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Effect of Explant and Sucrose on Microtuber Induction in Potato Cultivars

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ABSTRACT

Effect of explant source was studied in indigenous potato variety PARS-70 and a Dutch introduction Santa for microtuber induction at different sucrose levels supplemented in Murashige and Skoog medium. *In vitro* grown shoots and nodal explants proved superior to shoot tips for shoot formation, shoot length, number of roots, root length, number of microtubers and microtuber weight in PARS-70 at 8% sucrose concentration in the medium. Cultivar PARS-70 also showed better response for above mentioned parameters at different sucrose levels than Santa. Such studies might help to reduce the cost of production of virus free potato seed from indigenous cultivars.

Key Words: Potato; Microtubers; PARS-70; *In vitro*

INTRODUCTION

Potato *(Solanum tuberosum L.)* is cultivated as a vegetable and cash crop in Pakistan. It produces the largest quantity of carbohydrates per day and per unit area among food crops (Zaag & Horton, 1983). Potato ranks second among the most important crops after maize in terms of number of producing countries and fourth after wheat, maize, and rice in global tonnage. In Pakistan, it is grown on an area of 104.5 thousand hectares with 1684.7 thousand tons production per annum (MINFAL, 2004). Environmental conditions are conducive for aphid (*Myzus persicae*) attack, the main insect vector for virus transmission. Potato cultivars are seriously infected with various virus and viroids. The potato leaf roll virus (PLRV) and potato virus Y (PVY) can cause upto 95% decrease in the yield of potato crop while potato virus X (PVX) has been reported to reduce tuber yield from 50-75% (Ahmed *et al.*, 1993).

In Pakistan, potato growers are using imported virus-free seed. The storage and transportation charges of seed bulk increases the cost of seed. Among the components of total cost of production, the seed share is ~47%. The yield potential of foreign introductions is below the desired levels, probably, because they were developed under different agro-climatic conditions. Methodologies for *in vitro* culture have contributed to vegetative propagation of many plant species (Murashige, 1977; Abo-el-Nil, 1977), and have emerged as an alternative to reduce the cost of production of virus free seed needed to be produced year around (Abbot & Belcher, 1986). The clonal progeny of microtubers can be raised in the green house or in the field as basic seed. The aim of this study was to develop a protocol for microtuber production of indigenous variety PARS-70 and new Dutch introduction Santa. PARS-70 is an approved cultivar which can be stored under improvised room conditions (Khan, 1991). Both PARS-70 and Santa are very high yielding cultivars. The protocol shall help to reduce the cost of production for the two new cultivars.

MATERIALS AND METHODS

Mother tubers of potato cultivars PARS-70 and Santa were selected. For explant preparation (shoot tips and nodes) the mother tubers were grown in three different ways. Two media were used for this purpose; a) agar solidified MS basal medium supplemented with 8% sucrose, and b) agar solidified MS medium supplemented with different levels of sucrose viz. 3, 6, 9 and 12%.

Etiolated shoots. Mother tubers were washed with water, followed by 15 min dip in 95% alcohol and then packed in the craft papers bags which were kept in dark for one month at 21°C constant temperature in the growth chamber. Etiolated shoots emerged were used as explant source (Fig. 1). Etiolated shoots were surface sterilized by washing for half an hour in tap water before *in vitro* culture.

***In vitro* grown shoots.** The shoots were raised from nodal explants of etiolated shoots cultured on MS medium supplemented with 3% sucrose concentration.

Green house grown shoots. Mother tubers were planted in pots in the green house. After 60 days from sowing the nodes and shoot tips were taken.

Fig. 1. Etiolated sprouts emerging from tubers of PARS-70 kept at 21°C for one month.

