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OPTIMIZATION OF MICROTUBERIZATION IN INDIGENOUS POTATO CV. DESIREE

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ABSTRACT

In vitro generated microtubers of potato are an ideal source for germplasm conservation and its exchange as a disease free material. The investigation reported here was conducted to evaluate different factors affecting microtuberization in potato variety Desiree. Initially the in vitro plantlets of potato were established from meristems. These in vitro generated plantlets were transferred to MS media containing different levels of sucrose (0, 4, 8, 10 and 12 %), kinetin (0, 1 and 2 mgl^{-1}) and photoperiod (8 and 16 hours) for the induction of microtuberization. During this study the data was collected for parameters as days required for tuber induction, number of tubers per plant, frequency of tuber induction and their mean weight. Statistical analysis was carried out and among the evaluated factors high sucrose concentration (8%), together with short photoperiod (8 hour) without hormones was found the most effective regime for the low cost mass production of high quality microtubers in potato variety Desiree.

INTRODUCTION

The extension of potato micropropagation is the induction of microtubers in *in vitro* generated plantlets. Although traditional tubers and microtubers are similar in morphology and biochemical characteristics, microtubers are easy to store, transport and exchange as healthy potato seed due to their small size and weight (Nistor et al., 2010). The usual size of a microtuber is about a pea in various shapes (round, oval or elongate) and colors (yellow, brown or green) (Ranalli, 2007).

The *in vitro* induction of microtubers followed by high rate multiplication provides thousands of potato seeds within a very short time period eventually reducing the process of potato production up to 3-4 years. Conventionally, potato is propagated vegetative by tubers. On one hand this strategy is very effective to maintain the uniformity of this edible crop in terms of genetic potential; on other hand this method of propagation has a drawback. This vegetative multiplication of crop accumulates viral infections. The accumulated viruses are transmitted from seed stock to progeny and if this seed stock is not maintained properly, the viral contaminations can attain a level of 100% (Nistor et al., 2010). Alternatively, when in vitro plantlets are generated from virus free mothers these will produce virus free minitubers. Thus, the in vitro generated material will be maintained as virus free material giving rise to virus free seed stock which will be ultimately utilized for the potato field propagation. Using such virus free minitubers as seed stock every time will improve the potato production. Thus, the *in vitro* propagation methods are suitable for rapid mass production, maintenance of elite cultivars and generation of new breeding lines and conservation of existing germplasm.

The performance of microtubers in the field, during storage and/ or in germplasm conservation procedures mainly depend

on their quality i.e. health and size. In general larger microtubers behave better as compared to smaller ones. Hence, the production of desired material needs optimization and modification of the in vitro methodology focusing the cultivar of interest. The process of microtuberization is very complex and mainly depends on genotype, state of explant and environmental factors (Uranbey, 2005; El-sawy et al., 2007). Environmental factors can be extended as culture medium, temperature, photoperiod, light intensity, minerals, concentration of sucrose and growth hormones. Although the production cost of one microtuber is higher than that of an *in vitro* plantlet, optimized *in vitro* methods can generate thousands of minitubers within months. In addition, this complexicity of the process suggests the importance of optimizing culture conditions for cost effective microtuberi-zation in different genotypes. The present study was tended to evaluate some of the most important factors controlling the micro tuberization process (as sucrose, kinetin, photoperiod) and implementing important findings for the cost effective production of microtubers in potato variety Desiree.

MATERIALS AND METHODS

Virus free tubers of cultivar Desiree were received from the Horticultural Research Institute, Mirpurkhas and were processed as described by Yasmin et al., (2011). The surface sterilized meristems were planted on MS media (Murashige & Skoog, 1962) containing 1.0 mg/l pantothenic acid and 0.5 mg/l gibberellic acid (Yasmin et al., 2011). The cultures were kept at $22\pm2\ ^{0}C$ for 16 hours photoperiod. After 6-weeks of multiplication there was enough stock of *in vitro* generated plantlets ready to be induced for microtuberization.

