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Factors Influence Shoot Proliferation and Short-Term Conservation of *Pyrus betulaefolia* Rootstock

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Abstract: *Pyrus betulaefolia* is the most tolerant decline of pear, exhibits good adaptability to several environmental conditions. It is high tolerance to drought, salt and cold. The aim of this work was to establish an efficient protocol for shoot proliferation of *Pyrus betulaefolia* as the first step of micropropagation of this valuable rootstock in Egypt and enhance the procedure of shoot preservation *via* synthetic seeds in order to provide a continuous source of shoots with reducing the cost. Shoot proliferation was affected by the strength of the nutrient medium, the growth regulators concentration and combination, the sucrose percent as well as the incubation condition especially the light intensity. The maximum shoot proliferation (51.0 shoots/shoot tip) and the valuable growth parameters; shoot length, nodes number and growth vigor were obtained when full or 3/4 MS strength was implemented and supplemented with 4% sucrose + 1.5 mg/l BA + 1.5 mg/l Kn + 100 µg/l Ancymidol and incubated in 1000 lux (as light intensity). Synthetic seeds employed for *in vitro* conservation of *P. betulaefolia*; the composition of synthetic seeds played as an artificial endosperm. Three composition were examined, MS, MS + 0.5 mg/l BA and MS + 0.5 mg/l BA + 100 µg/l Ancymidol. The presence of BA in the composition of synthetic seeds promoted germination (after 44.33 days), shoot proliferation (19 shoots/synseed) and enhance the ability of storage to 36 weeks with a moderate number and percent of vital synseeds (11.67syneeds, 48% vitality, respectively). While synthetic seeds which contained both BA and Ancymidol delayed the germination of synthetic seeds (77 days) and depressed the proliferation in the early period of conservation and promote both the number of shoot proliferation (28.33 shoots/synseed) as well as the number and percent of vital synseeds in the late period of conservation (24.67 vital synseeds and 97.33% vitality, respectively). Results may due to the suppressed effects of Ancymidol (the anti-gibberellin) which inhibited plant growth, retarded synthetic seeds germination and configured the encapsulated explants to store; this behavior may aid in the late period of conservation to maintain vital with high ability to shoot proliferation.

Key words: *Pyrus betulaefolia* · Micropropagation · BA · Kn · Ancymidol · Synthetic seed · Preservation · Light intensity · Sucrose

INTRODUCTION

There is a real need for Pyrus rootstocks because of the variation in the environmental conditions in addition to the lake of water and the diseases sensitivity of the economic scions. *Pyrus betulaefolia* is the most tolerant decline of pear, exhibits good adaptability to several environmental conditions. It is high tolerance to drought, salt and cold. This is due to its superior ability to regulate osmotic potential, in addition, the high unsaturation fatty acids which present in biological membranes of *P. betulaefolia* [1]. *P. betulaefolia* seeds are exported from abroad, which costs a lot of money beside its germination troubles as a result of bad storage or transportation conditions. Hence, tissue culture can be a supernatural solution if there is an efficient protocol for micropropagation and successful procedure for plant preservation.

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In plant tissue culture, media composition is the factor which determines the enhancement of plant growth. Micropropagation influenced by manipulating the plant growth regulators as well as MS media strength which could affect the obtained number of shoots [2-4]. Woody rosaceous species and cultivars used MS medium as a mineral medium for re-generation and/or proliferation, subsequent for rooting, BA was the supplement to MS to induce shoot proliferation. Also, agar was used for medium solidification alone or with a mix of gellan gum and sometimes double-phase culture system; solid and liquid layer [5-7]. The most multiplication methods used full or half strength of MS basal nutrient medium with slight modifications [8 and 7]. On contrary, the lower concentration of major elements in Woody Plant Medium (WPM); was more suitable for micropropagation of some pear genotypes [9, 10].

Cytokinin is the most important hormone in plant proliferation. It plays many roles in the development of the plant like the promotion of the cell division and expansion, stimulation of plant protein synthesis and some enzymes activities [11]. For pear micropropagation, BA is the most cytokinin frequently used [10]. Increasing the concentrations of BA to 1 mg/l in MS medium resulted in significantly increasing in the number of micro-shoot/explants and the highest shoot proliferation was obtained from 1 mg/l BA, but the concentration of BA which was higher than 1 mg/l lead to the reduction of new micro-shoot number per explant [12, 13]. Other report concluded that BA augmented shoot proliferation of "Sebri" pear cultivar, 3 mg/l BA was more effective than 2 mg/l on the number of shoots [14, 15].

Shoot developed and shoot proliferation were affected by carbohydrates through affecting the growth and the frequencies of shoots [16]. In pear culture, sucrose is classified as the most carbon source. Increasing the concentrations of sucrose in tissue culture medium to 38g/l promoted shoot proliferation [17]. Agar, mixtures of agar and gellan gum or corn starch and gellan gum are used as a solidifying agent [18, 19]. Ancymidol; a type of pyrimidines, is a plant growth retardant extensively used in foliage plant production. Its strength as a member of growth retardant is intermediate between the strongest plant growth regulators, such as the triazoles and, the weaker ones, like daminozide or chlormequat [20]. It inhibits a step of biosynthetic pathway of the gibberellin, which responsible of the oxidation of ent-kaurene to form ent-kaurenoic acid, by an enzymatic active site disruption [21]. Ancymidol implemented in tissue culture as anti-gibberellin, which may aid in push plant to store or form storage organs like microtubes or microcormes as well as enhance *in vitro* growth vigor of shoots [22].

