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In Vitro Micropropagation and Micro-tuberization Potential of Selected Potato Varieties

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ABSTRACT

Six potato varieties, *viz*. Kufri Jyoti, Kufri Megha, Kufri Giriraj, Kufri Girdhari, Kufri Himalini and Kufri Himsona were evaluated for their responses to *in vitro* propagation and tuberization. Kufri Giriraj performed well for most of the growth parameters and exhibited superiority over other cultivars for both stem elongation (10.4 cm) and microtuber formation (no of micro-tubers-14 and weight 3.10 g) during micro propagation. Rooting efficiency was good for all the varieties except Kufri Megha. No difference was observed among the varieties Kufri Girdhari, Kufri Himalini, Kufri Himsona and Kufri Jyoti in terms of the total micro-tuber number and weight except Kufri Megha, which recorded lowest yield. The present study clearly shows that different cultivars respond differently towards tissue culture conditions.

Keywords: Potato, Micropropagation, Micro-tubers

INTRODUCTION

Potato is largely a vegetatively propagated crop. In a conventional system of seed potato production, a single healthy virus free tuber is clonally multiplied and requires at least 4-5 years to produce a breeder quality seed potato. With the advent of various tissue culture techniques, mass multiplication of different crops under laboratory condition has become a possibility. Potato is readily amenable to tissue culture manipulations. It allows large scale asexual multiplication of pathogen free potato cultivars. As a result tissue culture based potato multiplication has successfully been incorporated in high quality potato seed production programme. Meristem culture, in combination with thermotherapy and chemotherapy, is now routinely used to obtain pathogen free potato microplants. These potato microplants can be multiplied through nodal cuttings or can be utilised for microtuber production. Studies on different crops have shown that growth of explants under laboratory condition is affected by many factors including genotype, media composition and growth conditions (Baciu et al. 2007; Shukla et al. 2007; Naresh et al. 2011). Thus it becomes necessary to evaluate new varieties for their response to micro-propagation before undertaking any large scale multiplication programme. In the present study, an attempt has been made to compare the micropropagation and micro-tuberization potential of six potato varieties found suitable for the North Eastern Himalayan region.

MATERIALS AND METHODS

The material for the present study comprised of micro-plants and micro-tubers of six potato cultivars namely Kufri Giriraj, Kufri Jyoti, Kufri Megha, Kufri Girdhari, Kufri Himalini and Kufri Himsona produced from in vitro raised virus free micro-plants. The virus free microplants were produced through meristem culture (Hossain 2005). Two noded micro-cuttings of about 10 – 15 mm were taken from in vitro raised shoots as explants in aseptic condition. The nodal cuttings were cultured on Murasighe and Skoog (1962) basal nutrients supplemented with D-calcium pantothenate (2 mgL^{-1}), gibberellic acid (0.1

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mgL^{-1}), α-naphthaleneacetic acid (0.01 mgL^{-1}) and sucrose (30 gL^{-1}), solidified with agar (7.0 gL^{-1}, Sharma et al. 2011). These cultures were incubated under 16 hours photoperiod from cool white fluorescent lights (approx 50-60 $\mu molm^{-2}s^{-1}$ light intensity) at 24° C. The experiment was conducted using randomized block design with 5 replications. One test tube consisting of three micro-plants constituted a replication. Observations were recorded on five morphological traits, viz. micro-plants stem elongation, number of nodes, inter-nodal length, rooting efficiency and leaf size after 6 weeks of culturing. The rooting efficiency was scored on a 1-5 scale where 1 meant light root growth and 5 meant vigorous root growth. Test tubes having average root length of >5cm were assigned score of 5 while those having average root length of <3cm were assigned score of 1. Test tubes having average root length between 3 to 5 cm were assigned score of 2. The leaf size was scored as 1, 2 and 3 for small, medium and large sized leaves based on the proportion of larger leaves in each test tube. Test tubes having more leaves of length >2.5 cm were assigned score of 3 while those having more leaves of length <1.0 cm were assigned score of 1. Test tubes having more leaves of length between 1 to 2.5 cm were assigned score of 2.