The *in vitro* established plantlets were transferred to MS media supplemented with different levels of sucrose (0, 4, 8, 10 and 12 %), kinetin (0, 1 and 2 mgl^{-1}) and photoperiod (8 and 16 hours). The 30 treatments were named as T1, T2, T3 to T15 and T1a, T2a, T3a to T15a treated under 8 and 16 hours photoperiod, respectively. Plantlets were grown on above mentioned treatments for 8-weeks and data was collected.

Data was recorded for tuber induction frequency/ treatment, days taken to induce tuberization, number of microtubers/ plantlet, and weight of microtubers. The experiment was performed in four replicates with 10 explants/ treatment. ANOVA was used to assess the data and Duncan's Multiple Range Test was performed to analyze the means for significant differences at 95% confidence level.

RESULTS AND DISCUSSIONS

In the present study the microtuberization was induced in the potato cv. Desiree and the effectiveness of different factors was evaluated. The optimal growth of microtubers (Mt) was observed in T3, 8% sugar without growth regulator in 8-hour photoperiod.

a. **Frequency of Microtuberization Induction (MI):** The MI was found in the range of 0-100%. Negative control (MS medium without sugar and kinetin) did not show any sign of MI, whereas 100% MI was observed when MS was supplemented with sucrose (8 and 10%) and kinetin (1 and 2 mgl^{-1}) in short day regime (8hours). 100% MTI was observed in T8, T9 and T13 whereas T3, T4, and T14 showed the 96.7, 93.3 and 93.3%, MI respectively (Table 1). The observed MI in all these treatments was non-significant when analyzed by DMRT ($p<0.05$). During the 8-week of study none of the *in vitro* plantlets showed 100% MI evaluated under 16-hours day period. In presence of

growth regulator Kinetin the MI frequency was improved as presented in Table 1. DMRT revealed these increments as insignificant ($p<0.05$). In summary T3, T4, T8, T9, T13 and T14 out of thirty treatments showed the highest frequency of MI in short days.

b. **Days taken to induce Mt:** Although the stolons were formed within first few days at sucrose concentrations 8 and 10%, the microtubers were visible as early as 9 days at 10% sucrose (T8). The MI was seemed to be mainly effected by sucrose concentration as in presence of sucrose and kinetin the increase in MI was insignificant. T8 (9days), T3 (9.5days) and T4 (10.5 days) took minimum time to induce Mt. the observed mean differences are insignificant at the $p<0.05$.

c. **Number of Microtubers:** A higher number of Mt was observed at higher concentration of sugar i.e. 8 and 10%. Number of Mt was found in the range of 0-5 microtubers/plantlet. According to the mean values of the numbers of microtubers (Mt) 2.37 Mt/ plantlet (T8) was the highest followed by 2.27 (T3), 1.97 (T9 and T14), 1.87 (T13) and 1.80 (T4). The mean differences between T8 and T9 are insignificant ($p<0.05$).

d. **Weight of Microtubers:** The average weights of obtained Mt after 8 weeks of culture are expressed in table 1. The highest weight of Mt was observed in treatment T3 (312.4mg.) followed by T9 (301.3mg.), T8 (300.8mg.), T13 (300.01 mg.), T10 (295.1mg.) and T14 (289.6mg.). The mean differences among above mentioned treatments are insignificant.