In vitro shoots need frequent transfers (subculture), renewed medium which need preparation, follow-up for the incubated plants in the growth room, human efforts and a lot of money, however, all of these procedures can be reduced by storage the cultures in a cold or cool environment or with reducing the nutrients [23]. *In vitro storage* is an important technique for germplasm in an active gene-bank. Tropical and sub-tropical plants can be preserved for long periods at sub-normal temperatures (15-18°C) [24].

The synthetic seed technology provides advantages such as short-term and long-term preservation, simplify distribution or translocation, as well as protect tissues from injuries. Also, encapsulation plays a role as an endosperm-containing, nutrients, growth regulator and carbon source [25]. MS medium fortified with 2 mg/l IAA possessed the highest synthetic seed germination [26, 25]. On the contrary, in cultivars of pear (*Pyrus communis*), an increasing of BA concentration led to produce a higher number of shoots and the maximum frequency of conversion of encapsulated into plantlets [27, 28]. Storage duration can vary greatly according to genus, woody plants possessed longer storage duration than herbaceous ones. The growth medium as well as the growth regulators used may influence storage period [29-31].

This investigation aimed to establish an efficient protocol for shoot proliferation of *Pyrus betulaefolia* as the first step of micropropagation of this valuable rootstock in Egypt and enhance the procedure of shoot preservation *via* synthetic seed in order to provide a maintenance source of shoots with reducing the cost.

MATERIALS AND METHODS

Plant Materials: One-year old nursery plants; from South El tahrir Horticultural Research Station, were employed as a source of explants. In the beginning of break dormancy, swelling buds were aseptic excised from cutting shoots.

Establishment the *In vitro* Culture

The Explants Sterilization: Cuttings, including swelling buds before bloom, were carefully washed and air dried. In laminar air flow, cuttings were dropped into ethyl alcohol (70%) for 60S then it was burned to remove the

out ward ness layers which covered the buds according to Ahmed *et al.* [32]. Then the buds were carefully separated and cultured on the medium. The free contaminated shoots were used in the following experiments. *In vitro* shoot tips were used as explants for the investigations.

Preparation of Culture Medium: Murashige and Skoog [33] medium (MS) was used in all investigations. The pH was adjusted as 5.7 prior to add the gelling agent (2 g/l gelrite). Then the medium was distributed into 350ml jars (50 ml/jar). The medium was sterilized in autoclave.

Incubation Conditions: All investigations were incubated at 20±2°C and photoperiod 16/8 h light/dark, if its not mention or examined factor in the investigation.

Shoot Proliferation:

Effect of MS Salt Strength and Light Intensity on Shoot Proliferation of *P. betulaefolia* Rootstock: Effect of MS salt strength (Full, 3/4 and 1/2 salt strength) in combination with light intensity (2000, 1000 lux and total darkness) on shoot proliferation was examined. Each treatment contained five jars and each jar contained four shoot tips. The cultures were incubated in growth room at 20±2°C. The number of shoots/shoot tip, shoot length (cm), nodes number/shoot and growth vigor as described by Pottino [34] were recorded after six weeks.

Effect of Concentrations of BA in Combination with Kn and/or Ancymidol on Shoot Proliferation of *P. betulaefolia* Rootstock: Shoot tips of *P. betulaefolia* rootstock were cultured on 3/4 MS medium supplemented with different concentrations of benzyl adenine (BA) (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) individual or in combination with 1.5 mg/l Kinetin (Kn) and/or 100µg/l Ancymidol (Anc) (the growth retardant) were examined. Four shoot tips were cultured in each jar and each treatment included five jars. The cultures were incubated in growth room at 20±2°C and 2000 lux. The number of shoots/shoot tip, shoot length (cm) and growth vigor were recorded after six weeks.

Effect of Sucrose Percentage in Nutrient Medium in Combination with Light Intensity on Shoot Proliferation of *P. betulaefolia* Rootstock: The recommended MS salt strength and growth regulators combination from the previous experiments were used in this investigation. Different concentrations of sucrose (20, 30, 40 and 50 g/l) (2, 3, 4, 5%) in the nutrient medium in combination with light intensity (1000, 2000 and total darkness) were investigated. Four shoot tips were cultured in 350 ml culture jar and each treatment included five jars as a replicate. The response of sucrose concentrations and light intensity on growth parameters of *P. betulaefolia* rootstock were recorded as a number of shoots/shoot tip, shoot length (cm) and nodes number/shoot after six weeks.

