

ROOT FORMATION IN TRUE POTATO SEED PARENTAL LINES BY IBA APPLICATION

Nizam-ud-Din*, Bushra Mirza*, Maqsood Qamar** and Abdul Khabir***

*Department of Biological Sciences, Quaid-i-Azam University, Islamabad.

**Wheat Program, National Agricultural Research Centre, Islamabad.

***Tissue Culture Lab., Directorate of Agriculture, Gilgit.

An experiment was conducted to study the response of dipping time and various concentrations of IBA (indole-3-butyric acid) on days to root initiation, number of roots per stem cutting and root length of stem cuttings of four TPS (true potato seed) parental lines, Atzimba, LT-8, MF-I and TPS-67. Root formation was significantly earlier in the cuttings treated with 150 ppm IBA than cuttings treated with 0 (control), 100 and 200 ppm IBA at seven days after planting. Similarly, Root initiation was significantly delayed in the cuttings that were treated with 0 ppm (control) IBA than in the cuttings treated with 100, 150 and 200 ppm IBA at 10 and 14 days after planting. Main effect of TPS parental lines and dipping time was significant on root initiation at 10, 14, and 17 days after planting. Cuttings dipped for 5, 10, 15 seconds in IBA solution initiated roots earlier than the cuttings of control. Main effect as well effect of parental line x IBA concentration interaction was significant on both number of roots per stem cutting and on root length. Under the most favourable combination (Atzimba x 150 ppm) number of roots per stem cuttings reached to 7.5. 150 ppm IBA concentration gave comparatively better results for root length (5.94 cm) and number of roots per stem cutting (5.47). LT-8 produced maximum roots (4.60) per stem cutting while the longest roots (4.71 cm) were noticed in cuttings of Atzimba.

Key words: IBA (Indole -3-butyric acid), potato, TPS lines, number of roots, root length

INTRODUCTION

Potato (*Solanum tuberosum* L.) belongs to family Solanaceae and is the fourth most important cash crop after maize, wheat and rice (FAO, 1995). The maintenance and disease free true potato seed lines is very important for both tuber production and breeding purposes. There is a well developed seed production system at Tissue Culture Laboratory, Directorate of Agriculture, Gilgit and three techniques of potato multiplication i.e. stem cutting, nodal cutting and leaf bud cutting are used to produce disease free planting material. Among these three techniques, stem cutting is mostly used technique. According to Cole and Wright (1967) mother plant can be cloned and recloned to provide a sizeable increase of stock and thousands of rooted propagules can be produced from a single plant. Tissue culture techniques show promise for rapid propagation but are not yet in practice (Singh and Naik, 1993).

Many research workers adopted different methods of producing potato tubers. Singh and Naik (1993) produced tubers from single node cuttings. Meguel *et al.* (1981) produced tubers from leaf bud cuttings. Bryan *et al.* (1981) produced seed tubers from apical bud cuttings respectively for rapid increase of selected stock. All these workers conducted experiments to produce tubers but the method in relation to hormone dose and dipping time are yet to be standardized.

The objective set in this experiment was to identify optimum dipping time of TPS lines in IBA concentration for quick initiation of roots.

MATERIALS AND METHODS

An experiment was conducted at Tissue Culture Lab and Greenhouse, Gilgit to investigate the optimum dipping time of TPS parental lines with IBA solutions.

The parental Stock consists of three female parental lines viz. Atzimba, LT-8, MF 1 and one male line TPS-67. The stem cuttings of these TPS parental lines were dipped into IBA solutions for a period of 5, 10, & 15 seconds. The mother plant from where the stem cuttings were obtained, originated from a plant derived from meristem of *in-vitro* tubers of TPS parental Line. Test tube plants of parental lines were planted in well-drained pots filled to half of the pot 12x20 cm with proper sterilized and fertilized peat moss. The apical growing points of the parental lines were removed to stimulate the growth of all the lateral shoots from the auxiliary buds from each node. After 15-20 days of removal of the apical growing point, lateral shoots from auxiliary buds were ready for harvest.

