The Comparison of Plant Regeneration between Jerusalem Artichoke and Purple Potato Cultured on MS Media with Different Concentrations and Combinations of Plant Growth Regulators

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***Abstract*—A deeper understanding of the influence of culture media and different germplasm is crucial to propagate the plants *in vitro* conditions. To focus this, two tuberous plant species; Jerusalem artichoke (*Helianthus tuberosus)* and Purple potato (*Solanum tuberosum)* were selected. In the present study, stem segments from two species selected as explant source were cultured on Murashige and Skoog's media containing various concentrations (0.2 and 0.5 mg L^{-1} NAA) (0.2 and 0.5 mg L^{-1} IAA) of auxin and (0.2 and 0.5 mg L^{-1} BA) (0.2 and 0.5 mg L^{-1} KIN) of cytokinins. Callus induction from stem segments of Jerusalem artichoke occured on most of the media tested, but the most callus formation (100%) took place on MS media containing only NAA for explants of purple potato. When the level of NAA increased in the medium, bulblet formation and shoot proliferation decreased for both species. On the other hand, lower concentrations of KIN induced shoot formation for the explants of both species. The present study reports on the effective regeneration protocol in tuberous plants tested and the outcome provides for further genetic research on Jerusalem artichoke and purple potato.**

***Index Terms*—*Helianthus tuberosus, solanum tuberosum,* plant regeneration, plant growth regulators.**

I. Introduction

Plant growth regulators induce plant development of callus, shoot, bud, root etc. Each type of phytohormone has a different effect on plant development and this effect may differ when such a hormone is combined with another growth regulator. Two types of plant growth regulators called cytokinins (Kinetin, 6-Benzylaminopurine, Zeatin, etc.) and auxins (2,4-Dichlorophenoxyacetic acid, Indole-3-acetic acid, etc.) have different effects on plants in terms of growth and development. In general, while cytokinins stimulate cell division, auxins are effective for cell elongation and division in bud and young leaves. At certain concentrations, the presence of both leads to the formation of callus.

Sterile culture without bacteria or fungal contamination provides more efficient medium environment for plants to see the effects of growth regulators independent from weak growth factor of those contaminations. The other advantage is that more reproduction is seen in a short period with optimal conditions such as light or heat factor. [1].

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This study focuses on the effects of different media with variable growth regulator concentrations on different tuberous species cultured *in vitro.* Furthermore, this study provides a preliminary data for any further research focused on these plants.

II. Material and Methods

A. Plant Material and Disinfection

Stem segments of two different species; Jerusalem artichoke (*Helianthus tuberosus)* and purple potato (*Solanum tuberosum)* were used for this study. Tuber samples of Jerusalem artichoke were obtained from the local market in Turkey and tubers of purple potato were obtained from USA (Fig. 1).

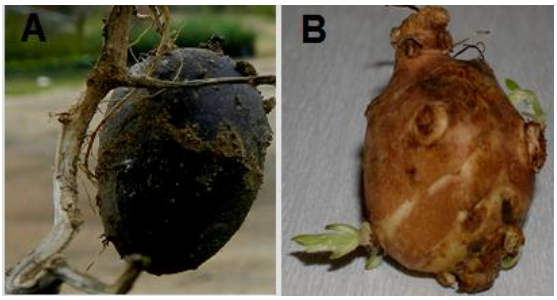

Fig. 1. (A) General view of purple potato's tuber, (B) General view of Jerusalem artichoke's tuber

Shoots of both species from greenhouse grown plants were used exclusively as explants in the present study.

Shoots were cut from about 1 cm from the plants grown in the greenhouse and then the leaves were removed. The explants (shoots) were surface-sterilized by dipping into 70% ethanol (3 mins) and were kept in 10% sodium hypochlorite for 10 minutes. The explants were then rinsed thrice consecutively with sterile distilled water in a laminar flow cabinet. After rinsing, surface-sterilized stems were cut into 5mm long stem explants.

The stem explants were cultured on Murashige and Skoog's media (MS) [2] with GA_3 (1 mg L^{-1}) to induce further stem formation for four weeks. After a month, three replicate petridishes with 6 stem explants each were placed on MS basal media containing different concentrations of various plant growth regulators shown in Table I.

B. Culture Media and Cultivation Conditions

Stem explants were cultured on regeneration media

containing different concentrations of NAA (0.2 and 0.5 mg L^{-1}), BA (0.2 and 0.5 mg L^{-1}), KIN (0.2 and 0.5 mg L^{-1}), and IAA (0.2 and 0.5 mg L^{-1}) (see Table 1 for concentrations and combinations) for organogenesis.

C. In vitro Culturing

After sterilization, stem pieces were placed on MS basal media solidified by 7.0 g L^{-1} agar and different concentrations of NAA, BA, KIN, IAA with 3% (w/v) sucrose were added.