Conservation of *P. betulaefolia* Rootstock *Via* Synthetic Seeds:

Synthetic Seeds Formation: Sodium alginate was prepared by mixing with liquid MS medium, then the explants were mixed with the Na-alginate solution and dropped into a solution of calcium chloride ($CaCl_2.2H_2O$), where the ion exchange reaction occurs and sodium ions were replaced by calcium ions forming calcium alginate beads (Fig. 1) as described by Hamza [35].

Effect of Several Compositions of Synthetic Seeds on Germination, Shoot Proliferation and Vital Number and Percent of Synthetic Seeds along the Conservation Period of *P. betulaefolia* Rootstock: Sodium alginate (4% w/v) was dissolved in various, constituents; MS, MS + 0.5 g/l BA or MS + 0.5 g/l BA + 100 mg/l Anc., where the MS medium supplemented with 40 g/l sucrose and the pH adjusted at 5.7, Then 100mM $CaCl_2.2H_2O$ was prepared and all solutions were sterilized in autoclave prior to use. The shoot tips were encapsulated with one of the previous prepared sodium alginates then dropped in 100 mM $CaCl_2.2H_2O$ for 15 min.; to allow occurrence of ions exchange, then seeds were collected and washed twice with sterilized distilled water then air dried in the laminar air flow on sterilized tissues. Five synthetic seeds were cultured in 110 ml jars, which were incubated in growth room at 18°C and light intensity 1000 lux. Each treatment contained five jars as replicates (total initial number of synseeds was 25 synseeds/treatment) and repeated three times. Period to seed germination (day) (Fig. 1), number of shoot proliferation/synthetic seed after various periods of conservation as well as number of vital seeds and seed vital percent after 36 weeks were recorded.

Statistical Analysis: All investigations were arranged in a completely randomized design. According to SAS Institute [36], the differences among the investigated treatments were compared using LSD at 5%. The ANOVA and LSD were determined by the MSTAT free analysis software.

Fig. 1: A: Ions exchange and synthetic seed formation B: Synthetic seed germination

RESULTS

Effect of MS Strength and Light Intensity on of Shoot Proliferation of *P. betulaefolia* Rootstock: Salt strength of MS nutrient medium affected shoot proliferations of *P. betulaefolia* (Table 1 and Fig. 2). The maximum number of shoot proliferation obtained on 3/4 MS salt strength followed by full MS salt strength (7.56 and 6.00 shoots/shoot tip, respectively). Also, light intensity of the incubation affected number of shoot proliferation. Incubated in 1000 lux possessed the highest number of shoot proliferation (7.78 shoot/ shoot tip) followed by cultures which incubated in 2000 and darkness condition (5.56 and 2.78 shoots/shoot tip, respectively). The interaction between MS salt strength and light intensity demonstrated that 3/4 MS salt strength incubated in 1000 lux gave the highest number of shoot proliferation (12.67 shoots/ shoot tip). Regarding the shoot length, full MS strength positively affected shoot length. Also, high light intensity increased the shoot length. Full MS salt strength which incubated in 1000 and 2000 lux significantly enhanced shoot length (7.03 and 7.60 cm, respectively). Also, 1/2 MS salt strength incubated in 2000 lux maximized the shoot length (7.50 cm). Nodes number related to the salt strength of the nutrient medium, the highest nodes number resulted from full and 3/4 MS salt strength with no significant difference between them (3.89 and 3.67 nodes/shoot, respectively). Light intensity of 1000 lux was superior on nodes number compared with darkness. Interaction between salt strength and light intensity revealed that 3/4 MS with 2000 lux and 1/2 MS with 1000 lux increased the nodes number (5.00 nodes/shoot for each). Adversely relationship was observed between MS salt strength and growth vigor with no significant differences among all values of growth vigor. Incubation in 1000 lux significantly induced growth vigor (4.11). Full MS salt strength incubated in 2000 or 1000 lux and 3/4 MS salt strength incubated in 2000 lux gave the maximum growth vigor (4.67 for each).

Effect of Concentrations of BA in Combination with Kn and/or Ancymidol on Shoot Proliferation of *P. betulaefolia* Rootstock: Growth parameters of *P. betulaefolia* rootstock were affected by the addition of growth regulators to MS medium (Table, 2 and Fig. 3). Number of shoot proliferation were enhanced when MS supplemented with 2.0 mg/l BA + 1.5 mg/l Kn+ 100 µg/l Anc or 1.5mg/l BA+ 1.5mg/l Kn + 100µg/l Anc (23.0 or 21.0shoot/shoot tip, respectively), with no significant difference between them. On contrary, basal MS medium or MS + 100 µg/l Anc resulted in suppress the shoot proliferation. Concerning the shoot length of *P. betulaefolia*, basal MS medium, MS + 0.5 mg/l BA or MS + 1.5 mg/l BA+1.5 g/l Kn significantly induced shoot length (11.0, 10.67 and 10.0 cm, respectively), followed by MS + 1.5 mg/l BA, MS +2.0 mg/l BA+1.5mg/l Kn +100 µg/l Anc and MS + 1.5 mg/l BA +1.5 mg/l Kn + 100 µg/l Anc (9.67, 9.33 and 9.00 cm, respectively). Most of growth regulators treatments augmented growth vigor except MS + 100 µg/l Anc and MS + 2.0 mg/l BA + 100 µg/l Anc.