Three to four cm long stem cuttings of TPS parental lines were obtained by giving a smooth cut with razor blade and the basal portion of these parental lines stem cuttings were dipped into IBA solutions for a period of 0, 5, 10 and 15 seconds and planted in peat moss in plastic trays. For control the stem cuttings

were dipped into the distilled water and planted in peat moss in plastic trays. 300 stem cuttings of TPS parental lines were treated in each treatment. Complete Randomized Design (CRD) with three replications was followed.

Five stem cuttings from each treatment were uprooted starting from 7, 10, 14 and 17 days of interval for record of data regarding days to root initiations. After 17 days of planting five stem were up rooted their root length, number of roots, measured counted and average was computed, and rooted seedlings were transferred to field for planting for hybrid seed production. Following data were recorded during the course of study.

1. Days to root initiation per stem cutting of TPS parental lines
 2. Number of roots per stem cuttings of TPS parental lines
 3. Root length per stem cuttings of TPS parental lines.
- Data was analyzed by using analysis of variance technique according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Root Initiation

Except the interaction between IBA concentration and parental lines at 10 days after planting all types of interaction were non-significant (Table 1). In control, difference among parental lines for root initiation was non-significant while variation among parental lines for the percentage of roots noticed at the cuttings that were treated with IBA was significant (Fig.1). For

200 ppm IBA concentration maximum number of cuttings (81.67 %) of LT-8 induced roots.

Dipping time had non-significant effect on days to root initiation at 7 days after planting and 17 days after planting (Table 1) while it showed significant effect at 10 days and 14 days after planting. Response of cuttings on days to root initiation was similar at 5, 10 and 15 seconds dipping time but at 0 second of dipping time, the lowest number of cuttings initiated roots (Table 2). The results here are in agreement with those of Hossain *et al.* (1998) who also observed the effect of dipping time on days to root initiation.

Main effect of parental lines on days to root initiation was significant at 10, 14 and 17 days after planting while it was non significant at 7 days after planting (Table 1). Percentage of roots were significantly lower in cuttings of TPS-67 than the cuttings of remaining parental lines at 10 days after planting but the response of Atzimba, LT-8 and MF-I was at par (Table 2). At 14 days after planting, 81.67 % cuttings of Atzimba produced roots while the lowest percentage of roots (63.33 %) was observed in the cuttings of MF-I (Table 2).

Main effect of IBA concentration on days to root initiation was significant at all observation taking days (Table 1). Initiation of root was significantly the highest (5.42 %) on the cuttings treated with 150 ppm at 7 days after planting (Table 2). At 10 days after planting, 69.1 % and 74.16 % cuttings that were treated with 150 ppm and 200 ppm respectively induced roots, which were significantly higher than the percentage of roots produced by cuttings treated with 100 ppm IBA

Table 1. Mean square values for percentage of root initiation per stem cuttings

Source of variation	df	7 DAP	10 DAP	14 DAP	17 DAP
Concentrations (A)	3	211.11*	47700.00**	36485.41**	980.55**
Parent line (B)	3	83.33 ^{NS}	1772.22*	3246.52**	691.66*
AxB	9	94.44 ^{NS}	1327.77*	940.97 ^{NS}	286.11 ^{NS}
Dipping time (C)	3	33.33 ^{NS}	4916.66**	1518.75*	25.00 ^{NS}
AxC	9	14.81 ^{NS}	1020.37*	516.89 ^{NS}	56.48 ^{NS}
BxC	9	12.96 ^{NS}	448.14 ^{NS}	218.75 ^{NS}	26.85 ^{NS}
AxBxC	27	16.66 ^{NS}	183.95 ^{NS}	313.19 ^{NS}	45.98 ^{NS}
Error	128	58.33	543.75	547.91	183.33

* Significant, ** Highly significant, ^{NS} Non-significant

example, at 100 ppm IBA concentrations, the cuttings of Atzimba initiated the highest %age (63.31%) of roots while the induction of roots were the lowest (30 %) in the cuttings of MF-I. The maximum (81.66 %) number of cuttings of MF-I induced roots at 150 ppm IBA but at

and control. At 14 days after planting, more than 80 % of cuttings treated with different IBA concentrations produced roots while only 29.58 % of cuttings that were not treated with IBA produced roots. At 17 days after planting, 100 % cuttings which were treated with 200 ppm had produced roots. The results here are in

Table 2. Effect of IBA concentrations, parental lines and dipping times on days to root initiation of Stem cuttings of TPS parental lines.

