Improvement of Micro-tuber Production Protocol for Potato Variety Granola to Increase Quality and Quantity of Micro-tubers

K.R.S.S.Gunasingha, L.M.H.R. Alwis,
Uva Wellassa University, Badulla, Sri Lanka
H.M.P.S.Kumari *and* J.A. Samanthi
Agriculture Research Station, Sita Eliya, Nuwara-Eliya, Sri Lanka

Introduction

Potato (*Solonum tuberosum L.*) is a starchy tuberous crop which belongs to the family solanaceae. Granola is a German potato variety with a high yielding and medium maturity and is recommended to grow in Sri Lanka by Department of Agriculture. *In vitro* induction of micro-tubers is a one type of micro propagation approach of potatoes. *In vitro* micro-tuberization of potato constitutes the transitory phase between *in vitro* multiplication and establishment of cultures in the field. They are considered as the miniature seed potatoes which represented an intermediary phase between "*in vitro*" plantlets and mini-tubers (Andreena & Champeanu, 2010). They are also called as the first generation of potato seeds from tissue culture (Struik and Wiersena,1999). *In vitro* tuberization found to be controlled by a number of physical and chemical factors including growth regulators, carbon source, photoperiod, culture conditions and genotype.(Hussey and Stacey,1984;Seabrook *etal.*, 1993). Among those conditions, sucrose used as the carbohydrate and BAP (6-Benzylaminopurine) acts as the first generation synthetic cytokinins that elicit plant growth and development responses. Therefore, this study was carried out to improve the protocol for microtuber production to increase quality and quantity.

Methodology

The experiment was set up according to the three factors CRD (Completely Randomized Design). Eight weeks old 6 to 10 cm height *in vitro* single nodal stem cuttings were used to obtain stock plants for the experiment. They were cultured on Murashige and Skoog medium (1962)supplemented with 0.5 mgl^{-1} BAP and 30 gl^{-1} Sucrose to maintain the stock plantlets. Then cultures were exposed to 22±2 ^{0}C temperature, 16hrs light/8 hrs dark photoperiod in 75% relative humidity in a culture room for six weeks. Plantlets of above stocks were treated with 30 ml of MS liquid medium supplemented with four different levels of sucrose(80,100,120,140 gl^{-1})and three different BAP levels of (4.5,5,5.5 mgl^{-1})in all possible combinations to promote microtuberization. Then each of them were kept in three levels of photoperiods (0 light/24hrs dark,8 hrs light/16 hrsdark,16hrs light/8hrs dark) under the 22±2 ^{0}c temperature and 75% relative humidity for one month and they were transferred to 0 light/24hrs dark condition. The effect of different culture conditions on growth of potato invitro plantlets were observed where time required for tuber initialization (days), Tubersize (garth), Number of tubers per bottle were recorded. The readings for above three parameters were obtained at the end of each week for a period of 12 weeks. Results were analyzed using MINITAB statistical package.

Results and Discussion

According to the analysis, the level of sucrose, level of BAP and different levels of photoperiods was statistically significant for the time required for the microtuber initialization, microtuber size and number of microtubers harvested at $p<0.000$.

The maximum amount of microtubers yielded per bottle (20) resulted in combination $4.5gl^{-1}$, with 80 gl^{-1} of sucrose under the 16 hrs light / 8 hrs dark conditions. And the least amount of microtubers harvested per bottle (7) was resulted in combination 4.5 gl^{-1} BAP,140 gl^{-1}sucrose Under 0 light/24hrs dark photoperiod. The findings were agreed with Chapman(1958) who stated that in long day photoperiod the tuber stimuli moves basipetally and induces the tuberization.It was also agreed with the findings of Borah and Milthrorpe (1967) who stated that when high light radiation is applied, it increases the concentration of carbohydrates at the stolon tip and favors the tuberization process.

Table 1: Tuber Initialization Time versus TRT

Source	DF	SS	MS	F	P
TRT	35	6995.3	199.9	6.86	0.000
Error	130	3789.9	29.2		
Total	165	10785.3			
S = 5.399 R-Sq = 64.86% R-Sq (adj) = 55.40%					

Table 2: Tuber Size (Gerth) mm versus TRT

Source	DF	SS	MS	F	P
TRT	35	1357.6	38.8	0.000	0.000
Error	130	1302.9	10.0		
Total	165	2660.4			
S = 3.166 R-Sq = 51.03% R-Sq(adj) = 37.84%					

Table 3: No. of Tubers Harvested versus TRT

Source	DF	SS	MS	F	P
TRT	35	1258.0	35.9	2.22	0.001
Error	130	2105.1	16.2		
Total	165	3363.1			
S = 4.024 R-Sq = 37.41% R-Sq(adj) = 20.55%					

