academicJournals

Vol. 7(7), pp. 203-210, July 2015
DOI: 10.5897/JPBCS2015.0507
Article Number: 9EBDDF454276
ISSN 2006-9758

http://www.academicjournals.org/JPBCS

Journal of Plant Breeding and Crop Science

Full Length Research Paper

High efficiency macropropagation of potato (*Solanum tuberosum* L.) cv. Kufri Jyoti in Kumaun Hills

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Received 2 March, 2015; Accepted 19 June, 2015

The present study demonstrated the use of various PGR combinations for efficient *in vitro* regeneration of cv. kufri jyoti in kumaun hills. Best callus induction and proliferation was observed in MS medium supplemented with 13.59 µM 2,4-D alone and 2,4-D + kinetin (9.06 + 1.16 µM) out of different concentrations of 2,4-D (4.53 to 18.12 µM) alone and 2,4-D (0 to 18.12 µM) with kinetin (1.16 µM). Leaf explants were more efficient in producing callus as compared to internodes. Medium supplemented with BA + GA_3 (8.88 µM + 1 µM) initiated shoot induction out of various combinations of BA (4.44 to 13.22 µM) and GA_3 (1 µM) after 7 days of incubation with significantly high average number of shoots, average shoot length and average number of leaves per explant. MS medium supplemented with different concentrations of zeatin (4.56, 9.12 and 13.68 µM) with IAA (5.71 µM) and GA_3 (8.49 µM) was tried for direct regeneration of shoots through nodes out of which zeatin + IAA + GA_3 (13.68 µM + 5.71 µM + 8.49 µM) served to be the best combination and the raised plantlets were found to produce microtubers in a period of 8 to 10 weeks. 2.45 µM IBA in full strength basal MS medium induced highest number of roots. In addition to an efficient regeneration protocol, the microtuber production was also studied in the present piece of work. The research protocol may also be utilized for *Agrobacterium tumefaciens* mediated transformation towards the biotic and abiotic stress tolerant potato crop.

Key words: *In vitro*, potato, callus, direct regeneration, microtubers.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the world's most economically important tuber crop belonging to the family Solanaceae. It plays an important role in the food chain, as it ranks 4th in importance after rice, wheat and maize (Solomon and Barker, 2001). Potato is a good, cheap source of carbohydrates, vitamins, minerals and proteins. It has multipurpose use in daily consumption and also industrial purpose (Hoque, 2010). cv. Kufri jyoti is well adapted to North and South Indian hills, parts of Bihar, Gujarat, Karnataka, Madhya Pradesh, Maharashtra, Punjab, Uttar Pradesh and West Bengal. It persists medium to long tuber dormancy, low storage losses and medium to high tuber dry matter. Appropriate combinations and concentrations of PGR in the culture media are required for rapid plant regeneration from explants (Ehsanpour and Jones, 2000a). *In vitro* regeneration of potato is easily done from different explants on MS medium supplemented with different PGR for diseases free good quality seeds and pathogen free planting materials (Hossain, 1994; Rabbani et

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al., 2001; Zaman et al., 2001). Successful *in vitro* plant regeneration of potato has been achieved from explants of different organs and tissues of potato such as leaf, stem, tuber discs and unripe zygotic embryos (Shirin et al*.,* 2007). The success of plant biotechnology relies on several factors which include an efficient tissue culture system for regeneration of plants from cultured cells and tissues (Khalafalla et al., 2010**)**. Tissue culture based potato multiplication has successfully been incorporated in high quality potato seed production programme (Srivastava et al., 2012).

Microtubers (*in vitro* developed tubers) are miniature seed potatoes and they represent an intermediary phase between *in vitro* plantlets and minitubers. The use of microtubers in storage and exchange of germplasm and seed potato production is advantageous (Seabrook et al., 1993; Rannali et al., 1994). Microtubers are the first generation of potato seed from tissue culture, being used to solve the problems of transplanting the plantlets from *in vitro* to *in vivo* conditions. They can be planted directly in the soil and they can be produced in any period of the year (Nistor et al., 2010).