Concentrations	7 DAP	10 DAP	14 DAP	17 DAP
	% age of roots	% age of roots	% age of roots	% age of roots
Control	2.08 b*	5.00 c	29.58 b	93.33 bc
100 ppm	0.42 b	46.66 b	85.00 a	98.78 ab
150 ppm	5.42 a	69.16 a	84.58 a	90.41 c
200 ppm	2.08 b	74.16 a	84.58 a	100.00 a
LSD $P=0.05$	3.09	9.42	9.45	5.47
Parental lines				
Atzimba	3.33 a	51.67 a	81.67 a	99.58 a
LT-8	3.75 a	53.75 a	72.91 ab	97.50 a
MF-I	2.08 a	49.58 a	63.33 b	94.58 ab
TPS-67	0.83 a	40.00 b	65.83 b	90.83 b
LSD $P=0.05$	NS	18.84	9.454	5.469
Dipping Times				
Control	1.25 a	33.75 b	63.75 b	95.41
5 second	2.91 a	55.83 a	77.50 a	95.00
10 second	2.91 a	53.33 a	71.25 ab	95.41
15 second	2.91 a	52.08 a	71.25 ab	96.66
LSD $P=0.05$	NS	9.418	9.484	NS

*Values sharing a letter common in columns are non-significantly different.

accordance with those of Hossain *et al.* (1998) who observed that root induction in the potato stem cuttings was quicker at a higher IBA concentration (50 g L^{-1}) than at lower IBA concentrations ($< 50 \text{ g L}^{-1}$).

Number of Roots

Response of various concentrations of IBA, dipping time and TPS parental lines is presented in Table 3. Except the interaction between IBA concentrations x parental line, all types of interactions were non-significant. Mean value of IBA concentrations x parental line was tested for their significance by using LSD at $P=0.05$ (Table 4). The maximum number of root per stem cutting (6.15) was produced by LT-8 at 150 ppm IBA followed by the MF-I (5.77) at 150 ppm IBA. The least number of roots per stem cutting were produced by TPS-67 (1.27) at control (Fig. 2). The result here is in accordance with those of Kayim and Koc (1992) who observed specific effect of varieties on number of roots per stem cutting at different levels of IBA in potato. Dipping time had non-significant effect on number of roots per stem cutting. The results of this experiment are contradictory with those of Hossain *et al.* (1998) who reported a significant effect of soaking time on number of roots per cutting. They soaked stem cuttings of potato in IBA + NNA solutions for 1, 5, 10, 20, 40 and 60 seconds and 1, 5, 15, 30 and 60 minutes. The soaking for five second produced highest number of roots per cutting. The significant

difference in soaking times in the study of Hossain *et al.* (1998) might be because of IBA and NNA interaction.

Table 3. Mean square values for number of roots and root length per stem cuttings

Source of variation	df	No. of roots	Root length
Concentrations (A)	3	58.691**	56.839**
Parent line (B)	3	8.385..	14.540**
AxB	9	3.040..	4.947**
Dipping time (C)	2	0.811 ^{NS}	0.472 ^{NS}
AxC	6	0.571 ^{NS}	2.957**
BxC	6	0.487 ^{NS}	1.015 ^{NS}
AxBxC	18	1.101 ^{NS}	0.912 ^{NS}
Error	96	0.746	0.685

** Highly significant, ^{NS} Non-significant

Main effect of parental line and IBA concentrations showed a highly significant effect on number of roots per stem cutting (Table 3). The parental line LT-8 produced significantly the highest number of roots per stem cutting (4.61) and TPS-67 produced the lowest (3.45) number of roots per stem cutting (Table 4). Ishtiaq *et al.* (1989) had also found variation among peach varieties in producing roots per stem cutting in *in-vitro* condition. Thus, it appeared from the results that the number of roots per stem cutting is genetically

controlled character and is also affected by environmental conditions (Nicoll, 1993).

