Usage of potato micro tubers in slow growth conservation

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Introduction

Potato is a tetraploid, highly heterozygous plant and segregates on sexual reproduction. Therefore, genetic integrity of the crop is maintained via vegetative propagation. Micro tubers, which originate from *in-vitro* plants and more tolerant to light and temperature variations than *in-vitro* microplants. They do not have the requirement of frequent sub-culturing instead of maintaining microplants. Micro tubers are convenient for handling, storage and exchange of germplasm than microplants (Tovar *et al*., 1985). However, their use in germplasm conservation is not common (Gopal *et al*., 1998). Micro tubers can be stored for a long period of time without losing their viability. Therefore, it represents a better alternative to microplants as a means of conservation and crop improvements (Ashmore, 1997). Potato germplasm has been conserved in the form of *in-vitro* cultures as a mandatory duty and national responsibility of the Plant Genetic Resources Centre (PGRC) of the Department of Agriculture as a national responsibility (PGRC manual, 1985). But *in-vitro* microplants have short subculture period of 4-6 months, resulting frequent transferring, which is cumbersome and costly. Therefore, the current research was conducted as an initial step to understand the use of micro tubers for slow growth conservation with long term goal of applying for other accessions as well.

Materials and methodology

In-vitro grown shoots of three potato accessions, Bio Technology Potato (BTP) 16 (CIP 800959), BTP 02 (CIP 390478.9), BTP 07 (CIP 397012.22) were used. Cultures were multiplied in 40 m*l* of potato multiplication medium (PMM), Murashige and Skoog (1962) medium (MS) supplemented with 0.1 mg/*l* NAA, 0.5 mg/*l* BAP, 0.1 mg/*l* Gibberelic Acid (GA_3), 30 g/*l* sucrose and 8 g/*l* agar. Cultures were incubated at 24 ^oC temperature, 60 to 65% relative humidity under 16 /8 hour light/ dark photoperiod and 42-46 $\mu molm^{-2}S^{-1}$ (Philips 36W/54) light intensity. The cultures were further multiplied in the same medium in liquid form under same condition. Each treatment consisted of ten replicates and each replicate consisted of ten microplants. 3 months incubation period was given for culture multiplication. Then the cultures

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were transferred into 40 m*l* of tuberization medium, MS with 5 mg/*l* of BAP and 80 g/*l* sucrose (*in-vitro* conversation labora-tory, PGRC). The cultures were incubated at 15 ^{o}C temperature under continuous dark for 90 days. After three months, tuberized cultures were hardened at room temperature for 10 days. Tubers were harvested under the laminar flow cabinet and tubers per bottle were counted and tuber diameter was recorded. Harvested tubers were dried for ten days and tubers were sorted under four different categories, < 0.3 cm, 0.3-0.5 cm, 0.5-1.0 cm, 1.0 cm according to diameters. Then, five tubers each was placed in sterilized 5ml test tubes and kept under three different temperatures, 1 °C, 4 °C and 24 °C. Each treatment combination was consisted of five replicates. Tuber regeneration was tested in three months after conservation. *In-vitro* tuberization trial was laid out as Completely Randomized Design and ANOVA performed to analyze data. Effects of accessions on tuber sizes were analyzed using categorical data analysis. Treatment means were compared using Duncan's Multiple Range Tests (DMRT) and analysis performed with SAS potable 9.

Results and discussion

In *in-vitro* tuberization, analysis of variance showed that the micro tuber production was significantly different among selected three accessions at probability level p=0.05. BTP 16 (CIP 800959) showed the highest and mean number of tuber production as 2.47 (tubers/micro plant) while the lowest resulted in accession BTP 07 (CIP 397012), it was 1.32. 1.56 mean number of tubers per microplant accession BTP 02 (CIP 390478.9) was produced. Gopal *et al*. (2004), highlighted that the genotypic differences were positively correlated with micro tuber production in potato. Islam (1995) reported that micro tuber initiation time varied with genotype. Tuber sizes were significantly different among accessions. The biggest micro tuber size 04, (>1.0 cm) was produced by accession BTP 07 (CIP 397012.22) followed by accession BTP 02 (CIP 390478.9). However, BTP 16 did not produce tubers >1 cm. BTP 16 produced the highest number of tubers in the tuber diameter categories of 0.3-0.5 cm and 0.5-1.0 cm. BTP 02 also produced more tubers in the same categories as well. Similar mean number of tubers was resulted in < 0.3 cm category from BTP 02 and BTP 16. BTP 07 produce the lowest tuber numbers in 0.5-0.1 cm range while its highest tuber numbers were resulted under >1 cm category (Figure 1). Nasiruddin and Rafiul (2018) were reported that tuber size depends on culture media and its genotype.

