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A STUDY OF TWO DIFFERENT METHODS FOR INDUCTION ON *IN VITRO* TUBERIZATION IN *SOLANUM TUBEROSUM* L.

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Two different methods for *in vitro* tuber induction in four cultivars of *Solanum tuberosum* L. var Ultimus, Desiree, Local Hunza and Cardinal were tested. Four formulations with MS medium were tested for each method. In principle the cultivar response for each medium was favourable. In the first method average of eight shoots used as an explant source, maximum no. of tubers i.e. 17 tubers/flask were produced in MS in combination with 5.0 mg/1 BAP (Benzyl amino purine) and 1000mg/1 CCC (Chlorocholine chloride). In the second method "Single node cuttings" of *in vitro* shoots were cultured on MS medium supplemented with different concentrations of BAP. It has been observed that maximum no. of tubers produced in the medium in combination with 4-5mg/1 BAP.

Key words: Tuberization, Chlorocholine chloride, Benzylamino purine.

Introduction

The potato *Solanum tuberosum* L. is conventionally multiplied through vegetative propagation. However, the disadvantages with such a system is that the seed tubers are easily affected by hundreds of diseases caused by viruses, bacteria, nematodes, mycoplasma, fungi. These pathogens give erratic results on the yield and quality of seed tubers. Moreover the tubers are perishable and quite expensive to store and transport.

In recent years techniques have been developed which allow tubers to be produced *in vitro*. These *in vitro* tubers have the advantages of being small, light and are produced under aseptic conditions [1]. They have the same morphological and biochemical characteristics as field produced tubers and added the advantages of being easily stored they can be used as an alternative method for germplasm distribution [2]. Thus, their year round availability is ensured with the advantage of economy of space for storage with very small transport coasts. Many national potato programme such as in Brazil, Kenya, China, Phillippines, Ecduador, Belgium, Mexico are studying *in vitro* tuber induction and the use of this material within a seed multiplication programme [2]. In Taiwan, National Potato Programe successfully developed an *in vitro* culture system to supply virus free mini seed tuber as an alternative source for certified seed potato [3].

There are some reports available [1,3,5-7] for induction and utilization of *in vitro* tubers in broad range of genotypes with factors affecting their formation. Some authors [1-3] reported *in vitro* mass tuberization and their utilization in fields.

The present paper reports studies on two different methods for *in vitro* tuber induction with number of potato cultivars, breaking of their dormancy and transplantation in pots at green house.

Materials and Methods

The explants were *in vitro* plantlets of potato cultivars Ultimus, Desiree, Local Hunza and Cardinal. Two different methods were used for tuber induction.

Method I. In vitro plantlets with 10-20 nodes were cultured on liquid media (MS + 0.5mg/1 BA + 0.4 GA_3 + 0.01 NAA) in 250 ml culture flasks (Fig. 1) . The cultures were maintained on reciprocal shakers at 60 rpm and incubated at a temperature of 25° ± 2° with a 16 hrs. photoperiod. Profuse growth of shoots was noted within 3 weeks of culture, after which the medium was replaced with a fresh medium for tuber induction (Table 1). The cultures were reincubated in dark where a temperature of 18° ± 2° was maintained.

Fig. 1. *In vitro* tuber induction of Solanum tuberosum L.

Method II. Individual nodes from *in vitro* plantlets were fragmented and cultured on media for tuberization (Fig. 2 and Table 2). The cultures were incubated in dark where a temperature of 18° ± 2° was maintained.

TABLE 1.

Method	Constituents with modifications
I	(a) MS, 5.0 BAP, 1000.0 CCC
"	(b) MS, 5.0 BAP, 750.0 CCC
"	(c) MS, 5.0 BAP, 500.0 CCC
"	(d) MS, 5.0 BAP, 250.0 CC
	Sucrose = 8%
II	(a) MS, 4.0 BAP
"	(b) MS, 5.0 BAP
"	(c) MS, 6.0 BAP
"	(d) MS, 7.0 BAP
	Sucrose = 6% Agar = 0.8%

All media were Based on medium of Murashige and Skoog (MS) 1962 (4). The addition of all there growth regulators is taken in mg/lit.