All cultures were kept on shelves in a growth chamber at 25±1 ℃ and were exposed to a 16 h photoperiod for 4 weeks due to the final logarithmic phase of growth. For control groups, MS basal media without any growth regulators were used in this study. For each experiment, 18 explants were used. Regenerating explants were subcultured on fresh media. The numbers of bud, callus and shoot per stem were calculated weekly and the growth of the cultured explants was observed every 5 days by stereomicroscope for morphological observations. Rooting of the regenerated shoots were spontaneous without any growth regulator treatment.

III. Results and Discussion

A. Plant Regeneration

The experiment was set as a total of 36 treatments for both Jerusalem artichoke and purple potato; each treatment was carried out in triplicates containing 6 explants in each culture medium. The regeneration ability of each genotype was scored every five days for a month period. The data were collected (Table I).

In our study, by applying KIN to the media, callus formation from the explants of Jerusalem artichoke increased, whereas the media containing IAA alone or in combination with KIN never triggered callus induction for purple potato explants. In all media containing 0.2 and 0,5 mg L^{-1} NAA, high callus formation (100%) from purple potato's stems was observed (Table I).

The highest bulblet formation (39%) for purple potato was obtained on MS media containing solely 0.2 mg L^{-1} IAA and combining with 0.2 mg L^{-1} KIN after 3 weeks.

Explants after 3 weeks of culture formed shoot structures and continued to grow however, callus formation was not observed at this stage; in contrast, by involving the control groups of purple potato on the MS media free from plant growth regulators, bulblets formation was hardly observed. 20 days after culture initiation, some explants (80%) of purple potato produced purple calli on nearly all media tested. In addition, bulblet formation from the callus of purple potato's stems was observed (Fig. 3).

After 4 weeks in the culture, there was little further development on the stems of purple potato compared to Jerusalem artichoke. For regeneration of shoot and bud, growth regulators tested in this experiment were not so effective for purple potato [Table 1]. On the other hand, the shoot formation of the purple potato (50%) was higher than Jerusalem artichoke (44%) on the media including solely high concentrations (0.5 mg L^{-1}) of KIN. According to our results, Jerusalem artichoke gave better results than the purple potato did. (Fig. 4).

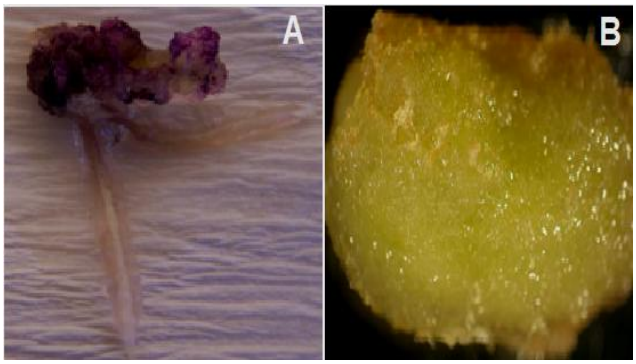

Fig. 2. Callus developed from stem explants of (A) purple potato, (B) Jerusalem artichoke after 3 weeks of culture initiation on MS medium including solely 0.2 mg L^{-1} NAA

Fig. 3. *In vitro* bulblet proliferation from callus of purple potato's (arrows: roots)

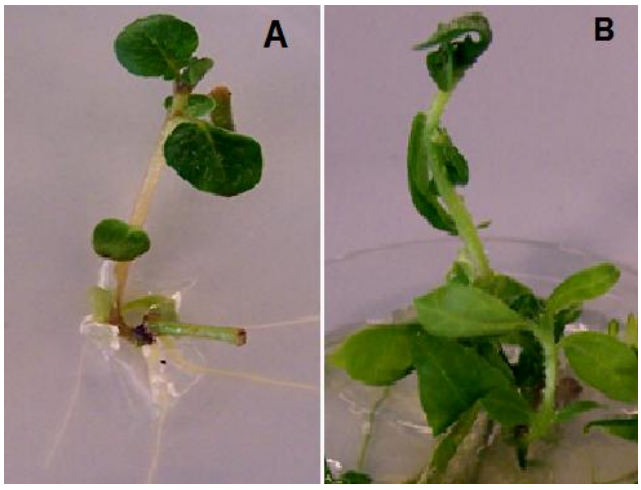

Fig. 4. *In vitro* shoot development on MS medium with soleley 0.5 mg L^{-1} KIN, (A) A month old shoots of purple potato; (B) A month old shoots of Jerusalem artichoke

Regeneration of purple potato's stems did not exceed 44% (Table I); in contrast, Jerusalem artichoke reached 61% (Table I).

