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EFFECT OF INOCULATION DENSITY ON POTATO MICROPROPAGATION

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ABSTRACT

The present study was conducted to examine the effect of inoculation density on potato micropropagation var. Desiree and investigate the nutrients competition among potato micro plants. The different growth factors were examined, *i.e*., shoot length, numbers of roots and numbers of nodes of each microplant. A number of 1-5 treatments of micro plants was examined with 10 repetitions of each treatment. In this experiment 1, 2, 3, 4 and 5 nodal cuttings were transferred in each test tube. The morphology of the plantlets during this experiment was observed, and remained same whether single inoculated plantlets or five nodal cuttings were inserted in a test tube. Post two weeks of culture certain numbers of nodes/microplant, roots/micro plant and shoot length were obtained from each culture tube. The numbers of nodes and roots were more in T_5 as compared to other treatments. During this experiment it had been observed that the inoculation density did not affect the normal growth of the plantlets. So the inoculation density effect did not affect (*i.e.*, p=0.05) the plantlets. Four week post explant culture the plantlets showed maximum growth under artificial condition of growth chamber. So, this experiment showed that the inoculation density has no significant effect (*i.e.*, p=0.05) on potato microplants.

Key words: potato**,** micro-propagation, inoculation density, nutrients competition.

INTRODUCTION

The Potato (*Solanum tuberosum* L) is characteristically a crop of cool temperate region. In Pakistan the climate of Hunza and other high hills of Northern areas are excellent conditions for the production of potatoes (NARC). Potato is an important and the chief ingredient crop of the world. Potatoes are the largest non-grain spacious crop of the world. The potato production was increasing with time throughout the world in both developing countries and developed countries due to the use of modern agro-techniques for potato seed germination, utilization and postharvest management. But still so much has to be done in developing countries to increase the production of potatoes. No doubt potato is a nutritional gold mine due to the dietary elements present in it and is a major cash crop for farmers.

The biotechnological approaches play a major role in the promotion of the crop. Micropropagation is the artificial growth of potatoes (*in vitro*) and it is a true type of multiplication of plants under controlled conditions in an artificial media. Micropropagation is rapid multiplication technique used to obtain high quality potato seed tube (Bryan 1981). Micropropagation can be carried out throughout the year and it can be accumulated in less space. The meristem culture is the only way to obtain virus-free biological material (Loebnstein et al., 2001). The recommendation of International Potato Center (CIP 1984) was to place two explants in 16×125 mm tubes, three in 18×150 mm test tubes, five in 25×150 mm test tubes and 20-30 in magnetic vessels. The in-vitro propagation is suitable for quick large scale manufacturing biomass, maintainnance of cultivars of the new breeding and conservation of existing germplasm. (Yasmin et al., 2011)

The effect of inoculation density on different growth parameters of potato such as stem, leaves, roots and nodes has been extensively studied so far. The present study was conducted to investigate the effect of the inoculation density on the numbers of roots, numbers of nodes and shoot length of potato plant in cultured test tubes. The method of the micropropagation has permitted the large numbers of plants in a little developing range of area in a relatively less time (Makawy et al., 2008). The in-vitro propagation system by utilizing sprouts and cuttings of nodes are more reliance for keeping the heredity integrity of the multiplied clones (Liljana et al., 2012).

MATERIALS AND METHODS

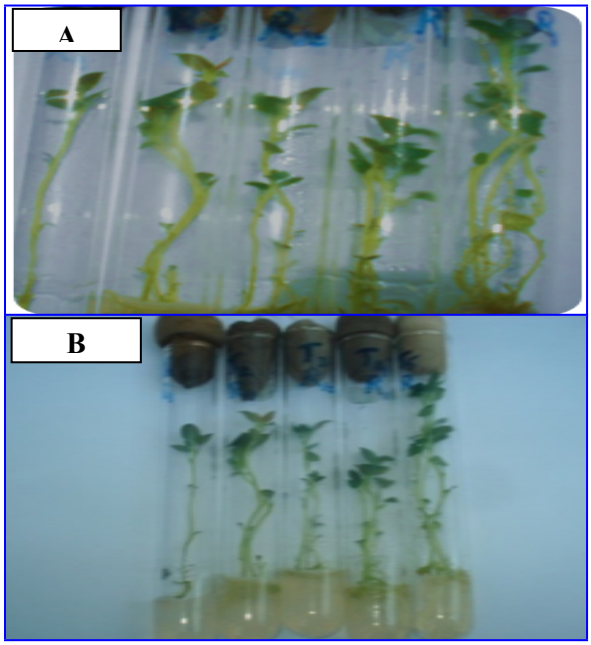
The nodes of genetically modified potato plantlets (*Solanum tuberosum* L. var. Desiree) were used as explants in single cultured tubes on MS medium (Murashige and Skoog, 1962) with $CaCl_2$ (8.800g), KNO_3 (38.800g), NH_4NO_3