Considering the main problems of potato cultivation in hills of Uttarakhand including biotic and abiotic stress, lack of seed agency who provide the quality seed potato and lack of technology intervention, the present study was undertaken to develop efficient protocol for *in vitro* regeneration of cv. kufri jyoti in kumaun hills directly through nodes and via callus using leaf and internodes as explants. This protocol may serve as a highly useful technique for crop improvement through *Agrobacterium tumefaciens* mediated transformation via rapid multiplication of plantlet production as well as virus free seed potatoes or microtuber formation.

MATERIALS AND METHODS

Plant material

cv. Kufri jyoti was obtained from Government Breeding Garden, Kashipur, Uttarakhand and grown in pots (20 × 15 cm) containing soil and farmyard manure in a ratio of 3:1 over a period of 10 to 15 days. All the explants were taken from these donor plants for the present research work. Explants such as juvenile leaf, nodes and internodes were initially washed with Tween-20 and then with distilled water 3 to 4 times to remove the traces of the chemical applied. Thereafter they were treated with bavistin (fungicide) solution (0.5%, 15 min) to avoid fungal contamination. For surface sterilization explants were subjected to $HgCl_2$ (0.1%, 1 min) and thoroughly washed with distilled water for 2 to 3 times under laminar airflow. Leaves were dissected into small pieces and trimmed, nodes and internodes were cut into small pieces (approx. 5 mm). After a quick dip in 70% alcohol explants were then washed with sterile distilled water.

Preparation of culture media and growth condition

Murashige and Skoog medium (1962) was used with 3% sucrose and solidified with 0.7% agar. For root development clarigel (0.24%) was used as solidifying agent for clear analysis. Plant growth regulators used were 2,4-D, zeatin, kinetin, IAA, GA_3, BAP and IBA. All experiments were carried out in 250 ml jam bottles /flasks containing 50 ml of culture medium. The pH of media was adjusted to 5.8 using 1N NaOH prior to autoclaving at 121°C at 15 lbs pressure for 20 min. Cultures were incubated under 16 h photoperiod with photosynthetic photon flux density of 40 µ mol m^{-2} s^{-1} fluorescent lamps.

Callus induction and shoot regeneration

For callus induction juvenile leaf sections and internodes with cut ends were placed on MS medium with different concentrations of PGR like 2,4-D (4.53 to 18.12 µM) alone and 2,4-D (0 to 18.12 µM) with kinetin (1.16 µM). Callus initiated after 15 to 20 days of incubation. Calli were subcultured in every 15 days. Well differentiated calli were placed on MS medium supplemented with various combinations of BAP (4.44 to 13.22 µM) and GA_3 (1 µM) for shoot regeneration. All cultures were maintained at 25 ± 2°C with 16 h photoperiod. Shoot regeneration initiated in 7 days.

Direct regeneration of shoots

For direct regeneration of shoots explants taken were nodes. Explants were cut into small sections of 2 to 5 mm size and inoculated in the MS medium supplemented with different concentrations of zeatin (4.56, 9.12 and 13.68 µM) with IAA (5.71 µM) and GA_3 (8.49 µM) for shoot multiplication. Cultures were kept at 25 ± 2°C with 16 h photoperiod. Shoot induction initiated in 3 to 4 days of incubation.