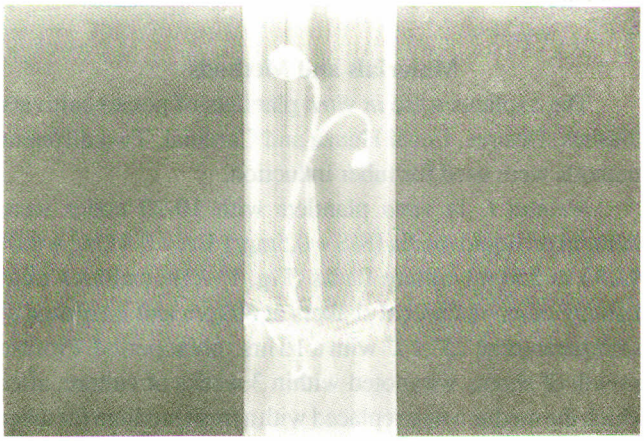

Fig. 2. *In vitro* tuber induction of Solanum tuberosum L. (Method II).

TABLE 2a. Method I

Media	No. of tubers/flask No. of tubers*
a	17 A**
b	10 C
c	15 B
d	8 D

* Average of five flasks with eight elongated shoots each.
** Means indicated by different letters are significant at 5% level.

Storage and dormancy breaking. The tubers produced were harvested under aseptic conditions and stored in autoclaved peteri dishes and tubes in refrigerator at 4°. Within 4 months these tubers initiated sprouting. A method was also used for early dormancy breaking in which minitubers were inoculated on plane MS medium with 1% agar, within one month they formed roots and shoots (Fig 3).

Plantation in green house. Sprouted tubers were planted in pots at a depth of 2-3cm containing sterile peatmoss with sand at ratio of 3:1 and covered with polythene bags to maintain high humidity levels (Fig 4).

TABLE 2b. Method II

Media	No. of tubers/24 test tubes No. of tubers*
a	21 A**
b	21 A
c	17 B
d	20 A

* Average of 24 test tubers, each tube containing "Single node cuttings".
** Means indicated by different letters are significant at 5% level.

Fig. 3. Mother plant raised from in vitro tuber.

Fig. 4. Pots covered with polythene sheet.

Results and Discussions

In both the methods complete tuberization was observed within 4-5 weeks. Different genotypic origins of the cultivars used resulted in tubers of different size and shapes. The tuber production response was the same in all cases as mentioned by R.Estrade *et.al.* [1]. In both methods used, the culture were maintained at a temperature of 18° ±2° in complete obscurity.

The rate and the number of tubers obtained by both the methods is summarized in Tables 2a and 2b.

In the first method significant difference was observed in the numbers of tubers/flask in all four combinations used. In medium with combination I a more tuber production was observed (Table 2a). In the second method there is no significant difference in all the medium combinations tested, with 4-5mg/1 BAP (Table 2a,b) comparatively better results were obtained (Table 2b). The tubers produced in the first method are longer in size (7-10mm) than the second method (3-7mm).

The harvested tubers were stored in sterile container at 4° temperature in referigerator. It has been observed that tubers induced in total darkness have longer natural dormancy period than tubers produced in light [2]. The method used for earlier promotion of sprouts of freshly harvested tubers, it has been shown that within one month 60% minitubers produced shoots and roots without any callus formation. On transference to pots in green house it was noted that within two months vigorously growing mother plants were produced for which cuttings were taken and planted in nursery beds in green house. Both methods for *in vitro* mini tuber induction proved useful for production of pre basic seeds in shorter time and less space. These tubers can be used as the initial pathogen tested material in a seed multiplication programme and their yield is much higher than those of field produced seed tubers [3].

References

1. R. Estrada, P. Tovar and H. Dodds, Plant Cell, Tissue and Organ Culture, **7**, 3 (1986).
2. P. Tovar, R. Estrada, L.S. Rentschler and H. Dodds, CIP Circular, **13**, 4 (1985).
3. Po-Jen Wang and Ching Yeh Hu, American Potato, J, **59**,, 33 (1982).
4. G. Wattimena, B. McComn and G. Weis, American Potato J., **60**, 27 (1983).
5. A.J. Abbott and A. Belcher, Plant Tissue Culture and its Agricultural Applications, 113, 121 (1986).
6. G. Ortiz Monteil, and H. LOzoya Saldans, American Potato J. , **64**, 535 (1987).
7. G. Hussey , N.J. Stacey, Annulas of Botany, **53** (4), 565 (1984).