Effect of Sucrose Concentrations in Combination with Light Intensity on Shoot Proliferation of *P. betulaefolia* Rootstock: MS nutrient medium in 3/4 salt strength fortified with different sucrose percentage affected the number of shoot proliferations and growth parameters of *P. betulaefolia* (Table 3 and Fig. 4). Medium fortified with 4 or 5% sucrose motivated the shoot proliferation and produced the highest shoots number (32.33 and 30.50 shoots/ shoot tip, respectively). Also, light intensity as physical incubation conditions affected shoot proliferation, 1000 lux was superior and produced 34.75 shoots/ shoot tip compared with 10.50 shoots/shoot tip in the case of 2000 lux. The interaction between sucrose percentage and light intensity showed a priority

Table 1: Effect of MS salt strength and light intensity on shoot proliferation of *P. betulaefolia* rootstock

	Shoot number/ shoot tip				Shoot length (cm)			
	Light intensity (lux) (B)				Light intensity (lux) (B)			
MS salt strength (A)	2000	1000	Dark	Mean (A)	2000	1000	Dark	Mean (A)
Full MS	6.67	8.00	3.33	6.00	7.03	7.60	2.73	5.79
3/4 MS	7.33	12.67	2.67	7.56	5.67	4.33	2.30	4.10
1/2 MS	2.67	2.67	2.33	2.56	7.50	3.10	2.20	4.27
Mean (B)	5.56	7.78	2.78	---	6.73	5.01	2.41	---
LSD at 5%	A: 1.09	B: 1.09	AxB: 1.89	---	A: 0.51	B: 0.51	AxB: 0.89	---
	Nodes number/shoot				Growth vigor			
	Light intensity (lux) (B)				Light intensity (lux) (B)			
MS salt strength (A)	2000	1000	Dark	Mean (A)	2000	1000	Dark	Mean (A)
Full MS	4.33	4.33	3.00	3.89	4.67	4.67	2.30	3.88
3/4 MS	5.00	4.00	2.00	3.67	4.67	4.33	2.33	3.77
1/2 MS	2.00	5.00	2.67	3.33	3.33	3.33	2.33	3.00
Mean (B)	3.89	4.44	2.56	---	3.56	4.11	2.32	---
LSD at 5%	A: 0.23	B: 0.23	AxB: 0.40	---	A: NS	B: 0.56	AxB: 0.97	---

where: Growth vigor according to description of Pottino [34]: 1=weak, 2=moderate, 3=good, 4=very good, 5=excellent

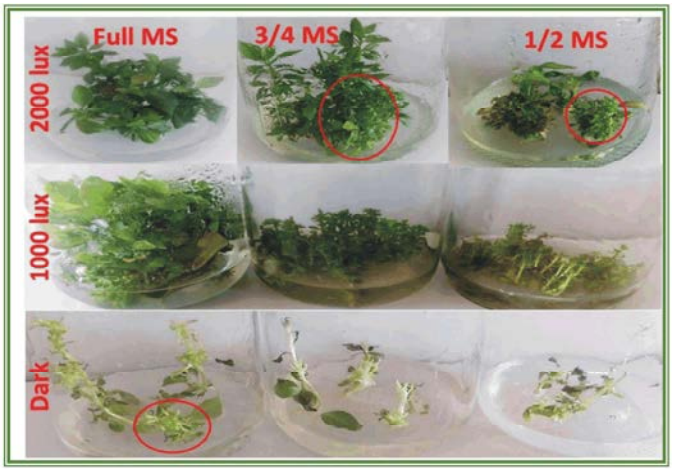

Fig. 2: Effect of MS salt strength and light intensity on shoot proliferation of *P. betulaefolia* rootstock:

Table 2: Effect of concentrations of BA in combination with Kn and/or Ancymidol on shoot proliferation of *P. betulaefolia* rootstock

Growth regulators					
BA conc. (mg/l)	Kn mg/l	Ancy. (µg/l)	Shoot number/ shoot tip	Shoot length (cm)	Growth vigor
Control			1.0	11.0	4.33
0.0	0.0	100	1.0	3.33	3.66
0.5	0.0	0.0	3.33	10.67	4.33
1.0	0.0	0.0	4.3	8.33	4.33
1.5	0.0	0.0	7.0	9.67	3.86
1.5	1.5	0.0	9.0	10.00	4.33
1.5	0.0	100	15.7	4.67	4.67
1.5	1.5	100	21.0	9.00	4.33
2.0	0.0	0.0	9.7	6.67	4.33
2.0	1.5	0.0	9.3	6.33	4.67
2.0	0.0	100	12.3	4.33	3.33
2.0	1.5	100	23.0	9.33	4.67
LSD at 5%			2.06	1.6	0.94

where: Growth vigor according to description of Pottino [34]: 1=weak, 2=moderate, 3=good, 4=very good, 5=excellent

