia et al., 2014). While, Yagi and Al-Abdulkareem (2006) found that Arg significantly increased leaf chlorophyll content in *Eruca sativa* shoots.

Acclimatization

The acclimatization of *in vitro* plants to natural conditions is a critical step for many species and requires time and expensive facilities, which restrict the commercial application of micropropagation processes (Fila et al., 1998). It is evaluated by percentage of the surviving plants, which was clearly affected by the

additives PG and Arg. The results indicated that acclimatization of rooted shoots in the new environment in the greenhouse ranged between 65 and 95%. The highest percentage of survival plants with new leaves was achieved 95% when cultured on medium with 20- 40mg/l PG, followed by 40 mg/l Arg which gave 85% survival percentage. On the other hand, the survival percentage of plants was the lowest (65%) when cultured on WPM medium without supplementation of PG and Arg(control). PG had a positive effect on rooting and *in vitro* acclimatization of the shoots of papaya var. Maradol Roja (Laisyn et al., 2016). PG is a growth regulator that is a product of the degradation of phloridzin and precursor in the biosynthetic pathway of lignin. It is a phenolic compound known for its properties as a promoter of plant growth, even though its effect as usually masked by other similar compounds (Teixeira da Silva et al., 2013). Its positive effect on lignification has been demonstrated (Ross and Castillo 2009, 2010). PG had a narrower pith, better xylem development, and increased lignification (Ross and Castillo, 2010). PG had a positive effect on rooting and acclimatization of rooted shoots (Alvine et al.,2020). Polyamines and their precursor Arg have been implicated as vital modulators in a variety process in higher plants, such as growth, physiology, and development, as well as in plant response to various stress factors. It has also been recorded that both endogenous and exogenous Arg play a role in plant stress response (Liu, et al.,2015; Winter et al.,2015).

IV. Conclusion

In conclusion, White Poplar can be successfully micropropagated using stem nodal segments and shoot tips as explants. The results also indicated that the application of additives; ADS and PG have the greatest effect on the development of White Poplar shoot and root *in vitro* and improvement of the survival during acclimatization.

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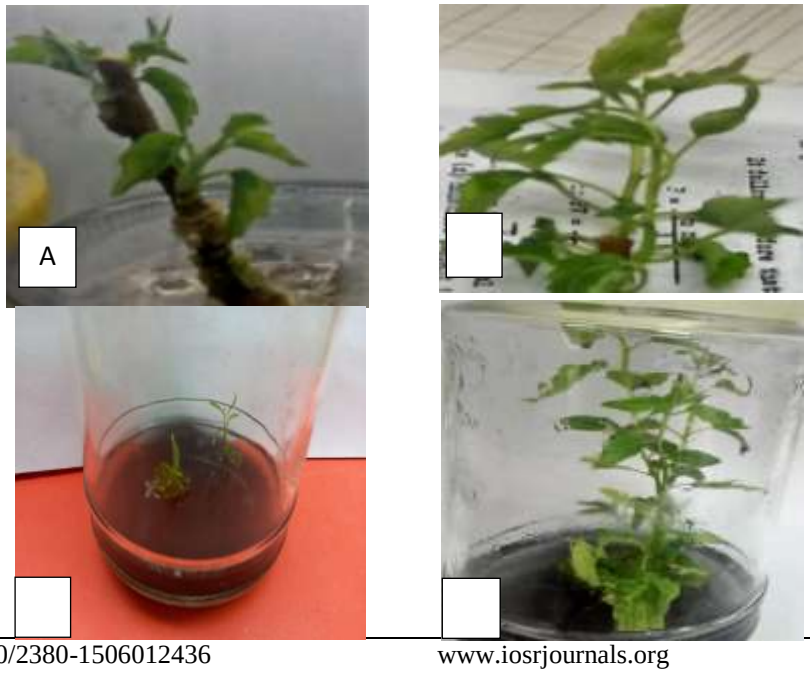

Figure 1. Culture initiation of White Poplar. A. stem nodal segment after two weeks and B. After six weeks. C. shoot tip after two weeks and D. after six weeks.

Figure2. Shoot multiplication of White Poplar on WPM medium with3mg/l BA and 0.5 mg/l IAA.

Figure 3. Enhancing shoot multiplication of White Poplar on WPM medium containing 3mg/l BA and 40mg/l ADS.

Figure 4. Rooted plantlets of White Polar in WPM medium containing NAA, IBA and IAA.

Figure 5. Root development of White Polar in WPM medium supplemented with 3mg/l IBA in addition to 40mg/l PG.

Figure 6. Hardened White Poplar plant under greenhouse conditions.