According to the obtained results, it is quite obvious that using very low or high sugar concentration for optimal microtuberzation was unsuitable. The very low sugar concentration is not enough to induce minitubers and very high concentration increased the osmotic properties of medium which distrubed the pH and balance of nutrient. Dodds *et al*. (1992) also reported the same observation that low and high sucrose concentrations influenced microtuberization negatively and less minitubers were produced. Potato shoots growing on MS medium with 2-3% sucrose can be induced for microtuberization by transferring them to MS medium with 8% sucrose (Nistor et al., 2010). In agreement to this, it was a continuous observation that the higher sucrose concentration 8 and 10% showed the positive results for MTI frequency, day taken to induce MT, no. of Mt and weight of Mt. It is demonstrated that the starch synthesis is regulated by the osmolarity of the media which can be increased by mixing high amount of sucrose in the media (Oparka and Wright, 1988). Alternatively, sucrose can work as inducer that stimulates some special genes in the potato plants for tuberization (Johnson and Ryan, 1990). Khuri and Moorby (1995) has proposed that the high sucrose levels in media are easily assimilated and converted to starch without interruption for the microtuberization. Keeping in view the studies of other researchers, it seems that sucrose contributes in microtuberization as an inducer, nutritive and osmoticum. Thus the higher sucrose concentrations trigger rapid starch biosynthesis and induction of microtubers. In addition according to Ranalli (2007) the high sucrose concentrations as 6-8 % (60–80 g l^{-1}) are necessary for the increase of biomass and dry matter of microtubers.

It is important to report the leaf wilting in plantlets grown on 10 and 12% sucrose during present study; this could be due to the high osmotic potential which suggests the use of 8% sucrose in media. Additionally, higher number of roots and root hairs were observed in 8% sucrose containing

media (data not shown). During this study the higher proportion of roots and root hairs on the stolon in the 8% sucrose may account for the greater number of microtubers (T8 and T3). It is known that gibberellins and cytokinins affect tuberization and are produced in the roots (Vreugdenhil and Struik, 1989). Roots also influence the amount of calcium present at the stolon tip (Vreugdenhil and Struik, 1989) which has a positive role in mediating the tuberization stimulus (Balamani et al., 1986). In summary T8 and T3 showed the best results. Although the presence of kinetin (T8) improved the growth of Mt, the mean differences among treatments with or without kinetin (T8 and T3) are insignificant statistically ($p<0.05$).

To reduce the cost of protocol our recommendation is to use 8% sucrose without kinetin in short photoperiod (8-hours). Potato tubers are underground stems and flourish in dark. Our data revealed that the short photoperiod (8-hours) is better for microtuberization as compared to 16-hours photoperiod (Table 1). There are many reports describing the affect of light on the yield of microtubers (Wang et al., 1982; Slimmon et al., 1989; Charles et al., 1993). According to Mahdi and colleagues (2004) complete darkness promoted aerial minitubers whereas low light induced green axiliary microtubers at the base of *in vitro* plantlets. In agreement to Hoque (2010) most of the minitubers produced in this study were green may be due to synthesis of the alkaloid solanin which is produced in light. The experimental obsevations of this study revealed the short photoperiod (8-hours) favourable for tuberization in cv. Desiree. Contrastly Mares et al., (1981) reported better microtuberization at 16 or 24 hours photperiod than 8 hours in exotic potato varieties. This could be due to genotypic differences of potato. The genes involved in such response towards photoperiod has to be explored yet.

Table 1: The Effect of Different Treatments on Microtuberization of Potato cv. Desiree in Two Photoperiods.