Regeneration of roots and development of elite plantlets

When shoots grew upto a height of 3 to 4 cm, they were aseptically removed, separated from each other and subcultured on half and full strength MS medium with varying concentrations of IBA for root induction. Root development initiated after 4 to 5 days of incubation. The completely rooted plants (2 to 3 weeks) were taken out carefully and gently washed under running water to remove excess clarigel. They were then potted in thermocole cups (12 × 8 cm) containing soil and farmyard manure (3:1, v/v); covered with transparent polythene bags with small holes to maintain humidity. These plants were placed inside growth chamber under 16 h photoperiod with photosynthetic photon flux density of 40 µ mol m^{-2} s^{-1} fluorescent lamps at 25 ± 2°C temperature. Plants were watered regularly and gradually acclimatized over a period of 1 month. The polythene bags were then removed and the established plantlets were subsequently transplanted to earthen pots (20 × 15 cm) and kept in a polyhouse for further growth (Figure 1i, j and k).

Production of microtubers

Well grown plantlets obtained from direct regeneration of nodes were maintained in culture room at 16/8 h light/dark condition and observed for the production of microtubers .

Statistical analysis

All the experimental observations were recorded at regular intervals. Mean values of various treatments were analysed by using one way ANOVA (Analysis of Variance) for statistical significance. Effect of different concentrations of plant growth

