Proceedings:
International Symposium on
Prospects of Horticultural Industry in Pakistan
28th to 30th March, 2007
Institute of Horticultural Sciences, University of Agriculture, Faisalabad

APPLE IMPROVEMENT THROUGH SOMACLONAL VARIATION VIA *IN VITRO* TECHNIQUE

Mir Saleem Khattak*, Muhammad Javed Khan, Sabir Gul Khattak, Muhammad Ibrar, A.Rauf and R.M Skirvin*
Agricultural Research Institute, Tarnab Peshawar, Pakistan
*Plant and Animal Biotechnology Building 1201 west Greory Drive, Urbana IL 61801, USA
*Email: mudasir_khattak@yahoo.com

Abstract

Embryos of three apple cultivars namely, Golden Delicious, USDA $_{4\text{-}20}$ and Liberty were subjected to BA (N^6-benzyle adenine) at 0, 20, 40 and 60 µM to induce somaclonal variation (dwarfism) via *in vitro* technique. The main objective of this study was to produce dwarf plants in apple, because dwarf plants are more yielder than standard size, while the labor cost is low on pruning, training, picking of fruit during harvesting and spraying chemicals to control diseases and insects. The result showed that 100% embryos emergence was noted only in USDA$_{4\text{-}20}$ at BA concentrations. BA at 60 µM took longer period (14 Days) of embryos emergence in Golden Delicious and USDA $_{4\text{-}20}$ and no sprouting in liberty. Shoot length was reduced remarkably (0.5 cm) at 60 µM of BA in USDA $_{4\text{-}20}$. At higher concentration of chemical (60 µM) equal root length (0.4 cm) was recorded in Golden Delicious in USDA $_{4\text{-}20}$. Numbers of leaves (9.0) were significantly higher at 60 µM (BA) in Golden Delicious. Greenhouse studies revealed that very dwarf plants (1.0 cm) and more number of roots (2) were observed in USDA $_{4\text{-}20}$ at 60 µM of BA. Survival percentage of very dwarf plants was maximum (3) in Golden Delicious at 60 µM of BA. The plants produced by BA under laboratory and greenhouse were slower in growth and dwarf in size than non-treated plants.

INTRODUCTION

Apple (*Malus domestica* Borkh) is propagated by tissue culture technology, which offers faster multiplication of true type colonel plant material via axillary bud proliferation (Lane et al., 1982; Zimmerman, 1984). The traditional method of apple propagation is budding/grafting onto a land-race rootstock, which is time consuming cumbersome, uneconomical and causes a spread of diseases between stock and scion (Jones, 1993). Plant tissue culture has facilitated the creation of somaclonal variation in plant species and cloned uniformity is now recognized as the exception rather than the rule (Skirvin, 1982). Somaclonal variation is variation among regenerated plants that occurs as a result of any type (make it clear). It may arise from pre-existing or induced variation. High concentration of growth regulators also can alter the frequency of ploidy changes vs point mutation (Skirvin , 1982). Tissue culture technology is also successful to separate dwarf apple cultivars from standard type. For instance Lane et al. (1982) reported that dwarf apple scion cultivar wijick is more resistant to high level of BA and TDZ (Thidiazoran) and summer land

(standard). Sawar and Skirvin (1982) stated that Regal Gala (standard) was less tolerant to BA than M26 dwarf and Macspur (Intermediate). Further confirmation was made by Khattak et al. (1997) and Khattak et al. (2004) who reported that BA and TDZ promoted dwarfism in apple cultivars. Dwarf apple cultivars are more yielder due to low vegetative growth, low labor cost on pruning and training, spraying chemicals to control insects and diseases (Roistacher, 1996). Dwarf plants are also easy to pick the fruits during harvesting. The objective of this study was to isolate dwarf apple cultivars from standard ones at early embryo stage by the application of *in vitro* technique.