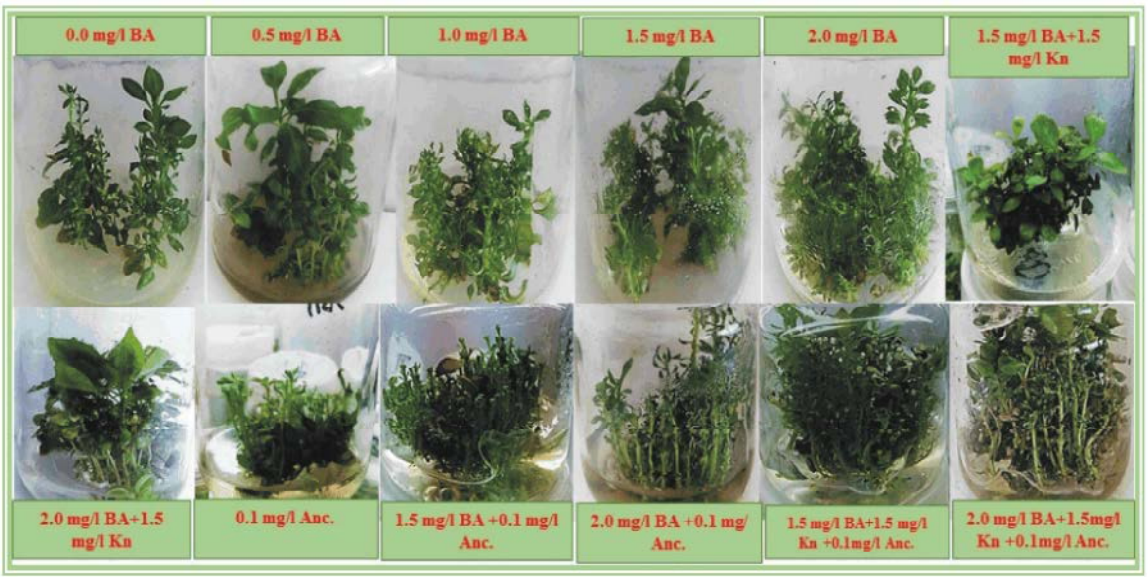

Fig. 3: Effect of concentrations of BA in combination with Kn and/or Ancymidol on shoot proliferation of *P. betulaefolia* rootstock

Table 3: Effect of sucrose percentage in nutrient medium in combination with light intensity on shoot proliferation of *P. betulaefolia* rootstock

	Shoot number/shoot tip			Shoot length (cm)			Nodes number/shoot		
	Light intensity (B)			Light intensity (B)			Light intensity (B)		
Sucrose (%) (A)	2000 Lux	1000 Lux	Mean (A)	2000 Lux	1000 Lux	Mean (A)	2000 Lux	1000 Lux	Mean (A)
2%	7.00	16.67	11.83	2.80	3.00	2.90	2.67	2.67	2.67
3%	8.33	23.33	15.83	3.77	3.60	3.68	4.33	3.67	4.00
4%	13.67	51.00	32.33	5.07	5.10	5.08	4.47	4.67	4.67
5%	13.00	48.00	30.50	3.77	3.73	3.75	2.33	3.00	2.67
Mean (B)	10.50	34.75	---	3.85	3.85	---	3.50	3.50	---
LSD at 5%	A: 2.17	B: 1.53	AxB: 3.07	A: 1.46	B: NS	AxB:2.07	A: 6.33	B: NS	AxB: 0.90

of 4 and 5% sucrose when incubated in 1000 lux (51.00 and 48.00 shoots/shoot tip, respectively) (Fig. 5). Shoot length also was affected by percentage of sucrose in the nutrient medium, 4% sucrose significantly increased shoot length (5.08 cm), while light intensity did not significantly affect shoot length. The interaction proved that 4% sucrose in the nutrient medium and 1000 or 2000 lux induced the shoot length of *P. betulaefolia* (5.10 and 5.07 cm, respectively). Nodes number significantly affected by percentage of sucrose in culture medium; 3 and 4% possessed the highest nodes number (4.00 and 4.67 nodes/shoot). On contrast, no significant differences were detected between the light intensity treatments. The interaction revealed that 4% sucrose in nutrient medium and 1000 or 2000lux light intensity ascending the nodes number (4.67 and 4.47 nodes/shoot, respectively).

Conservation of *P. betulaefolia via* Synthetic Seeds: Effect of Several Compositions of Synthetic Seeds on Germination, Shoot Proliferation and Vital Number and Percent of Synthetic Seeds along the Conservation Period of *P. betulaefolia* Rootstock: Composition of synthetic seeds affected the required period to beginning germination (Table 4 and Fig. 6); synthetic seeds contained MS+0.5mg/l BA +100µ/l Anc retard the germination to 77days, followed by both synthetic seeds contained basal MS without any additive components and MS contained 0.5mg/l BA (53.67 and 44.33 days, respectively). Results cleared that presence of BA accelerated the ability of germination while presence of Ancymidol suppressed the germination compared with basal MS. Concerning the number of shoot proliferation along the preservation period, data in Table 4 cleared that composition of synthetic seeds showed variation in