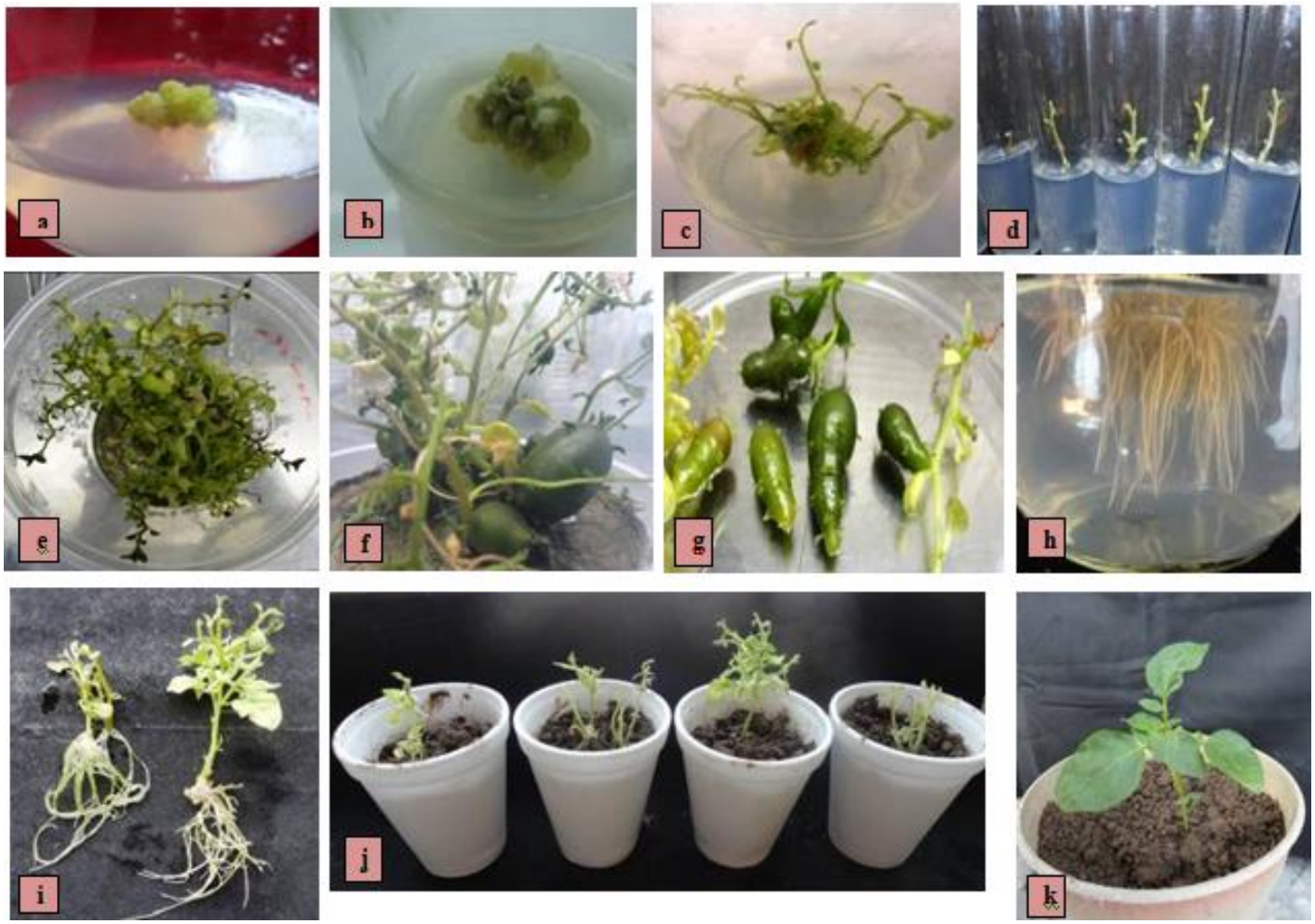

Figure 1. Different stages in macro propagation of *S. tuberosum* in full strength MS medium. (a) *In vitro* callus induction and proliferation with 2,4-D + kinetin (9.06 + 1.16 µM) after 14 of incubation;(b, c) *In vitro* shoot induction and proliferation from 8 weeks old callus with BAP (8.88 µM) + GA_3 (1.00 µM) after 7 and 14 days of incubation respectively; (d, e) *In vitro* direct shoot regeneration from nodes using different PGRs viz. zeatin + IAA + GA_3 (13.68 + 5.71 + 8.49 µM) after 7 and 49 days of incubation respectively; (f, g) Microtubers formation from *in vitro* direct shoot regenerated plantlets containing zeatin (13.18 µM) with IAA (5.71 µM) and GA_3 (8.49 µM) under 16/8 h light/dark photoperiod after 60 days of incubation. (h) *In vitro* root induction in microshoots of *S. tuberosum* in full strength MS medium containing 2.45 µM IBA after 15 days of incubation; (i, j) *In vitro* developed plantlets were transferred to thermocole cups containing soil and farmyard manure (3:1, v/v) for acclimatization and hardening in culture room conditions for 30 days (k) *In vitro* hardened plantlets were successfully transferred to polyhouse conditions.

regulators were determined on average number of shoots, average shoot length, number of nodes and average number of leaves per explant.

RESULTS

The explants showed callus formation in MS medium containing 2,4-D. Best callus induction and proliferation was observed after 15 to 20 days of incubation in MS medium with 13.59 µM 2,4-D alone and 2,4-D + kinetin (9.06 + 1.16 µM) (Figure 1a). The callus obtained was light green in colour. Increased concentration of hormones lead to browning of callus (Tables 1 and 2). This phenomenon was also supported by previous studies in other species. Auxin alone and in combination with cytokinin can produce callus but 2,4-D was found to be most effective for callus induction and proliferation (Shirin et al., 2007). But on the contrary, in the present study callus induction in leaf explants was more frequent in comparison to internodes as explants as observed after 30 days of incubation. Regular subculture of callus enhanced proliferation rate due to availability of nutrients before their exhaustion in the medium.

Shoot regeneration from the calli initiated in the medium supplemented with BAP + GA_3 (8.88 + 1 µM) after 7 days of incubation (Figure 1b). GA_3 has been reported to help in elongation of shoots. Combination of

Table 1. Effect of different combinations of 2,4-D + kinetin in full strength MS medium on *in vitro* callus induction in various explants (leaf, internodes) of *Solanum tuberosum* after 30 days of incubation.

PGRs	Concentration (µM)	Callus growth	Callus colour
2,4-D + kinetin	0.00 + 1.16	-	-
	4.53 + 1.16	+	Light yellow
	6.79 + 1.16	+	Light yellow
	9.06 + 1.16	**+++**	Light green
	11.32 + 1.16	++	Light green
	13.59 + 1.16	++	Light green
	15.85 + 1.16	+	Light green
	18.12 + 1.16	+	Light brown

+++, Assumed as 100% response; ++, assumed as 75% response; +: assumed as 50% response; **-,** for no callus.