Nodes and shoot tips obtained from the above mentioned sources of explant were surface sterilized by 1.5 min dip in 70% ethyl alcohol and 20 min dip in 10% chlorax solution plus two drops of Tween-20 as surfactant followed by 3-5 washings with double distilled autoclaved water (Wang & Hu, 1980). Nodes and shoot tips obtained were cultured on MS-medium supplemented with 8% and 3, 6, 9 and 12% sucrose concentration for microtuber induction. Cultures were placed in growth room at a temperature of 27°C under 16 h photoperiod with light intensity of 2500 lux. The experiment was laid out in completely randomized design (CRD). There were three replications with 10 observations per treatment per replication. The data were analyzed by Duncan's Multiple Range test (DMR) (Steel & Torrie, 1980).

RESULTS AND DISCUSSION

Analysis of variance revealed highly significant differences among the explant types than the explant sources. Mean comparison of explant types obtained from three different explant sources presented in Table I showed that nodal explant was better for shoot formation (100%), shoot length, number of microtubers (2.16) and microtuber weight (164.50 mg/tuber) while shoot tip explant gave significantly more number of leaves (6.61) and higher root formation (81.37%).

Maximum shoot development (100%) was observed from nodal explants obtained from all the three sources and shoot tip from *in vitro* grown and etiolated sprouts. While significantly (P>0.005) less shoot formation (51.25) was depicted by shoot tip explant obtained from greenhouse grown shoots. These observations are highly supported by Warch *et al.* (1989) who worked on North American potato cultivars and obtained similar trend. Maximum shoot length (5 cm) was observed in the shoot tip explant obtained from *in vitro* grown shoots followed by nodal explant from *in vitro* grown and etiolated shoots. Minimum shoot length was found from both explant types obtained from green house grown shoots. (Table I). The results are in line with the findings of Litz and Conover (1978) and Asokan *et al.* (1984). Similar trend was observed for number of leaves as maximum number of leaves were obtained form shoot tip and nodal explants excised from *in vitro* grown shoots followed by shoot tip and nodal explants from green house grown and etiolated shoots, respectively. Similarly large number of leaves was obtained from Desiree and Estima potato cultivars on MS medium (Amirouche *et al.*, 1985). Root formation percentage was significantly (P>0.001) better in shoot tip and nodal explants from *in vitro* grown and etiolated shoots ensued by nodal and shoot tip explants of the previously mentioned sources of explant, respectively, while green house grown shoots depicted minimum root formation. Significantly (P>0.001) higher number of microtubers (5.00) was yielded by nodal explant from *in vitro* grown shoots followed by shoot tip and nodal explant of the *in vitro* and greenhouse grown shoots; whereas, least microtuber formation was found from both explants of the etiolated shoots. On the other hand, maximum tuber weight was given by nodal explant from greenhouse grown shoots (480.60 mg/tuber) followed by etiolated shoots while minimum tuber weight was depicted by shoot tip explant obtained from *in vitro* grown shoots (Table I). Microtuber induction was found to be directly related to high sucrose concentration than the level of growth hormones in the medium and the findings are supported by Garner and Blake (1989).

Table I. Response of explant source for various growth parameters at 8% sucrose level

Explant Sources	Shoot Formation (%)		Shoot Length (cm)		Number of Leaves		Root Formation (%)		Number of Microtubers		Microtuber weight (mg/tuber)	
	Node	Shoot Tip	Node	Shoot Tip	Node	Shoot Tip	Node	Shoot Tip	Node	Shoot Tip	Node	Shoot Tip
In vitro grown shoots	100 a	100 a	3.14 b	5.00 a	8.62 a	10.31 a	62.75 b	100 a	5.00 a	1.25 b	196.80 c	106.10 d
Green house Grown shoots	100 a	51.25 b	1.45 c	0.69 c	3.14 c	6.78 b	1.25 c	0.25 c	1.25 b	0.25 c	480.60 a	190.40 c
Etiolated shoots	100 a	100 a	3.27 b	1.30 c	5.38 b	2.74 c	100 a	62.75 b	0.25 c	0.25 c	395.40 b	198.20 c
Means	100 a	83.75 b	2.71 a	2.33 a	5.71 b	6.61 a	54.66 b	81.37 a	2.16 a	0.66 b	357.60 a	164.90 b

*****Means sharing the same letters are statistically non-significant.

Table II. Effect of different sucrose levels on various growth parameters of Potato cultivars