MATERIALS AND METHOD

Embryos of three cultivars namely Golden Delicious, $USDA_{4-20}$ and liberty were disinfected with commercial bleach (10%) NaOC1 (V/V) supplemented with Triton × 100 (5 drops per 100 ml) for 10 min. These were then explanted in 30 ml of water agar medium containing only BA with 0, 20, 40 and 60 µM in culture jars (8.7x5 cm^2). The media were prepared by adding the appropriate amount of BA and agar (5 g l^{-1}) in culture flasks. Agar was boiled in an autoclaved for 5 min, dispensed into culture jars which were then autoclaved at 1.05 kg cm^{-2} for 20 minutes. All cultural manipulations were carried out aseptically in laminar air flow hood. The plant materials were maintained in a culture room at about 25ºC for 16 hours photoperiod with light intensity of 131 μMS^{-1} (cool white fluorescent). There were five jars (replicates) per treatment and 10 embryos were cultured in each jar. Data for various morphological characters were analyzed as mean ± standard deviation.

RESULTS AND DISCUSSION

It is obvious from Table 1 that BA at 60 µM presented maximum embryo emergence (100%) in USDA $_{4-20}$ as compared with other apple cultivars. BA at higher concentration (60 µM) took longer period of embryo emergence (14 days) in Golden Delicious and USDA $_{4-20}$. Smaller shoot (0.5 cm) and root (0.4 cm) was noted in USDA $_{4-20}$ at 60 µM of BA than Golden Delicious and Liberty. Maximum number of leaves (9) was produced by Golden Delicious at 60 µM of BA.

Table 1: Effect of BA concentrations on embryo emergence and growth of apple cultivars *in vitro*

S. No.	Cultivars	BA concentration (µM)	Embryo emergence		Plant growth parameters		
			Percent	Days	Shoot length (cm)	Root length (cm)	Leaf number
1	Golden delicious	0	90±11	7.0	3.0±0.6	3.6±2.0	6.4±1.1
		20	81.7±11.7	9.3±3.0	2.3±0.4	1.6±0.5	3.5±1.4
		40	71.6±3.6	11.7±3.2	1.4±0.3	0.8±0.3	8.1±1.0
		60	65±2.9	14.0	0.8±0.2	0.4±0.1	9.0±0.9
2	$USDA_{4-20}$	0	100	7.0	2.7±0.7	2.6±0.8	6.0±1.3
		20	100	10.5±3.8	1.6±0.4	1.0±0.3	4.0±1.2
		40	100	12.8±2.3	1.3±0.3	0.7±0.2	8.0±1.9
		60	100	14.0	.05±0.2	0.4±0.1	8.6±1.7
3	Liberty	0	100	7	2.8±0.6	3.2±1.0	6.2±1.5
		20	100	7	2.0±0.6	1.5±0.6	3.0±1.2
		40	100	14	1.5±0.5	1.0±0.5	6.3±1.0
		60	0	0	0	0	0

0 indicates no emergence; Mean of 5 replicates; Mean ± Standard deviation

The ex *vitro* apple cultivars were transferred to greenhouse and kept for 6 weeks. Table 2 reveals that the smaller shoot of 1.0 cm was produced in USDA $_{4\text{-}20}$ at 60 µM of BA. While, 2 roots were generated by the chemical at 60 µM in Golden Delicious. The chemical at higher concentration (60 µM) also produced shorter root (1.3 cm) in the cultivar. Survival percentage was more in Golden Delicious (3%) and USDA $_{4\text{-}20}$ (2%) by BA at 60 µM.

Table 2: Growth and survival percentage of ex-in vitro apple cultivars plantlets in green house (6 week studies)

S. No.	Name of Cultivar	BA concentration (µM)	Shoot length (cm)	Number of roots	Root length (cm)	Survival percentage
1	Golden delicious	0	8.1±2.1	7.3±2.5	1.8±0.6	100
		20	4.8±1.2	4.6±1.5	3.6±1.6	60
		40	3.9±1.0	2.6±1.3	2.6±1.0	30
		60	2.7±1.1	2.0±0.8	1.5±0.9	3
2	USDA$_{4\text{-}20}$	0	7.9±2.2	7.5±2.6	1.9±0.6	100
		20	4.5±1.3	3.6±1.5	3.0±0.7	50
		40	3.0±1.5	2.7±1.0	2.7±0.5	20
		60	1.0±0.5	1.0±0.3	1.3±0.6	2
3	Liberty	0	8.0±2.3	7.8±2.3	1.8±0.7	100
		20	3.5±2.0	3.7±1.6	2.8±0.9	50
		40	2.1±1.6	1.6±0.8	1.8±0.8	25
		60	0	0	0	0