Table 2. Effect of different concentrations of 2,4-D in full strength MS medium on *in vitro* callus induction in various explants (leaf, internodes) of *Solanum tuberosum* after 30 days of incubation.

	Concentration (µM)	Callus growth	Callus colour
2,4-D	0.00	-	-
	4.53	-	-
	6.79	-	-
	9.06	+	Whitish
	11.33	++	Light Green
	13.59	**+++**	Light Green
	15.85	+	Light Green
	18.12	+	Light Brown

+++, Assumed as 100% response; ++, assumed as 75% response; +,: assumed as 50% response; **-,** for no callus.

Table 3. Effect of various combinations of BAP and GA_3 in full strength MS medium on *in vitro* shoot regeneration from callus of *S. tuberosum* after 30 days of incubation [values are mean ± S.E. (n=3)]

SN	Treatments	Avg. no. of shoots explant^{-1}	Avg. shoot length (cm)	Avg. no. of leaves
1	Control	0.00	0.00	0.00
2	BA + GA_3 (4.44 + 1 µM)	0.88 ± 0.11	2.49 ± 0.53	5.55 ± 1.28
3	BA + GA_3 (8.88 + 1 µM)	2.99 ± 0.19	3.48 ± 0.19	7.02 ± 0.15
4	BA + GA_3 (13.22 + 1 µM)	0.55 ± 0.11	0.82 ± 0.09	1.55 ±0.11
	LSD (P≤0.05)	7.587724*	0.000108*	0.000177*

ns, Non significant; *, significant at $p < 0.05$; S.E, standard error of mean; LSD, least significant difference; SN, serial number.

BA + GA_3 (8.88 + 1 µM) produced significantly high average number of shoots, average shoot length and average number of leaves per explant as compared to other combinations (Table 3 and Figure 1c). The results of one way analysis of variance (ANOVA) showed that F-factor and P- value for most of the parameters were significant at 0.05% level (Table 4). Longest shoot obtained was 4.50 cm in height as observed after 20 to 25 days of incubation. This agrees with Haque et al. (2009) who observed the longest shoot by the treatment combination of BAP and GA_3 in other species of the plant . On the other hand direct regeneration of shoots with highest average number of shoots, nodes and leaves per explant took place in the medium supplemented with zeatin + IAA + GA_3 (13.68 µM + 5.7 µM + 8.49 µM) (Table 5 and Figure 1d). Longest shoot attained a height

Table 4. F-ratio and level of significance of one way analysis of variance (ANOVA) test for *in vitro* shoot regeneration from callus of *S. tuberosum* after 30 days of incubation on full strength MS medium containing various combinations of BAP and GA_3.

Source of variation	Avg. no. of shoots explant^{-1}			Avg. shoot length (cm)			Avg. no. of leaves explant^{-1}		
	df	MS	F	df	MS	F	df	MS	F
Between groups	3	5.15	110.22*	3	7.44	29.82*	3	32.70	26.02*
Within Groups	8	0.04		8	0.24		8	1.25	
Total	11			11			11		

Data were recorded after every 1 week of culture. All values are an average of 9 explants; individual treatments consisted of three replicates, one explants per flask and the experiment was repeated thrice. MS, mean square; F, *f* statistic; LSD, least significant difference; df, degree of freedom, ns, not significant; *, significant at 0.05 level.

Table 5. Effect of different concentrations of zeatin, IAA and GA_3 on *in vitro* direct shoot regeneration from nodes of *S. tuberosum* on full strength MS medium after 21 days of incubation [values are mean ± S.E (n=3)].