(33.00g), KH_2PO_4 (3.500g), $MgSO_4.7H_20$ (7.400 g), $MnSo_4.4H2O$ (0.338g) and H_3BO_3 (1.24g), $ZnSo_4.7H_2O$ (1.12g), $Na_2MO_4.2H_2O$ (0.50g), KI (0.166g), $CuSO_4.5H_2O$ (0.005g), $Cacl_2.6H_2O$ (0.005g), $FeSo_4.7H_2O$ (1.39g), Na_2EDTA (1.86 g) were also used in MS media. Myo Inositol, glycine (200 mg), nicotinic acid (50.00 mg) and pyridoxine (50.00 mg) were also used. Agar and sucrose were also added in MS media.

The single nodal cutting method as described by CIP were used to investigate the effect of inoculation density on the shoot length, numbers of nodes and numbers of roots. The microplants which were cultured in incubated room under highly conditions of laminar flow chamber were placed in growth chamber (16 hours light & 8 hours darkness) at 22°C. The microplants were placed in growth chamber for four weeks.

Inoculation densities: In this study five inoculation densities (treatments) had been tested which includes 1-5 different nodal cuttings in different cultured test tubes. Initially one nodal cutting was inserted down in different replicates. After that: 2, 3, 4 and 5 nodal cuttings were transferred into test tubes. Each of the treatment had 10 replications. Therefore, five treatments that were used in this experiment had total 50 replications.

Data collection and analysis of statistics: The effect of inoculation density on potato micro-plants was observed on micro shoot length, numbers of roots and numbers of nodes per microplant. The data was recorded twice in four weeks.



The data were analyzed by using analysis of variance after testing Randomized complete Block AOV. After this least significance difference ($LSD_{0.05}$) was applied on variance of randomized complete block AOV. The software used for the $LSD_{0.05}$ to check the effect of inoculation density on potato micro plants was Statistix 8 version 8.1.

RESULTS AND DISCUSSION

It had been observed that the inoculation density did not effect the normal growth of the plantlets growing in the growth chamber. The plantlets grow rapidly during these four weeks under artificial conditions given in the growth chamber.

Effect of inoculation density on shoot length: The inoculation density has not effect the shoot length of the plantlets growing in the growth chamber. The maximum length of the shoot has been recorded after two weeks in cultured test tubes. In the fourth week of culturing the plantlets becomes double in length as compare to the second week. It had been recorded that the homogeneity rate was same in the means of all treatments according to second and fourth weeks of readings. So, it was conducted that the shoot length of the plantlet do not depend upon the increasing or decreasing numbers of nodal cuttings that were inserted in the test tubes.

Effect of the inoculation density on roots: In the second week the thin roots have been recorded during the observation.When the reading was taken in the fourth week maximum roots has been observed. The homogenous rate was observed same during the experiment.

Effect of inoculation density on numbers of nodes: The effect of the inoculation density on the nodes of the growing plantlets has also been observed. The treatments which got more nodal cuttings, *i.e.*, 3, 4 or 5 nodal cuttings of explants became mature into plantlets after an appropriate period of time and produce various nodal cuttings. The homogeneity rate was in two groups, *i.e.*, A and AB in second week reading due to a little difference in mean of T_1 (Fig. 1, Table 1 and Graph 1a) while in fourth week the homogeneity rate was same in all the treatment (Fig. 2, Table 2 and Graph 2b).

Fig. 1A and B:. Plantlets two weeks post culturing.