Treatments			Photoperiod 8 hours b				Photoperiod 16 hours a			
No.	Sucrose %	Kinetin mgl^{-1}	MT I F %	Days to MT I	No. of MT/ plantlet	Weight of MT (mg)	MT I F %	Days to MT I	No. of MT / plantlet	Weight of MT (mg)
1.	0	0	00 a	00 a	00 a	00 a	00	00	00	00
2.	4	0	23.3 b	22.5 e	0.53 ab	178.4 c	20.7	35.0	0.33	134.1
3.	8	0	96.7 f	9.5 b	2.27 c	312.4 f	73.3	23.5	0.80	153.3
4.	10	0	93.3ef	10.5 b	1.80 c	280.7 ef	76.7	25.0	1.13	141.7
5.	12	0	53.3 c	18.5 de	0.83 b	217.1 cd	66.7	29.5	0.60	125.5
6.	0	1	6.7 ab	34.0 f	0.13 a	105.3 b	3.0	48.5	0.27	85.6
7.	4	1	53.3 c	13.5 bc	0.87 b	260.6 de	43.3	29.0	0.30	149.3
8.	8	1	100 f	9.0 b	2.37 c	300.8 ef	80.0	19.5	0.67	179.6
9.	10	1	100 f	11.0 b	1.97 c	301.3 ef	73.3	21.5	0.93	186.5
10.	12	1	70.0cd	15.5 bc	1.13 a	295.1 ef	46.7	33.5	0.87	163.1
11.	0	2	1.0 a	29.5 f	0.10 a	90.7 b	6.7	51.0	0.10	59.7
12.	4	2	73.3d	18.5 de	1.03 b	278.1 ef	50.0	31.0	0.53	104.9
13.	8	2	100 f	12.5 bc	1.87 c	300.01 ef	80.0	21.5	0.87	147.7
14.	10	2	93.3ef	11.5 b	1.97 c	289.6 ef	76.7	23.0	0.77	159.1
15.	12	2	76.7de	19.0 de	0.93 b	253.5 de	63.3	32.5	0.13	126.3

Different letters with each mean value (representing10 explants/ treatment in 3-replicates) indicate significant differences of the mean values at $P<0.05$.

In conclusion here we report a protocol for commercial rapid and cost effective *in vitro* tuberization of potato cv. Desiree based on MS basal media with 8% sucrose

without kinetin in short photoperiod (8-hours). Pakistan is spending a lot of foreign exchange to import the healthy potato seed every year. Studies like this can contribute in solving the potato seed problem of Pakistan.

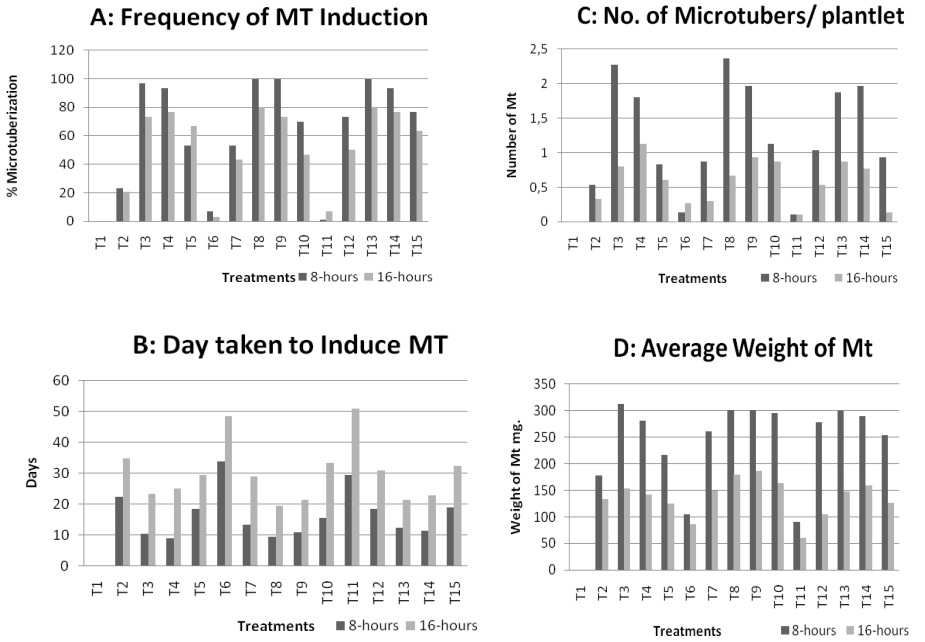

Figure 1: The Effect of different treatments (T1-T15) and photoperiod on different growth traits (A-D) to evaluate the microtuberization (MT) potential of potao variety Desiree.

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