Sucrose Levels (gL^{-1})	Shoot Regeneration		Shoot Length (cm)		Number of Leaves		Number of Roots		Root Length (cm)		Number of microtubers	
	PARS -70	Santa	PARS-70	Santa	PARS- 70	Santa	PARS-70	Santa	PARS- 70	Santa	PARS-70	Santa
3	1.23 a	0.74 b	5.29 a	2.04 b	5.17 a	3.24 b	3.74 b	2.79 c	4.33 a	1.05 c	2.21 c	0.21 d
6	1.51 a	0.75 b	4.81 a	2.19 b	4.62 a	3.57 b	4.03 b	2.22 c	5.01 a	1.05 c	12.50 a	0.50 d
9	1.06 a	0.70 b	4.05 ab	2.75 b	4.76 a	3.85 b	6.18 a	4.37 b	3.48 b	2.04 b	4.58 b	0.26 d
12	1.06 a	0.87 b	2.90 b	2.11 b	3.73 b	3.29 b	2.53 c	2.56 c	1.43 c	1.02 c	2.32 c	0.29 d
Means	1.29 a	0.76 b	4.26 a	2.27 b	4.57 a	3.29 b	4.12 a	2.56 b	3.58 a	1.30 b	5.40 a	0.31 b

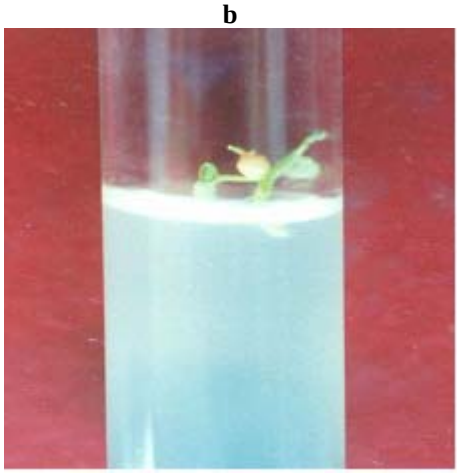
*****Means sharing the same letters are statistically non-significant.

In second part, effect of different sucrose concentrations was studied for two different potato cultivars PARS-70 and Santa for microtuber induction and other growth parameters. Mean comparison revealed that PARS-70 cultivar of potato was found significantly better for all the growth parameters studied including shoot regeneration, shoot length, number of leaves, number of roots, root length and number of microtubers (Table II; Fig. 2a; b and Fig. 3).

Fig. 2. Microtuber induction from a) nodes and b) shoot tip of test tube grown seedlings of PARS-70

a

b



Six percent sucrose concentration was much better for good shoot regeneration and root length while 3% sucrose for shoot length and average number of leaves for PARS-70. PARS-70 at 6% sucrose concentration displayed maximum number of roots (Table II). In cultivar Santa, 12% sucrose was found significantly better for shoot length (2.75 cm), number of leaves (3.85), root formation (4.32%) and root length (2.04 cm) at P>0.005. Overall, 6 and 9% sucrose levels were found much better for PARS-70 and Santa, respectively, for all the growth parameters studied. Significantly higher number of microtubers was induced using 6% sucrose concentration in the MS medium while further increase in sucrose level reduced the number of microtubers whereas no microtuber induction was observed in Santa. The microtubers obtained are further used for the multiplication and formation of minitubers in the green houses (Fig. 4) The results obtained are in conformity with Lipe and Skinner (1979), Wareing and Jenning (1980), Garner and Blake (1989), Zaag and Zaag (1990) and Khan *et al*. (2003). The indigenous potato cultivar PARS-70 was better for all the parameters studied than foreign introduction Santa and the results are supported by the

Fig. 3. Microtuber formation from nodal explant in PARS-70

Fig. 4. Minitubers of cultivar PARS-70

findings of Mumtaz and Quraishi (1989), Khan (1991), Ahmad and Khan (1993) and Khan *et al*. (2003). Such studies are helpful in reducing the cost of production of virus free potato seed from indigenous cultivars and to save the foreign exchange every year to import seed.

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