S/N	Treatments	Avg. no. of shoots explant^{-1}	Avg. Shoot length (cm)	Avg. no. of nodes explant^{-1}	Avg. no. of leaves explant^{-1}
1	Control	1.00 ± 0.00	1.12 ± 0.12	0.00	1.00 ± 0.40
2	Zeatin+IAA+GA_3 (4.56+5.71+ 8.49 µM)	0.99 ± 0.13	1.35 ± 0.29	0.91 ± 0.39	3.87 ± 0.95
3	Zeatin+IAA+GA_3 (9.12+5.71+ 8.49 µM)	1.41 ± 0.08	3.17 ± 0.34	2.10 ± 0.39	8.55 ± 1.47
4	Zeatin+IAA+GA_3 (13.68+5.71+ 8.49 µM)	1.49 ± 0.21	4.10 ± 0.27	2.90 ± 0.29	12.37 ± 1.90
LSD (P≤0.05)		0.036155*	0.000011*	0.000110*	0.000320*

ns, Non significant; *, significant at p < 0.05; S.E., standard error of mean; SN, serial number.

Table 6. F-ratio and level of significance of one way analysis of variance (ANOVA) test for *in vitro* direct shoot regeneration from nodes of *S. tuberosum* on full strength MS medium containing different concentrations of zeatin, IAA and GA_3 after 21 days of incubation.

Source of variation	Avg. no. of shoots explant^{-1}			Avg. Shoot length			Avg. No. of nodes explant^{-1}			Avg. no. of leaves explant^{-1}		
	df	*MS*	*F*	*df*	*MS*	*F*	*df*	*MS*	*F*	*df*	*MS*	*F*
Between groups	3	0.28	3.93*	3	8.28	27.32*	3	6.93	17.52*	3	101.10	13.96*
Within groups	12	0.07		12	0.30		12	0.39		12	7.23	
Total	15			15			15			15		

Data were recorded after every 1 week of culture. All values are an average of 12 explants; individual treatments consisted of four replicates, one explants per flask and the experiment was repeated thrice with qualitatively similar results. MS, Mean square; F, *f* statistic; LSD, least-significant difference; df, degree of freedom, ns, not significant; *, significant at 0.05 level. S.E, Standard error of mean.

of 6 cm after 15 to 20 days of incubation. Profound shoot proliferation was obtained after 7 to 8 weeks (Figure 1e). Direct shoot regeneration is preferred since it reduces the possibility of somaclonal variation (genetic variation) common in plants regenerated from cultured cells or tissues (Misra and Datta, 2001; Dayal et al., 2003). Results of this experiment are also proved to be significant using ANOVA (Table 6). Observations recorded were observed for different parameters viz. average number of shoots, average shoot length, number of nodes and average number of leaves per explant.

Plantlets were found to produce microtubers in a period of 8 to 10 weeks (Figure 1f and g). Cytokinins are believed to have strong promotive effects on tuberization, and to constitute major part of the tuberization stimulus, either alone or in combination with other substances (Pelacho and Mingo-Castel, 1991). The average weight of the tubers obtained was found to be 0.20 g with an average number of 3 tubers per explant. The microtubers obtained were green in colour. For root induction, out of different concentrations of IBA tried with basal MS medium and half strength basal MS medium, 2.45 µM IBA induced highest number of roots after 15 days of incubation in full strength basal MS medium (Figure 1h) as well as in half strength basal MS medium as compared to other concentrations. IBA has proved to be efficient in promoting root induction (Sakthivel and Manigandan, 2011). The mean value of *in vitro* rooting

Table 7. Various parameters studied under the effect of different concentrations of IBA in half strength and full strength MS medium on rooting response in *in vitro* multiplied shoots of *S. tuberosum* after 15 days of incubation [values are mean ± S.E (n=3)]