For micro-tuber production, five double node cuttings were cultured for 21 days in a conical flask containing modified MS medium (liquid) for initial propagation of micro-plants except addition of agar, and incubated under 16 hours photoperiod from cool white fluorescent lights (approx 50-60 $\mu molm^{-2}s^{-1}$ light intensity) at 24° C. After 21 days of incubation, the liquid propagation medium was discarded from the conical flask and 40 ml of microtuber induction medium based on MS basal nutrients supplemented with N^6-benzyladenine ($10mgL^{-1}$), chlorocholine chloride ($500mgL^{-1}$) and sucrose ($80gL^{-1}$) was added. Each flask was then incubated in complete darkness at 25° C. The experiment for microtuberisation was conducted using randomized block design with 5 replications. One conical flask containing five micro-plants constituted a replication. Observations were recorded on the total number and weights of different grades of micro-tubers viz., <4mm, 4-8mm and >8mm diameter after 60 days of induction.

RESULTS AND DISCUSSION

Micropropagation potential of potato varieties

Potato micropropagation through double node cutting is an efficient way of mass multiplication of potato microplants under tissue culture conditions (Rai et al. 2012). The potato varieties showed varied response toward micro-propagation under laboratory condition (Table 1).

Stem height

Among the six potato cultivars, Kufri Giriraj exhibited the maximum stem elongation (10.4 cm) followed by Kufri Girdhari (9.1 cm), Kufri Himalini (9.1 cm) and Kufri Himsona (8.5 cm). Shortest stem height was observed in Kufri Megha (6.5 cm) and Kufri Jyoti (5.1 cm), indicating slower growth rate. Stem elongation of explants has two important effects during the initial regeneration stage. Firstly, it provides a clear distinction between fast and slow regenerating cultivars. Secondly, the longer the stem height the more number of nodal cuttings can be achieved per plantlet. Thus, all the varieties except Kufri Megha and Kufri Jyoti grew at a faster rate

Table 1: In vitro growth potential of six potato cultivars

Variety	Stem Elongation (cm)	No. of nodes	Internodal length (cm)	Rooting efficiency*	Leaf Size#
K Giriraj	10.4	6.9	1.5	5.0	3.0
K Girdhari	9.1	7.4	1.2	4.8	2.0
K Himsona	8.5	8.5	1.0	5.0	2.0
K Himalini	9.1	8.5	1.1	5.0	2.0
K Jyoti	5.1	4.8	1.1	5.0	2.8
K Megha	6.5	5.0	1.3	3.4	1.2
C.D. (5%)	0.9	0.6	0.2	0.4	0.3
C.V. (%)	8.0	6.7	9.6	6.5	11.6

* Poor -1, medium- 3 and vigorous – 5;
# Small -1, medium -2 and large -3

which meets the requirement for achieving a specified target at a specified time.

Number of nodes

The number of nodes per plantlet is another important parameter in order to study the growth rate. A nodal cutting is one that has one node and one leaf and is usually 2-3 cm in length. Both Kufri Himsona and Kufri Himalini exhibited highest number of nodes (8.5) followed by Kufri Girdhari (7.4) and Kufri Giriraj (6.9). These cultivars could be classified as high multiplying cultivars because of their higher number of nodes which indicated that at least 3-4 cuttings per plantlet could be obtained. Kufri Jyoti and Kufri Megha on the other hand could produce only 2 cuttings in relation to their height and nodes, thus appeared to be slow multiplying cultivars.

Internodal length

Internodal length refers to the distance between two successive nodes. For a given plant height, the lesser the internodal length more will be the number of nodes which will again contribute to more number of nodal cuttings. Here, the maximum internodal length was observed in Kufri Giriraj (1.5 cm) followed by Kufri Megha (1.3 cm). Minimum internodal length was observed in Kufri Himsona (1.0 cm) followed by Kufri Himalini (1.1 cm) and Kufri Jyoti (1.1 cm).

The overall stem height of Kufri Giriraj (10.4 cm) was higher, thus it produced more number of nodes with longer intermodal length as compared to Kufri Megha. Kufri Himalini and Kufri Himsona however proved to be superior to all other varieties as it produced more number of nodes with shorter internodal length.