0 indicates no emergence
Mean of 5 replicates except last column
Mean ± Standard deviation

The laboratory/greenhouse test of *in vitro* or ex vitro clones indicated that plants of different growth habits were obtained. For instance, the embryo subjected to different levels of BA had compact and slow growth, dwarf in size, few and short root length than non-treated. The same characteristics of dwarf mutant plants were reported by Lapin (1976). The findings of this study are also in agreement with Sarwar (1996) who reported that dwarfism depends on the growth regulators to which the tissue is exposed as well as the genetic make up of the particular plants. The plant may have mutant cell in their somatic tissue, which have the ability to grow as dwarf plants after treatment to high levels of cytokinins. Dwarfism is also due to chimerism in which the parent consists of sector of both mutant (dwarf) and normal tissue growing together (Hartmann and Kester, 1968). This relationship was explained by Skirvin et al. (1994) who reported that growth regulators have shown implication in the induction of variability. The variation rates are increased as the over all concentrations of growth regulators rise. High growth regulator concentrations also can alter the frequency of ploidy change vs point mutation. This relationship is similar to Lane et al. (1982) that dwarf apple cultivar "Wijick" is more tolerant to the highest level of BA than "Macspure" (intermediate) and summer land (Standard). Silimar observations were also confirmed by Sarwar and Skirvin (1996); Sarwar (1996); Khattak et al. (1996); Khattak et al. (1997) and Khattak et al. (2004). They found that Red Delicious has shown resistant to higher level of TDZ. The same observations were also reported by Sarwar and Skirvin (1996) who revealed higher tolerance to BA. In the present studies high level of BA is recommended for induction of dwarfism in apple cultivar USDA $_{4\text{-}20.}$

REFERENCES

Hartmann, H.T and D.E. Kester. 1968. Plant propagation Principles and Paractices. 2nd Ed. Prentice Hall, Inc. Englewood cliffs, New Jersy, pp.203.

Jones, O.P. 1993. *In vitro* propagation of tree Crops. In plant Biotechnology. Soc. Exp. Biol. Seminar ser:18 (Mantell. A.H. and H. Smith. (Ed) Cambridge Univ. Press U.K.pp 139-155.

Lapins, K.O. 1976. Spur type growth habit in 60 apple progenies. J. Amer. Soc. Hort. Sci. 99:568-572.

Roistacher, C.N. 1996. A historical review of the major Graft-transmissible diseases of citrus. FAO, UNO, Cairo, Egypt, pp.89.

Sarwar, M. and R.M. Skirvin. 1996. The development of "Selective Ckulture Media to distinguish dwarf, intermediate and standard apple (Malus × domestica Borkh.) cultivars *in vitro*. Plant Cell Tiss. Org. Cult.

Khattak, M.S., R.M. Skrivin and M. Sarwar. 1996. Can tissue culture be used to distinguish dwarf and standard size Apple tree in segregation population of apples seedlings *in vitro*. Fruits Varieties J.

Khattak, M.S., R.M. Skirvin and A.G. Otterbacher. 1997. TDZ promotes dwarfism in segregating population of apple cultivars *in vitro*. Endeavours in biotech. Deptt. Botany, Univ. of Peshawar, Pakistan, pp.91-95.

Khattak, M.S., R.M. Skirvin and A.G. Otterbocher. 2004. Induction of dwarfism in apple cultivar USDA $_{4\text{-}20}$ via *in vitro* technique. Sarhad J. Agric. 20(2):203-206.

Sarwar, M. 1996. Cytokinin tolerance and *in vitro* selection for dwarfism in apple. Ph.D. Thesis, University of Illinois, Urbana Champaign, USA, pp.289.

Skirvin, R.M. 1982. Fuit crops. In: congr. BV(ED) cloning Agriculture plants via *in vitro* techniques. VRS press Boca Ration, FI. pp.51-139.

Lane, W.D., N.E. Lonney and F. Mage. 1982. A selective media for the growth of compact (dwarf) mutant of apple. Theor. Appl. Genetic. 61:219-233.

Zimmerman, R.H. 1984. Rooting apple cultivars *in vitro* interaction among light, temperature, phlorgucinol and auxin. Plant Cell Tiss. Org. Cul. 3:301-311.