Table 4. Effect of IBA at different concentrations on number of roots and root length per stem cutting of TPS parental lines.

Treatments	No. of roots per cutting	Root length (cm)
Concentrations		
Control	2.40 c	3.19 c
100 ppm	4.03 b	3.42 c
150 ppm	5.47 a	5.94 a
200 ppm	4.43 b	3.90 b
LSD $P = 0.05$	0.4041	0.387
Parental lines		
Atzimba	4.05 b	4.71 a
LT-8	4.60 a	4.22 b
MF-1	4.23 ab	4.32 b
TPS-67	3.45 c	3.22 c
LSD $P = 0.05$	0.404	0.38

*Values sharing a letter common in columns are non-significantly different.

Number of roots per stem cutting significantly varied among different IBA concentrations (Table 3). Maximum number of roots (5.47) was formed in the cuttings treated with intermediate concentration (150 ppm of IBA) (Table 4) while the minimum number of roots (2.40) was observed in the cuttings that were not

treated with IBA (Control). Hossian *et al.* (1998) also found similar results. The increase in number of roots per cutting treated with IBA might be due to cambial activity that resulted in root induction (Digby and Wangerman, 1965). Nymora and Mnzwa (1983) found that IBA increased elasticity of cambium and thus accelerated cell division and produced more number of roots in treated cuttings. IBA did not affect genetic structure of cells but slowly inhibited cell activity at higher concentration (Melik-Sarkisov *et al.*, 1994), hence number of roots was significantly lower in the cuttings (4.40) treated with 200 ppm IBA.

In this study, increasing level of IBA had a tendency to increase rooting of roots. However, at higher concentrations it was reduced. Ahmad *et al.*, (2003) reported that IBA enhanced rooting in peach cuttings at intermediate concentrations (3 mg l^{-1}) but inhibited at higher concentration (4 mg l^{-1}). In this experiment a intermediate dose (150 ppm IBA) proved better than higher regime (200 ppm IBA). Werner and Boe (1980) also supported these results.

Root Length (cm)

Three-way interaction and the interaction between parental lines and dipping time was non-significant (Table 3). Parental lines showed a highly specific effect on root length of stem cuttings at different concentrations of IBA (Fig. 3), for example root length of TPS-67 was significantly lower than MF-1 at control but difference in root length between TPS-67 and MF-1 was non- significant when the stem cuttings were

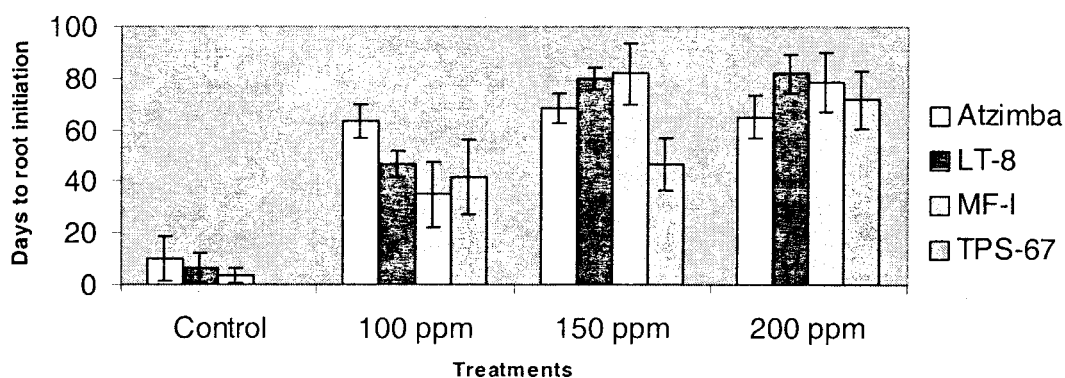


Fig. 1. Effect of IBA concentration on days to root initiation of stem cuttings of TPS parental lines.