Rooting efficiency

All the varieties exhibited good rooting pattern (> 4) except Kufri Megha which showed reduced rooting (< 4). This indicated that all the varieties were amenable to tissue culture multiplication. Good rooting pattern ensures better and early establishment and foundation of a plant thereby increasing the plants survival percentage in the field.

Leaf size

Green plant having large and numerous leaves have increased overall photosynthetic rate thereby increasing overall plant survival. Results have shown that Kufri Giriraj (3.0) and Kufri Jyoti (2.8) have larger leaves when cultured *in vitro* compared to other varieties. Kufri Megha was recorded to have the smallest leaf area (1.2). This may have an influence on the survival rate of the varieties under net house transplanting.

Microtuberization potential

There were significant differences among the varieties in terms of microtuber number and weight (Table 2). Highest number of micro-tubers were observed in Kufri Giriraj (14.00) followed by Kufri Girdhari (11.26) and Kufri Himsona (11.13). Kufri Giriraj also recorded maximum micro-tuber weight (3.89 g) followed by Kufri Girdhari (2.46 g) and Kufri Himsona (2.40 g). Kufri Jyoti produced highest number (5.83) and weight (0.47 g) of <4 mm micro-tubers. This may be due to delayed onset of micro-tubers in Kufri Jyoti. Highest number and weight of micro-tubers of >4mm diameter was observed in variety Kufri Giriraj followed by Kufri Girdhari and Kufri Himsona. Kufri Megha

Table 2: Microtuber production potential of six potato varieties

Variety	Average Microtuber Number				Average Microtuber weight (g)			
	4mm	6mm	8mm	Total	4mm	6mm	8mm	Total
K Giriraj	4.20	5.50	4.30	14.00	0.27	1.10	1.81	3.18
K Girdhari	5.00	3.33	2.93	11.26	0.31	0.71	1.44	2.45
K Himsona	4.73	3.47	2.93	11.13	0.43	0.76	1.21	2.41
K Himalini	4.00	3.23	2.00	9.23	0.25	0.67	0.70	1.62
K Jyoti	5.83	3.13	1.97	10.93	0.47	0.63	0.88	1.98
K Megha	3.07	2.47	1.23	6.77	0.24	0.40	0.56	1.20
C.D. 5%	0.20	0.21	0.22	0.50	0.02	0.05	0.10	0.14
C.V.	3.34	4.62	6.61	3.60	4.68	5.07	6.81	5.11

produced lowest number and weight in all the three classes of micro-tuber size. In terms of the total number and weight of micro-tubers Kufri Giriraj proved superior over all the varieties. Kufri Jyoti though produced more number of <4 mm micro-tuber, its total tuber number was less. Kufri Megha again showed a poor response towards micro-tuber production.

It was possible to induce *in vitro* micro-tuberization in all the 6 varieties and the micro-tubers appear to be similar in shape to the field produced tubers. It is not necessary that the cultivar which produces maximum number of micro-tubers may also have high average weight. This may be due to the period of harvest (Randhava and Chandra 1990). In class wise comparison of micro-tubers, sizes of >4 mm are most preferred as they have better survival ability when transplanted in field condition. Among all the cultivars, Kufri Giriraj was found to be the superior variety for above traits.

The genotypic dependence of micro-tuber production is well known (Gopal et al. 1998). Studies have shown that genotypes under the same cultural conditions showed a wide range of variations in their growth pattern which seemed to pertain to varietal differences (Hossain 2005). The variety Kufri Giriraj has shown to have the highest potential as a fast multiplier, high microtuber producer compared to all other varieties. Kufri Himalini, Kufri Himsona and Kufri Girdhari, which are newly released late blight resistant varieties for the region are also medium to fast multiplying varieties during the initial propagation stage, however their capacity to produce micro-tubers are not satisfactory. In the present investigation, Kufri Giriraj exhibited the best potential to produce micro-tubers. This may be due to inherent genetic potential of the genotype or due to culture conditions.

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