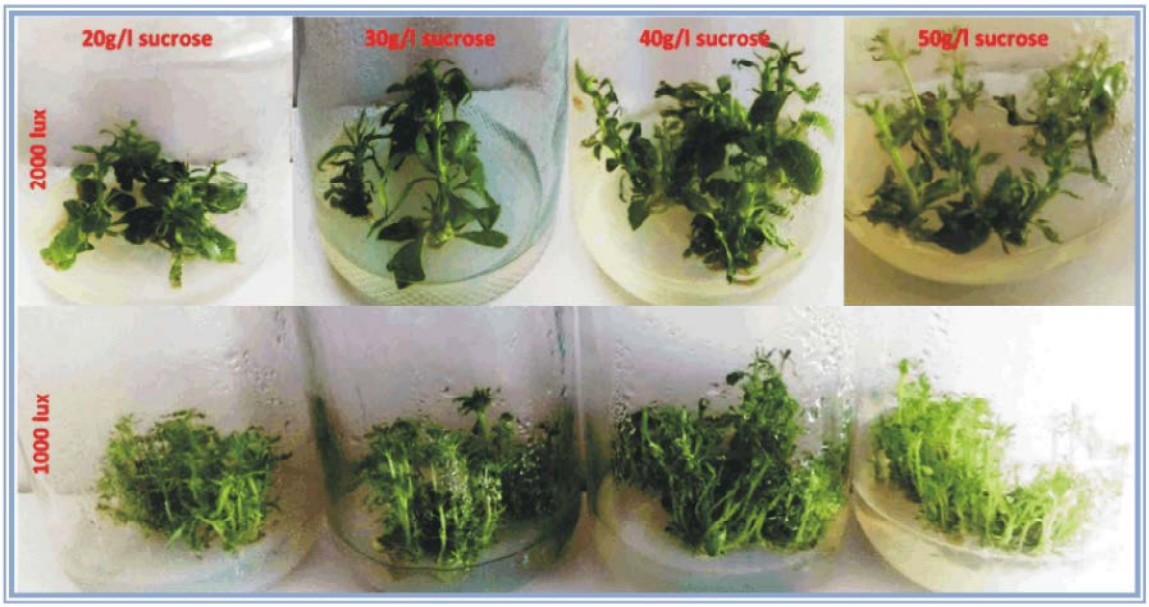

Fig. 4: Effect of sucrose percentages in nutrient medium in combination with light intensity of shoot proliferation of *P. betulaefolia* rootstock

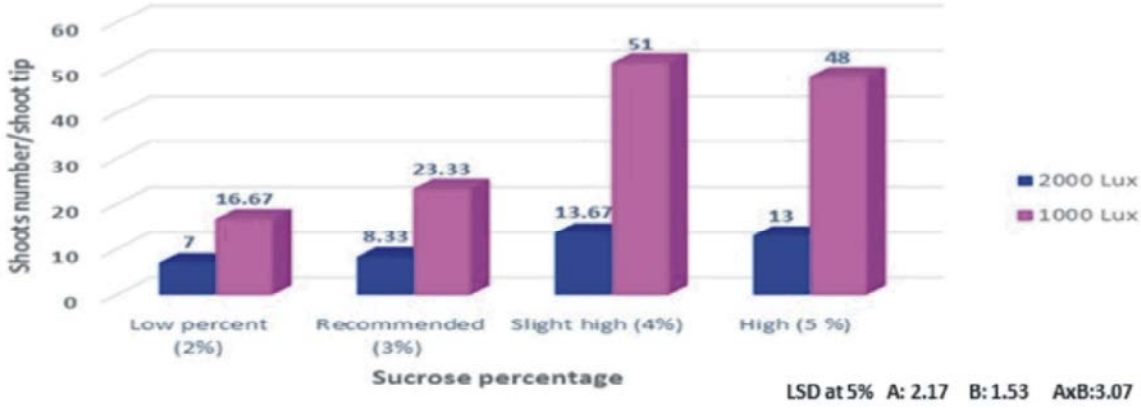

Fig. 5: Effect of interaction between sucrose percentages in nutrient medium in combination with light intensity on shoot proliferation of *P. betulaefolia* rootstock

responses along the conservation period. After 8 weeks, synseeds contained MS + 0.5 mg/l BA maximized the number of shoots followed by synseeds contained basal MS, while synseeds contained Ancymidol did not germinate until the 11th week. The same trend maintained until the 16th week of conservation, then synseeds contained MS + 0.5 mg/l BA + 100 µg/l Anc resulted in the highest number of shoot proliferation after 28 weeks of the conservation period. The maximum number of shoot proliferation was obtained from the synseeds contained MS + 0.5 mg/l BA+ 100 µg/l Anc after 36 weeks, followed by synseeds contained MS + 0.5 mg/l BA (28.33 and 19.00 shoots/synseed, respectively) (Fig. 7 and Fig. 8). While, decline in shoots began on those resulted from synseeds contained basal MS medium after 32 weeks. The number and percentage of vital synthetic seeds at the end of the conservation period cleared that vital number varied according to the composition of synseeds. The highest number and percentage of vital synthetic seeds recorded for synseeds contained MS + 0.5 mg/l BA+ 100µg/l Anc (24.67 vital synseeds and 97.33 vitality percent), while, synseeds contained basal MS medium possessed the lowest number and percent of synseeds vitality (8.67 synseeds and 33.33%) at the end of investigation.