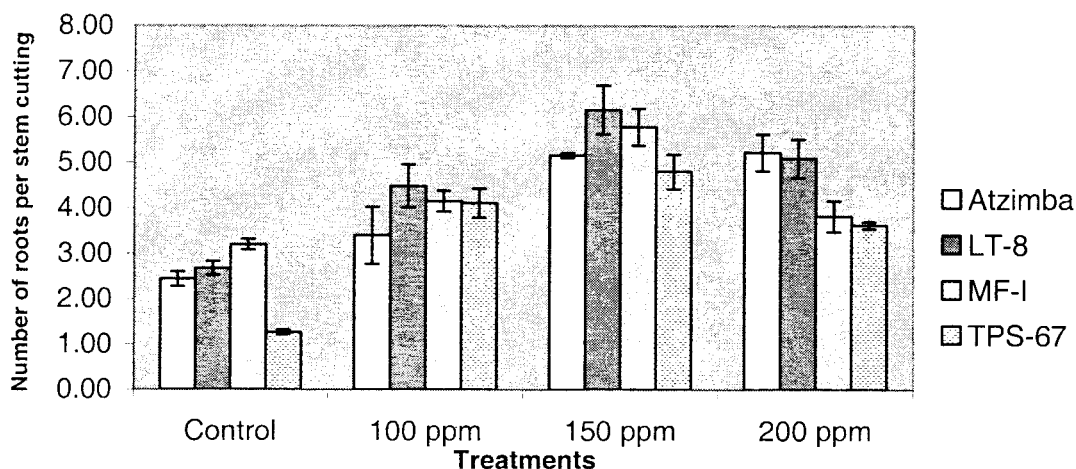


Fig. 2. Effect of IBA concentrations on on number of roots per stem cutting of TPS parental lines.

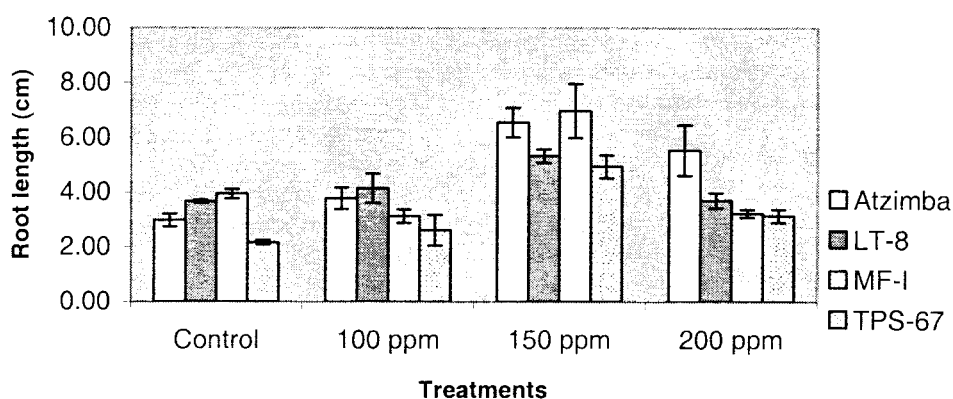


Fig. 3. Effect of IBA concentrationson on root length per stem cutting of TPS parental lines.

treated with 100 ppm IBA. Similarly, there was non-significant difference in the root length of Atzimba and LT-8 when the stem cuttings were treated with 100 ppm IBA, whereas they showed significant difference in root length of stem cuttings that were treated with 150 ppm IBA. The results here agree with those of Ishtiaq *et al.* (1989) who also found parental lines specific effect on root length of stem cuttings of peach when treated with different concentrations of IBA. The results suggest that the effect of different concentrations of IBA on root length is cultivar dependent (Holwerda and Ekanayake, 1991 and Wood and Coke, 1990).