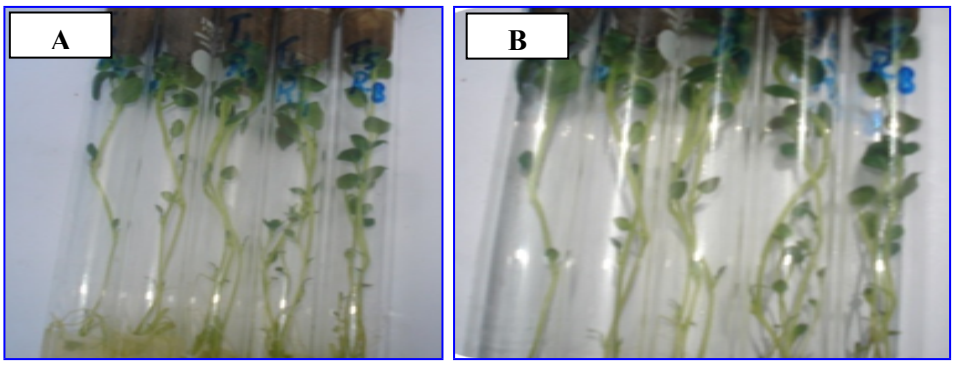


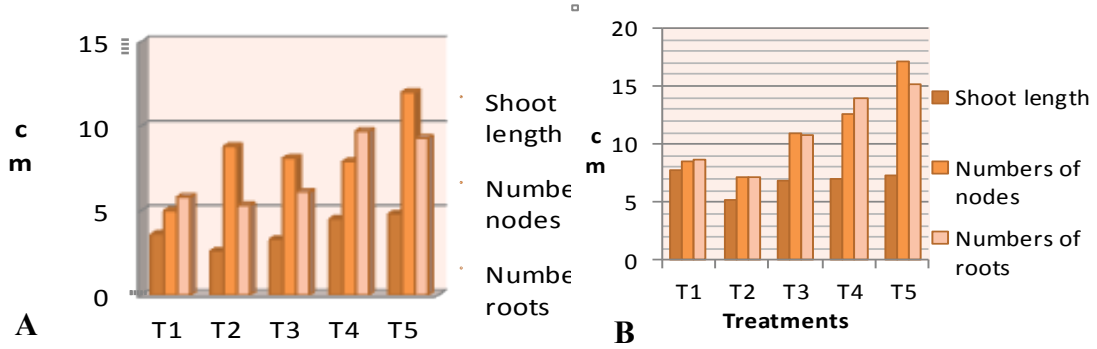
Fig. 2. Plantlets four weeks post culturing.

Table 1. Readings of second week of various treatments of potato microplant $LSD_{0.05}$.

Treatments	Micro shoot Length		Number of Nodes/Microplant		Number of Roots/Microplant	
	Mean	Homogenous	Mean	Homogenous	Mean	Homogenous
T_1	3.6	AB	5.0	AB	5.8	A
T_2	2.6	B	8.8	A	5.3	A
T3	3.3	AB	8.1	A	6.1	A
T4	4.5	A	7.9	A	9.7	A
T5	4.8	A	12.0	A	9.3	A
LSD 0.05	3.7		6.2		7.2	
Grand Mean						

Table 2. Readings of fourth week of various treatments of potato microplant$LSD_{0.05}$.

	Micro shoot Length		Number of Nodes/Microplant		Number of Roots/Microplant	
Treatments	Mean	Homogenous	Mean	Homogenous	Mean	Homogenous
T1	7.7	A	8.5	B	8.7	A
T2	5.2	A	7.2	B	10.3	A
T3	6.8	A	11.0	B	10.8	A
T4	7.0	A	12.6	AB	14.0	A
T5	7.3	A	17.2	A	15.2	A
LSD 0.05	6.8		11.3		11.6	
Grand Mean						



Graphs-1A and B: Growth parameters of various treatments of potato microplants.

It is reported plant population did not effect on the yield of maize as well as low and high density could not effects plant growth (Abuzar *et al.,* 2011). The inoculation has no effect on the number of nodule rhizobium as well as on the biomass of shoot and grain yields (George, *et al.,* 2007). The 60 and 90 explants were placed in a separate vessel (TIS). However the best results were obtained when less explants were placed in the vessel (Alonso *et al.,* 2007). In

another study *Eisenia foetida* was inoculated with 70% of the cattle manure, with 75% of pig manure and 65% with the mixture of these manure along with 8 Earth worms per 100 grams manure (dry weight) was inoculated. *Eisenia foetida* showed the highest rate of reproduction when inoculated in cattle manure. (Long *et al*., 2002)

The similar studies have been conducted by Sarkar *et al*., (1997) and showed that the growth of the cultured tubes did not change significantly in four weeks of culture as our studies demonstates. The effect of planting density and size of potato seed minitubers were studied. Four minitubers of various sizes (*i.e.,* 10, 10-15, 15-20 and 20-25 mm) and four planting densities (*i.e.,* 10, 20, 40 and 80 plants/m^2) were used (Karafyllidis *et al.,* 1997).

Filip *et al.,* (1989) observed 35 large firs and were inoculated with the fungal symbiont *Trichosporium symbioticum*. They had assumed that the *Trichosporium symbioticum* did not affect the inoculation density and no trees had been damaged due to inoculation density of fir with fungus. The impact of inoculum density and seed treatment for F. avenaceum and R. solani were noteworthy for seed yield and the emergence of seedling. The emergence in seedling and seed yield declined with expanding inoculum level of both *F. avenaceum* and *R. solani* (Chang et al., 2014)

Therefore, the results of this experiment demonstrated that the factor of inoculation density is very important to take full advantage of the capacity of the plantlets of potato growing in a single cultured tube without affecting any growth parameter phenotypically.

CONCLUSION: This study concluded that the inoculation density is very important to study the effect of inoculation density of various parts of the plants. This study is very useful to obtain maximum benefits, *i.e.,* to increase crop yield in less space or an area. The results of our experiment indicated that inoculation densities has no significant role on potato micropropagation, *i.e.,* from 1-5 nodal cuttings in a single test tube. This work constitutes that the inoculation density indeed did not affect the potato micro plants production so we can take maximum advantages of increasing numbers of potatoes microplants in less space.

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