Table 4: Effect of several compositions of synthetic seeds on germination, shoot proliferation and vital number and percent of synthetic seeds along the conservation period of *P. betulaefolia* rootstock

		Shoots proliferation number/synseed							
Synseeds composition	Period to germination (day)	After 8 weeks	After 12 weeks	After 16 weeks	After 28 weeks	After 32 weeks	After 36 weeks	No of vital seeds	Vitality %
MS	53.67	1.33	3.00	3.33	3.67	4.00	3.00	8.67	33.33
MS+0.5 mg/l BA	44.33	4.00	6.00	16.70	17.67	19.00	19.00	11.67	48.00
MS+0.5 mg/l BA + 100 µg/l Ancymidol	77.00	0.00	4.00	14.00	20.00	27.33	28.33	24.67	97.33
LSD at 5%	12.96	1.77	NS	7.05	2.88	3.78	3.99	6.45	6.00

where A, Na-alginate prepared in several constitutes; A: MS medium which began germination after 53.67 days, B: MS + 0.5 mg/l BA which began germination after 44.33 days C: MS + 0.5 mg/l BA + 0.1 mg/l Anc. which began germination after 77 days

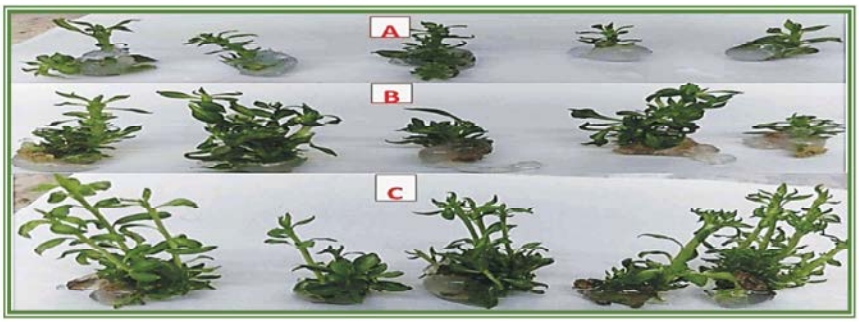

Fig. 6: Effect of composition of synthetic seeds on shoot proliferation at the beginning of each treatment

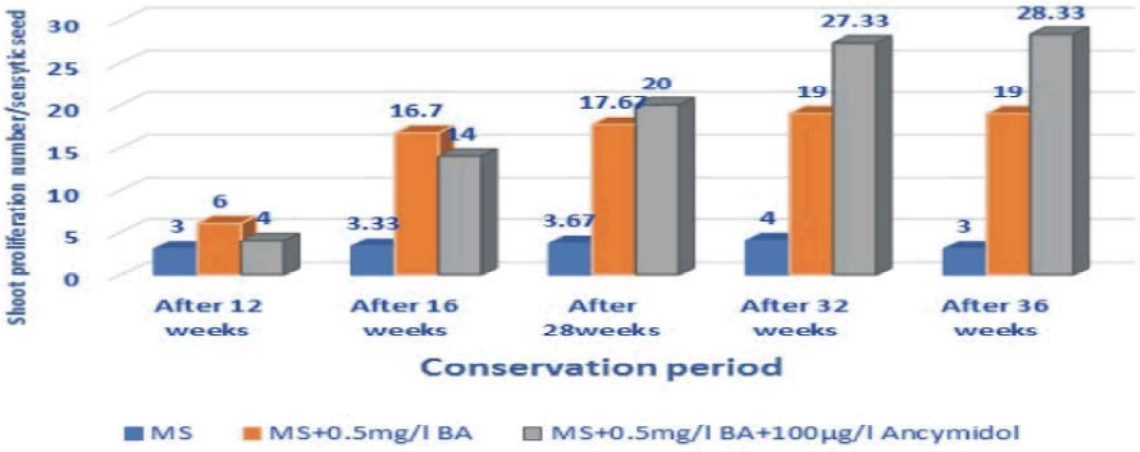

Fig. 7: Effect of composition of synthetic seeds on shoot proliferation along the conservation period

Fig. 8: Effect of composition of synthetic seeds on shoot proliferation along the conservation period