Dipping time x concentration effect was highly significant (Table 3). Difference between root length of cuttings treated with 100 ppm and 200 ppm of IBA was significant when the cuttings were dipped in the IBA for five seconds whereas when the cuttings were dipped for 10 and 15 seconds the difference in root length of stem cuttings at these concentrations was non significant (Fig.4). The results suggest that further investigation should be made to find out proper dipping x concentration combinations for TPS parental lines propagation.

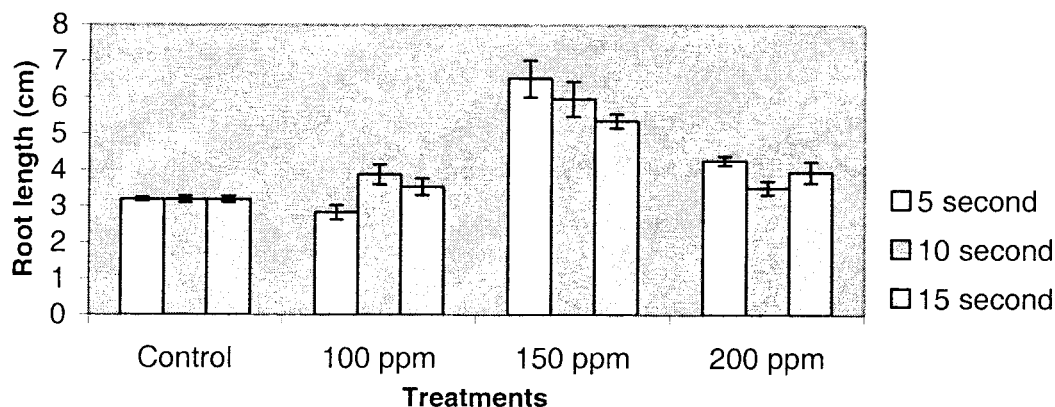


Fig. 4. Effect of IBA concentration on x dipping time on root length per stem cutting of TPS parental lines.

Main effect of parental lines was highly significant on root length (Table 3). Varieties mean showed that the longest root (4.71 cm) was formed in cuttings of Atzimba while the shortest roots (3.22 cm) were observed in TPS-67 (Table 4). The results here are in accordance with those of Wood and Coke (1990) and Ishtiaq *et al.* (1989) who found varietal difference in root length of stem cuttings in potato and peach, respectively.

The effect of concentrations of IBA on root was highly significant (Table 3). The longest roots (5.94) were formed in the cuttings treated with 150 ppm whereas the cuttings that were not treated with IBA produced the shortest roots. In apple (Badshah *et al.*, 1995) and Apricot (Ishtiaq *et al.*, 1988) root length greatly increased with increasing IBA concentration whereas in potato (Hossain *et al.*, 1998) root length was the greatest with intermediate concentration of IBA. The increase in root length of cuttings in this experiment may be due to effect of IBA on metabolic translocation (McDavid *et al.*, 1972, Ishtiaq *et al.*, 1989) and increased plasticity of the cell wall of cambium cells (Heyn, 1931, Digby and Wangerman, 1965, and Nymora and Mnzwa 1983). Shorter root length of cuttings at 200 ppm than 150 ppm IBA (Table 4) suggests that the IBA slowly inhibited cell activity at higher concentration (Melik –Sarkisov, 1994). These results also supported by findings of Ahmad *et al.*, (2003) who indicated that root elongation phase was very sensitive to IBA concentrations in apple rootstock and was inhibited at higher concentrations