Control groups of Jerusalem artichoke developed on the MS media free from plant growth regulators showed little developing calli and roots (Table I). On the media containing 0.2 mg L^{-1} IAA-0.5 mg L^{-1} KIN and solely 0.2 mg L^{-1} KIN, the highest percentage of shoot regeneration (61%) of Jerusalem artichoke was obtained after 4 weeks.

TABLE I: SHOOT REGENERATION, BULBLETS AND CALLUS FORMATION PERCENTAGES OF EXPLANTS ON THE MEDIUM CONTAINING DIFFERENT CONCENTRATIONS OF NAA (0.2 AND 0.5 MG L^{-1}), BA (0.2 AND 0.5 MG L^{-1}), KIN (0.2 AND 0.5MG L^{-1}), AND IAA (0.2 AND 0.5 MG L^{-1})

Explant	Plant Growth Regulators (mg L^{-1})				Percentage of calli/explant	Percentage of bulblets/explant	Percentage of shoots/explant
	BA	NAA	IAA	KIN			
	0	0	0	0	*22*	11	33
	0	0	0	0.2	*33*	22	61
	0	0.2	0	0	100	39	17
	0	0	0	0.5	61	22	44
	0	0.5	0	0	100	4	11
Jerusalem artichoke	0.2	0	0	0	67	6	22
Stem segment	0	0	0.2	0	67	17	33
	0.2	0.2	0	0	100	44	6
	0	0	0.2	0.2	44	0	44
	0.2	0,5	0	0	67	33	6
	0	0	0.2	0.5	100	22	61
	0.5	0	0	0	67	11	44
	0	0	0.5	0	72	28	44
	0.5	0.2	0	0	100	44	0
	0	0	0.5	0.2	72	50	22
	0.5	0.5	0	0	67	22	0
	0	0	0.5	0.5	100	28	50
	0	0	0	0	*0*	6	17
	0	0	0	0.2	*0*	11	44
	0	0.2	0	0	100	6	6
	0	0	0	0.5	0	6	50
	0	0.5	0	0	100	0	6
Purple Potato	0.2	0	0	0	0	6	22
Stem segment	0	0	0.2	0	0	39	44
	0.2	0.2	0	0	67	0	6
	0	0	0.2	0.2	0	39	44
	0.2	0,5	0	0	100	0	0
	0	0	0.2	0.5	0	6	0
	0.5	0	0	0	0	17	6
	0	0	0.5	0	0	28	33
	0.5	0.2	0	0	100	0	17
	0	0	0.5	0.2	0	11	6
	0.5	0.5	0	0	100	0	0
	0	0	0.5	0.5	0	17	17

For purple potato, explants which were cultured on the MS media free from plant growth regulators, did not show callus formation. A mass of small cells initiated the regeneration of the shoot meristems directly from explants *in vitro*.

As they continued to grow, they became yellow and did not develop into shoots. In addition, some demonstrated necrosis on the control groups.

After 2 weeks on the media, explants of two species developed into shoots and about 3 weeks culturing on MS media supplemented with 1 mg L^{-1} GA_3, root formation was observed. (Fig. 5).

Fig. 5. Plantlet regeneration; (A): The 10th day of culturing of stems, (B) 15th day of culturing stems; A and A1: Jerusalem artichoke's plantlets; B and B1: purple potato's plantlets

To date, there are some studies concerning the micropropagation of Jerusalem artichoke [3], [4] and purple potato [5]-[7], but literature on tuberous plant regeneration indicates that there is no published work that compares the differences between two species used in the present study.

The significance of the present study is the comparison of the plant regeneration of two economically important tuberous plants by applying various growth regulators.

Our study and the published studies [8]-[10] showed that each genotype differed in terms of regeneration.

According to the previous research outcomes from the abundant studies on the regeneration of plants' stems, NAA induces the callus formation [11], [12]. We also observed that the presence of NAA alone developed callus from the cultured stems.

When we examined under a dissecting microscope, the callus obtained from the stems of Jerusalem artichoke were white to yellowish and compact, in contrast, ones of purple potato were purple and friable (Fig. 2). It could be due to comprising anthocyanin. Some studies were conducted on the isolation of anthocyanin in purple potato [13].

A. Rooting

The regenerated plantlets were transferred to MS media with 1.0 mg L^{-1} GA_3 and the roots emerged from stems after 2 weeks. According to previous studies, GA_3 stimulated the root formation in tuberous plants. This is also similar to our results (Fig. 6).