S/N	Treatment	Avg. no. of roots shoot^{-1}	Avg. root length (cm)
	Half strength medium		
1	IBA (1.12 µM)	37.50 ± 12.50	7.50 ± 0.50
2	IBA (2.45 µM)	42.40 ± 7.50	7.25 ± 0.50
3	IBA (4.9 µM)	37.50 ± 2.50	7.50 ± 0.50
	Full strength medium		
4	IBA (1.12 µM)	34.00 ± 4.00	7.50 ± 0.00
5	IBA (2.45 µM)	43.50 ± 1.50	7.50 ± 0.50
6	IBA (4.9 µM)	31.50 ± 1.50	7.25 ± 0.25

S.E., Standard error of mean; SN, serial number.

response for all the parameters at different PGR concentration showed that average number of root (43.50) and average root length (7.50 cm) was observed to be maximum with 2.45 µM IBA in full strength MS medium. Longest root attained the length of 8.5 cm. Data analysed for average number of roots and average root length for different concentrations of IBA used is represented in Table 7.

DISCUSSION

Callus is an unorganized mass of plant cells. Reliable callus induction and regeneration of viable plants is considered as a limiting step to the successful use of modern techniques in genetic improvement of the major crops (Murphy, 2003). In the present study, different PGR combinations were checked for *in vitro* callus induction in explants of *Solanum tuberosum*. Callus induction was found to be successful using different concentrations of 2,4-D alone and in combination with Kinetin. The auxin 2,4-D, by itself or in combination with cytokinins, has been widely used to enhance callus induction and maintenance. Moreover, many researchers observed 2,4-D as the best auxin for callus induction both in monocots and dicots (Chee, 1990; Mamun et al., 1996). Role of 2,4-D in callus induction has been widely accepted and utilized for potato, tomato and many medicinal plants (Ashakiran et al., 2011; Ahmed et al., 2012; Lakshmi and Reddy, 2012; Mehta et al., 2012).

On the basis of regular observation it was concluded that the source of explant has a direct effect on callus induction. Results showed that leaf explant were more efficient for callus induction with 100% response as compared to internodes which gave only 50% response. This may be due to the presence of more meristematic activity in leaves as compared to internodes. This result supported the previous study by Haque et al. (2009) who found that callus length was affected by different explants and that the leaf explants produced significantly highest callus length in contrast to the shoot tip which produced least results in case of potato cv. Diamant. The interaction effect between explant and concentration of growth regulators were found to have significant differences on callus length in different researches. This result was also proved to be significant by Dobranaszki et al. (1999) and Fomenko et al. (1998) who also observed significant effects of explants of potato on callus length. In the present study different concentrations of 2,4-D and Kinetin showed significant differences in callus growth and colour. Rate of callus induction increased with the increasing concentration of 2,4-D alone upto 13.59 µm and in the combination of 2,4-D and Kinetin upto 9.06 µm and 1.16 µm respectively. Further increase in concentration lead to decrease in callus growth and resulted in browning of callus. Callus initiation on cut ends of *in vitro* cultured explants of potato could be observed in all 2,4-D levels (Khalafalla et al., 2010). Similar findings were also reported by (Fiegert et al., 2000; Jayasree et al., 2001; Yasmin et al., 2003).

Both callus induction and plant regeneration from explant require appropriate combinations and concentrations of plant growth regulators in the culture media (Ehsanpour et al., 2000b). In the present research work, best results for shoot regeneration from callus of *S. tuberosum* was obtained by using a combination of 8.88 µM BAP and 1.00 µM GA_3 with significantly high average number of shoots, shoot length and number of leaves per explant as compared to other combinations. BAP, Zeatin or Kinetin are known to help produce adventitious shoots. Martel and Carcia (1992) reported that both BAP and GA_3 at higher concentrations were necessary for shoot formation of potato. Shoot regeneration responses vary with the potato cultivar but in most cases cytokinin helps to enhance shoot production (Ghaffoor et al., 2003). Generally a low ratio of auxin to cytokinin is required for adventitious shoot development in case of potato (Anjum and Ali, 2004).