REFERENCES

- Ahmad, T., H.U. Rahman, CH.M.S. Ahmad and M.L. Laghari. 2003. Effect of culture media and growth regulators on micropropagation of peach rootstock GF 677. *Pak. J. Bot.*, 35(3): 331-338.
- Badshah, N., N. Rahman and M. Zubair. 1995. Effect of indolebutyric acid (IBA) on the cuttings of M-26 and M-27 apple rootstocks. *Sarhad J. Agri.* XI (4): 449-453.
- Cole, E. F. and N. S. Wright. 1967. Propagation of potato by stem cuttings. *Am. Potato J.* 44:301-304.
- Digby, J. and E. Wangerman. 1965. A note on the effect of the shoot and root apex on secondary thickening in pea radicles. *New Phytol.* 64: 168-170.
- FAO. 1995. Statistical summary of agricultural production in: Quarterly bulletin of statistics, food and Agriculture Organization of the United Nations, Rome. 5(1): 14-17.
- Heyn, A.N.J. 1931. Auxin and cell elongation. *Plant growth substances in Agriculture*. 1st Ed. P. 93. W.H. Freeman and Company San Francisco.
- Holwerda, H.T. and I. J. Ekanayake. 1991. Establishment of sweet potato stem cuttings as influenced by size, depth of planting, water stress, hormones and herbicide residues for two genotypes. *Scientia Horticulturae*. 48(3-4):193-203.
- Hossain, M.J., M.A.I. Khan and M.A. Hoque. 1998. Effect of IBA and NAA on rooting of potato stem cuttings. *J. Indian Potato Assoc.* 25(1&2): 53-56.

- Ishtiaq, H.M., K.D. Wazir and A. I. Haq. 1988. Initiation of roots in Trevatt and Nencape apricot cuttings as affected by indolebutyric acid (IBA). *Sarhad J. Agric.* 4(6): 793-798.
- Ishtiaq, H.M., I.U. Haq and A. Mohammad. 1989. Initiation of roots in peach rootstocks cvs. Peshawar local and nemaguard as affected by indolebutyric acid. *Sarhad J. Agric.* 5(1): 41-45.
- Kayim, M. and N.K. Koc. 1992. Obtaining of virus free potato (*Solanum tuberosum* L.) planting stock material through meri stem culture. *Doga, Turk Tarum ve Ormancilik Dergis.* 16(2): 380-391. Cukurova, University, Adana, Turkey.
- McDavid, C.R., C. Marshall and G.R. Sager. 1972. The effect of Auxin from the shoot on root development in pea. *New Phytol.* 71: 1027-1032.
- Meguel, Q. B., J. E. Brayan, M. T. Jackson and M. G. Nelson. 1981. Leaf bud cuttings: a rapid multiplication technique for potato's CIP slide training series, Guide Book 1(4): CIP, Lima Peru. P.24.
- Melik-Sarkisov, O.S., L.V. Cherezhanova and V.N.I. Ovchinnikova. 1994. Exogenous phytohormones as a factor in the cytogenetic variability of potato cells in *in-vitro* culture. *Sel'skokhozyaistvennaya-Biologiya* 1: 69-73.
- Nicoll, F. 1993. Genetic improvement of cherry for farm woodlands. *Q. J. For.* 87:187-194.
- Nymora, M.S. and Mnzwa. 1983. Rooting response to Juvenile and adult cuttings of apple (*Malus sylvestris* L.) and peach (*Prunus persica* L.) to IBA in Tanzania, *Beitrage Zuetropischen Landwirtschaft and veterinarmeddizin.* 20(2): 135-140 (*Hort. Absts.* 53(8):552).
- Singh, S. and P. S. Naik. 1993. Rapid seed multiplication in advances in Horticulture. Vol.7. Potato (K.L. Chadha and J. S. Grewal, Eds.) pp.557-75.
- Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics. McGraw Hill Book Co. Inc. New York.
- Wood, K. and L. Coke. 1990. Growth and tuberisation of five varieties of potato (*Solanum tuberosum* L.) in *in-vitro*. *Proc. Annual National Conf. On Science and Technology* (part 2). Scientific Research Council, Kingston, Jamaica. pp. 18-30.
- Werner, E.M. and A.A. Boe. 1980. *In Vitro* propagation of malling 7 apple rootstock. *Hort. Sciences*, 15: 509-510