DISCUSSION

Shoot proliferation of *P. betulaefolia* rootstock affected by multi factors including strength of MS nutrient medium, growth regulators concentration and combinations, sucrose percent in nutrient medium as well as physiological factors like light intensity. MS salt strength affected number of shoot proliferation of *P. betulaefolia* rootstock as well as shoot length, nodes number/shoot and growth vigor. Full and 3/4 MS salt strength possessed the highest shoot number and growth parameters with no significant differences between them in the most growth parameters. Light intensity affected shoot proliferation and other growth parameters; 1000 lux was superior in the most parameters. Total darkness depressed the most growth parameters, but it promoted the response of the cultures when it was sub-cultured to a new medium and incubated in light (data is not presented). Full MS salt strength incubated in 2000 or 1000 lux as well as 3/4 MS salt strength incubated in 2000 or 1000 lux promoted shoot proliferation and other growth parameters. Results harmony with the finding of Nobakht, *et al*. [2]; Fadel *et al*. [3] and Sianipar *et al*. [4] who reported that MS strength affected shoot proliferation. Also, results partially agree with Singha [8] and Bell and Reed [7] who stated that the most multiplication methods used full or half strength of MS basal nutrient medium with slight modifications. While, results were against the results of Thakur *et al*. [10] who demonstrated that the lower concentration of major elements in Woody Plant Medium (WPM); Lloyd and McCown [9], was suitable for some pear genotypes micropropagation. My results may be difference because the investigated species is difference, so, variants response to medium composition is expected according to the variation in genetic characteristic and nature of growth of each species.

The obtained results proved that BA and Kn in presence of 100 µg/l Anc improve the shoot proliferation number and growth parameter of *P. betulaefolia*. MS fortified with 1.5 mg/l BA + 1.5 mg/l Kn + 100 µg/l Anc or MS fortified with 2.0 mg/l BA + 1.5 mg/l Kn + 100 µg/l Anc gave the maximum number of shoot proliferation (21.0 or 23.0 shoots/shoot tip, respectively). Concerning the shoot length of *P. betulaefolia*, basal MS medium and MS medium contained BA individual or in combination with Kn promoted the shoot length, while, presence of Ancymidol reduced the shoot length. Most of growth regulators treatments augmented growth vigor except the basal MS contained Ancymidol individual (100 µg/l Anc.). Results supported with the finding of George *et al*. [11]; Thakur *et al*. [10]; Thorpe *et al*. [12]; Arab *et al*. [13] and Rehman *et al*. [15] who concluded that cytokinin is the most important growth regulator in tissue culture especially BA, it plays an important role in plant proliferation, it is a promotor for cell division and elongation so it affected the shoot multiplication as well as the shoot length. The results also supported by the finding of Emara *et al*. [22] who stated that Ancymidol implemented in tissue culture as anti-gibberellin, which may aid in enhance *in vitro* growth vigor of shoots.

Also, percentage of sucrose in nutrient medium and light intensity affected shoot proliferation and growth parameters, 4% sucrose and 1000 lux possessed the highest number of shoots proliferation (51.00 shoots/shoot tip), shoot length (5.10 cm) and nodes number (4.67 nodes/shoot). Results came in line with Pasqual *et al*. [17] and Kadota and Niimi [16] who reported that carbohydrates are affecting the growth and the frequencies of shoots. Sucrose is classified as the most carbon source in *in vitro* micropropagation of Pear. Increasing sucrose concentrations in tissue culture medium to 38 g/l promoted shoot proliferation.

The effect of synthetic seeds composition on *P. betulaefolia* conservation proved that presence of BA in composition of the synthetic seeds accelerated the ability of germination while presence of Ancymidol suppressed the germination compared with basal MS without BA or Ancymidol. Concerning the number of shoot proliferation along the preservation period, it can concluded that presence of BA in the composition of synthetic seeds promote germination (after 44.33 days) and shoot proliferation (19 shoots/synseed) and enhance the ability of storage to 36 weeks with moderate number and percent of vital synseeds (11.67 synseeds, 48% vitality). While synthetic seeds which contained both BA and Ancymidol retard the germination of synthetic seeds (77 days) and depressed the proliferation in the early period of conservation and promote both the number of shoot proliferation (28.33 shoots/synseed) and the number and percent of vital synseeds in the late period of conservation (24.67 vital synseeds and 97.33% vitality). Results may due to the suppressed effects of Ancymidol (the anti-gibberellin) which inhibited plant growth, retarded synthetic seeds germination and configured the encapsulated explants to store; this behavior may aid in the late period of conservation to maintain vital with high ability to shoot proliferation. Results supported with the finding of Freire *et al*. [27]; Faisal and Anis [28]; Ananthan *et al*. [26]; Kovalchuk *et al*. [29];

Mohanraj *et al*. [25]; Kovalchuk *et al*. [29]; Kovalchuk *et al*. [31] and Emara *et al*. [22] who reported that storage duration can vary greatly according to genus, the growth medium as well as the growth regulators. The encapsulation plays a role as an endosperm-containing, nutrients, growth regulator and carbon source. In cultivars of pear (*Pyrus communis*), an increasing of BA concentration led to produce a higher number of shoots and the maximum frequency of conversion.

CONCLUSION

In vitro shoot proliferation and growth parameters of *P. betulaefolia* was maximized on full or 3/4 MS salt strength supplemented with 1.5 mg/l BA+ 1.5 mg/l Kn +100 µg/l Anc + 40 g/l sucrose and incubated in 1000 lux. The synthetic seed composition should be contained MS + 0.5 mg/l BA + 100 µg/l Anc + 40 g/l sucrose to conserve the shoot tip of *P. betulaefolia* for 36 weeks without need to subculture.

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