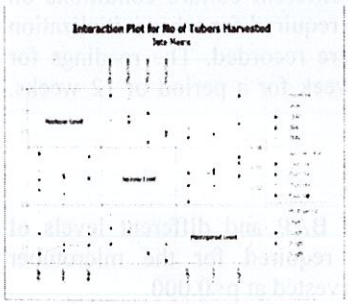

Figure 1: Interaction plot for number of tubers harvested

Figure 2: Interaction plot for tuber size (Garth) mm

Figure 3: Interaction plot for Plot for Time required for tuber

Plamer and Smith (1969) and Wang and Hu(1982) found that the potato plantlets tuberized at low sucrose concentrations of 6% to 8%.In the present study the maximum number of microtubers were harvested when MS medium was supplemented with BAP at 4.5 ml^{-1} . However with a variance of 0.7 with regard to the number of tubers harvested, the cultures with all three BAP concentrations (4.5 mgl^{-1}, 5 mgl^{-1} and 5.5mgl)behave similarly indicating similar performance under different BAP levels. Further, Okazawa and Chapman (1962) had stated that tuber formation rely on the interaction of several regulators rather than the concentration of a single factor. Therefore, as Vreugdenhil and Struik (1989) stated that proper tuberization may occur, if cytokinins interacted with other hormones.

The maximum microtuber size (21.697 mm) resulted when combined with 4.5 gl^{-1} BAP, 80 gl^{-1} sucrose under the 0 light/24 hrs dark photoperiod and the 5 gl^{-1} BAP with 120 gl^{1}sucrose under0 light/24hrs dark photoperiod. This shows that complete obscurity was an essential factor in tuber induction. Based on the finding of Nasim and Iqbal(1991) darkness enhances tuberonic acid synthesis, which induces the tuber formation. Tuberonic acid is a glycoside of 12-hydroxiljasmonic acid, involved in tuber induction.

In present study the lowest time period for microtuber initialization (35 days) resulted in combination of 4.5 gl^{-1} BAP with 140 gl-^{1}sucrose under the 0 light/24 hrs dark photoperiod where as the maximum time period for microtuber initialization (65 days) resulted when combined 5 mgl^{-1} BAP with 120 gl^{-1} sucrose under 16 hrs light/8 hrs dark photoperiod. The findings were agreed with the finding of Plamer and Smith (1969) and Mes and Menge(1954) which stated that high sucrose concentrations and total darkness stimulates the tuber initialization process.

Conclusion

Potato cv. Granola responded for *in-vitro* microtuberization differently at different levels of sucrose, BAP and photoperiods. The liquid MS medium supplemented with 4.5 mgl^{-1} BAP concentration with 80 gl^{-1} sucrose concentration under the 16 hrs light/8 hrs dark photoperiod level is the best condition for obtaining the highest number of microtubers of potato variety Granola under culture condition of 22±2 ^{0}C.The liquid MS medium supplemented with 4.5mgl^{-1} BAP and 80 gl^{-1} sucrose under the 0light/24hrs dark photoperiod level is the best condition to obtain the maximum microtuber size of potato variety Granola under culture condition of 22±2 ^{0}C.Minimum time period for microtuber initialization was at MS medium supplemented with 4.5 mgl^{-1} BAP with 140 gl^{-1} sucrose concentrations at 0 light/24hrs dark photoperiod level of potato variety Granola under culture condition of 22±2 ^{0}C

Acknowledgments

The author acknowledges Agriculture Research Station, Sita Eliya and Uva Wellassa Unviersity for the facilities and guidance given for the study.

References

Andreena, N. and Champeanu, G. 2010. Influences of Potato genotypes on "*in vitro*" production of microtubers. Romanian bioltechnological Letters.15,5317-5323

Borah, M.N. and Milthorpe, F.L. 1962. Growth of potato as influenced by temperature. Indian journal of Plant Physiology.5,53-72

Chapman, H.W. 1958. Tuberization in the potato plant. Physiologia Plantarum 11:215-224.

Hussey, G. and Stacey, N.J. 1984. Factors affecting the formation of *in vitro* tubers of potato(Solanum tuberosum L.).Annals of Botany 53,565-578.

MesMG, and Menge I. 1954. Potato shoot and tuber cultures in vitro physiologiaplantarum 7:637-649

Murashige,T.and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures,Physiologia Plantarum 15,473-497

Nasim,G. and S.H. Iqbal. 1991. Species of Glamus associated with non-root portion of some rhizomatous plants and characteristics of their mycorrhize. *Tranications of the mycological society of Japan*,32;541-545

Okazawa,Y. and Chapman, H.W. 1962. Regualtion of tuber formation in the potato plant. PhysiologiaPlantarum 15: 413-419

Plamer, C.E. and Smith, O.E. 1969. Cytokinins and tuber initiation in the potato Solanum tuberosum L. Nature 221,279-280

Seabrook J.E.A., Coleman, S. and Levy, D. 1993. Effect of photoperiod on *in vitro* tuberization of potato(Solonum tuberosum L.).Plant tissue Culture Org.Cult.34:43-51

Vreugdenhil, D. and Struik, P.C. 1989. An intergrated view of the hormonal regulation of tuber formation in potato(Solanum tuberosum). Physiologia Plantarum 75:525-531

Wang, P.J. and Hu C.Y. 1982. Invitro Mass Tuberisation and Virus free seed potato production in Taiwan.AM.Potato J. 59:33-37