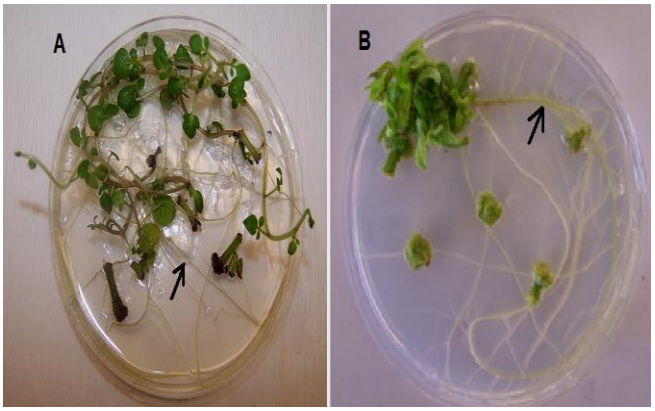

Fig. 6. *In vitro* root development on MS medium with 1.0 mg L^{-1} GA_3, (A) Rooted shoots from the explants of purple potato, (B) That of Jerusalem artichoke (arrows: roots)

IV. Conclusion

In vitro plant regeneration was achieved from stem explants of Jerusalem artichoke and purple potato.

We assume that the findings from the study presented here provide new insights for propagation of tuberous plants. The evolution of tuber development remains recondite. In addition, due to cross pollination, the plants which are research materials in the present study can be less tolerant to climatic changes. To deal with the problems related to evolutionary tuberization and genetic segregation, clonally propagated plants contribute to research on genome analysis with the facilitation of rapid determination of the ploidy level and genetic heredity for further genetic research and also cytological analysis in these plants. In addition, the data obtained from this study can be a good source for further genetic research regarding these species. Moreover, it is also important for *in vitro* production of valuable compounds from two selected species which have economic potential for production and human consumption.

References

[1] S. S. Bhojwani and M. K. Razdan, *Plant Tissue Culture: Theory and Practice*, a Revised Edition, The Netherlands: Elsevier, pp. 2, 1996.

[2] T. Murashige and F. Skoog, "A revised medium for rapid growth and bioassays with tobacco tissue cultures," *Physiologia Plantarum*, vol. 15, pp. 473-497, July 1962.

[3] C. Pugliesi, P. Megale, F. Cecconi, and S. Baroncelli, "Organogenesis and embryogenesis in Helianthus tuberosus and in the interspecific hybrid Helianthus annuus x Helianthus tuberosus," *Plant Cell, Tissue and Organ Culture*, vol. 33, pp. 187-193, December 1993.

[4] M. Fambrini, G. Cionini, A. Conti, V. Michelotti, and C. Pugliesi, "Origin and development in vitro of shoot buds and somatic embryos from intact roots of Helianthus annuus x H. tuberosus," *Annals of Botany*, vol. 92, pp. 145-151, April 2003.

[5] J. F. Shepard and R. E. Totten, "Mesophyll cells of potato," *Plant Physiol*, vol. 60, pp. 313-316, 1977.

[6] M. M. H. Molla, K. M. Nasiruddin, M. A. Amin, D. Khanam, and M. A. Salam, "Effect of Growth Regulators on Direct Regeneration of Potato," *IPCBEE*, vol.12, 2011.

[7] C. M. Raker and D. M. Spooner, "Chilean tetraploid cultivated potato, solanum tuberosum, is Distinct from the Andean Populations: Microsatellite Data," *Crop Sci*, vol. 42, pp. 1451-1458, 2002.

[8] J. S. Hulme, E. S. Higgins, and R. Shields, "An efficient genotype-independent method for regeneration of potato plants from leaf tissue," *Plant Cell, Tissue and Organ Culture*, vol. 31, pp. 161-167, April 1992.

[9] C. E. Green and R. L. Phillipes, "Plant regeneration from tissue cultures of Maize," *Crop Science*, vol. 15, pp. 417-421, Jan. 1975.

[10] S. E. Maddock, V. A. Lancester, R. Risiott, and J. Franklin, "Plant regeneration from cultured immature embryos and inflorences of 25 cultivars of wheat (Triticum aestivum)," *Journal of Experimental Botany*, vol. 34, pp. 915-926, October 1982.
[11] T. Fossen and O. M. Andersen, "Anthocyanins from tubers and shoots of the purple potato, Solanum tuberosum," *Journal of Horticulture Science and Biotechnology*, vol. 75, pp. 360-363, 2000.
[12] V. Gaba, E. Schlarman, C. Elman, O. Sagee, A. A. Watad, and D. J. Gray, "*In vitro* studies on the anatomy and morphology of bud regeneration in melon cotyledons," *in Vitro Cellular and Development Biology-Plant*, vol. 35, pp. 1-7, January 1999.
[13] V. T. Huan, T. Takamura, and M. Tanaka, "Callus formation and plant regeneration from callus through somatic embryo structures in Cymbidium orchid," *Plant Science*, vol. 166, pp. 1443-1449, June 2004.

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