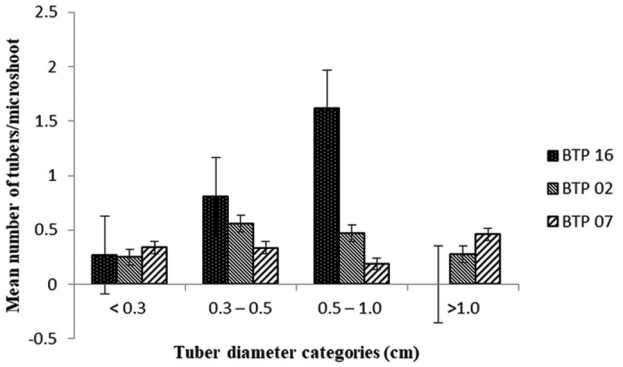

Figure 1. Accessions response for different sized micro tuber formation in MS + with 5 mg/*l* of BAP and 80 g/*l* sucrose medium.

There is a need for developing genotype specific protocol for efficient microtuberization and it is important in planting material production system (Gopal *et al*., 2004). However, it may be difficult to develop genotype specific protocols for germplasm conservation. Hence, optimized cost effective techniques such as *in-vitro* induced reproductive organs can be applied (Nasiruddin and Rafiul, 2018). After 90 days, it was observed that all the treatment levels conserved under 4 °C and 24 °C showed 100% auto germination but micro tubers conserved at 1 °C could not show auto germination. The observation was common for all tested accessions. Gopal *et al*. (2002), reported that fresh micro tubers could be conserved satisfactorily in 6±1 °C for 15 months. However, micro tubers stored under 1 °C showed 100% tuber germination ability in PMM by producing shoots representing all the tuber categories of all accessions.

Conclusion

Potato accession (BTP) 16 (CIP 800959) produced the highest total number of micro tubers than tested other two accessions, BTP 02 (CIP 390478.9) and BTP 07 (CIP 397012.22). Micro tuber sizes are accessesion dependent and the highest micro tubers resulted in 0.5 - 0.1 cm diameter categories. Selected accessions cannot be conserved neither at 4 °C nor 24 °C temperatures at least for 3 months. All the tuber categories of three accessions can be conserved at 1 °C temperature for three months with 100% survival. Effective conservation period for micro tuber conservation at 1 °C temperature is to be identified by testing of germination percentage in regular time intervals.

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References

Ashmore, S.E. 1997. Status report on the development and application of *in-vitro* techniques for the conservation and use of plant genetic resources. International Plant Genetic Resources Institute, Rome. 67.

Gopal J, A. Chamail and D. Sarkar. 2004. *In-vitro* production of micro tubers for conservation of potato germplasm: effect of genotype, abscisic acid, and sucrose. *In-vitro* Cell. Dev. Biol.—Plant 40:485–490.

Gopal, J., J.L. Minocha and H.S. Dhaliwal. 1998. Microtuberization in potato (*Solanum tuberosum* L.). Plant Cell Reports 17:794–798.

Gopal, J., C. Anjali and S. Debabrata. 2002. Slow-growth *in-vitro* conservation of potato germplasm at normal propagation temperature. Potato Research. 45(2-4): 203–213

Islam, M.S. 1995. Indigenous potato varieties of Bangladesh: Characterization by RAPD markers and production of virus free stock. Ph. D. Dissertation. Dept. of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706. Bangladesh

Murashige T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497.

Nasiruddin, M. and A.K.M.I. Rafiul. 2018. *In-vitro* slow-growth conservation for two genotypes of solanum tuberosum l. Bangladesh J. Bot. 47(3): 369-380.

Plant Genetic Resource Centre (PGRC).1985. PGRC Manual.

Tovar P., R. Estrada, L. Schilde-Rentschler and J.H. Dodds. 1985. Induction and Use of In- vitro Potato Tubers. CIP Circular 13. CIP, Lima, Peru. 1-5.