A decrease in all the parameters of shoot regeneration occurred after increase in the concentration of BAP after 8.88 µM. Similar effects of increasing concentration of BAP on shoot regeneration of potato cv. Asterix were observed by Molla et al. (2011) who observed that the length of shoot increased with increasing BAP concentration up to 3 mg l^{-1} and then decreased. Role of GA_3 in shoot elongation is well known and reported by many researchers. For rapid multiplication, addition of GA_3 to the MS media has been reported to improve growth and development of shoots. Farhatullah and Abbas (2007) also have reported that dosage of 0.248 mg l^{-1} of GA_3 in the MS medium boosted all morphological characters in *in vitro* raised potato plantlets. Ullah et al. (2012) also have reported that GA_3 is involved in cell elongation and its addition in MS medium enhanced shoot growth in *in vitro* propagated plants of potato variety "Desiree".

Direct regeneration system has an edge over regeneration after passing through callus phase to maintain the true-to-type nature of the regenerated plantlets and avoid somaclonal variation. Potato breeding programs can highly benefit from biotechnological tools, which are capable of surpassing some limitations found by traditional plant breeding methods and open new avenues for crop improvement. In the present study, attempts were made also made to induce direct regeneration of *S. tuberosum.* Explant used were nodes. Leaf discs and inter nodal tissues are the least responsive explants for direct regeneration. These explants underwent callus induction phase and then resulted in shoot regeneration indirectly in a study conducted by Hussain et al. (2005) on three potato cultivars viz., Cardinal, Altamash and Diamont. There are many advantages of taking nodal tissue as an explant, that is, a large number of aseptic plants can be obtained quickly and easily, and plants produced may remain true to type. Successful regeneration was obtained using hormonal combination of Zeatin, IAA and GA_3 in a concentration of 13.68, 5.71 and 8.49 µM, respectively. Role of Zeatin in regeneration has been reported by Wendt et al. (2001) who found that the internode explant of potato cultivar Macaca treated with Zeatin showed higher regeneration rate than those treated with BAP.

Roots were induced in microshoots using different concentrations of IBA, out of which 2.45 µM concentration emerged to be best with maximum average number of root (43.50) and a maximum average root length of 7.50 cm in full strength MS medium. IBA has been shown as a potent root inducer in many studies conducted on various tomato cultivars (Chaudhry et al., 2010; Khalafalla et al., 2010; Sakthivel and Manigandan, 2011).

Microtubers of *S. tuberosum* were obtained after incubating directly regenerated shoots at 16/8 h light/dark condition after 8 to 10 weeks. Microtubers obtained were green in colour. The green colour might be due to the presence of alkaloid solanin which is produced under light conditions. Microtubers may vary in their shape, colour, weight, diameter, length etc. (Rannali, 2007). This study also supports the similar findings of Hoque (2010). The edible part of the plant is the tuber, which is formed at the end of underground stems called stolon. Potato produced more protein and calories per unit area per unit time and per unit of water than any other major plant food. *In vitro* tubers can be produced throughout the year and thus holds benefit over conventional tubers (Hoque, 2010).

Conclusion

The present regeneration protocol could be useful for rapid *in vitro* regeneration, multiplication and virus free seed, that is, microtuber production. This piece of work may also be utilized for transformation techniques for production of biotic and abiotic stress tolerant potato crop which may in turn contribute to overcome major obstacle in potato farming especially in the Kumaun hills towards quality and efficient production of this major cash crop.

Conflict of Interest

The authors have not declared any conflict of interest.

Abbreviations: ANOVA, analysis of variance; **BA,** benzyl adenine; **GA_3,** gibberellic acid; **IAA,** indole acetic acid; **MS,** Murashige and Skoog (1962); **PGR,** plant growth regulators; **2,4-D,** 2,4-dichlorophenoxy acetic acid.

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