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Short communication

Modified medium for micropropagation of recalcitrant potato cv. Kufri Jyoti

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ABSTRACT

Experiments were conducted to devise an efficient micro-propagation medium for mass multiplication of a high yielding but recalcitrant potato cv. Kufri Jyoti using nodal explant with different concentrations and combinations of NH_4NO_3, GA_3 and NAA. Virus-indexed nodal cuttings established in full-strength Murashige and Skoog (1962) basal salts and vitamins, supplemented with 0.29 µM GA_3 and 0.05 µM NAA was used as explants for the experiment. Among the various media tested, MS medium with increased level of ammonium nitrate (25.79 mM) supplemented with GA_3 (0.58 µM) and NAA (0.1 µM) was found to be optimum as it significantly improved the shoot length, number of leaves, number of nodes and inter nodal length after 21 days of sub-culturing. The next higher concentration of ammonium nitrate (30.94 mM) supplemented with GA_3 (0.58 µM) and NAA (0.1 µM) also exhibited statistically at par response in most of the morphological characters but higher concentration (41.25 mM) retarded all the parameters studied.

Key words: Micropropagation, potato, NH_4NO_3, concentration.

Productivity of potato is constrained primarily by use of low quality seeds. Many field multiplication generations of vegetatively propagated basic seed result in build-up of seed-borne diseases (Chindi *et al*., 3). The rapid spread of pests and diseases and need for clean and quality planting material has stimulated it's production through aseptic micropropagation techniques. The major disadvantage of micropropagation in potato is that some of the cultivars require variety specific protocols for its successful mass multiplication. Among different cultivars released by ICAR-CPRI, Kufri Jyoti has been classified as recalcitrant based on the performance on the standard MS medium, the microplants during *in vitro* multiplication shows very slow growth, clumping of internodes, yellowing of basal leaves and premature senescence. Sotiropoulos *et al.* (11) proposed that the effective N uptake of *in vitro* plantlets depends on a balance between both nitrate and ammonium ions. In addition to this the role of balanced plant hormone is also important for maintaining the growth and physiology of potato microplants. The objective of present study was to investigate the morphological changes at elevated nitrogen level with different concentration and combinations of GA_3 and NAA in order to find best suited medium for micropropagation of recalcitrant potato cv. Kufri Jyoti.

The study was carried out at ICAR-Central Potato Research Institute, Shimla with the objective to improve the *in vitro* response of recalcitrant cultivar Kufri Jyoti. Accordingly, two double node cuttings derived from middle nodes of the microplants were cultured per tube (25 × 150 mm) in Murashige and Skoog (7) medium supplemented with 0.1 M sucrose and 4.19 µM D-calcium pantothenate and solidified with 0.2% gelrite. In order to hasten the growth and multiplication rate, concentrations of NH_4NO_3 was elevated from the standard level of 20.63 to 25.79, 30.94, 36.11 and 41.25 mM. Each NH_4NO_3 concentration was further augmented with different concentrations of GA_3 (0.0, 0.29 and 0.58 µM) and NAA (0.0, 0.05 and 0.1 µM). Different concentrations and combinations of ammonium nitrate, GA_3 and NAA used for the study are detailed in Table 1. The experiment was carried out in a factorial (5 × 5) CRD over a period of 28 days. Each treatment comprised of four replicates and each replicate consisted of five test tubes. The culture tubes were incubated under 16/8 h photoperiod (irradiance of 60 µmol m^{-2} s^{-1}) at temperature of 22 ± 1°C. After 28 days of culturing, observations were recorded on micro-plant height (cm); number of leaves, nodes and roots; inter-nodal and root length (cm); fresh as well as dry mass (mg). As the experiment was conducted twice, data were pooled over individual experiments. The two-way analysis of variance was done using the software AGRES and means were separated according to the least significant difference (LSD) at 0.05 level of probability.

The micro-plant morphological characters, *viz.,* micro-plant height, number of leaves, nodes and roots, inter-nodal and root length and fresh as well

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Table 1. Effect of different concentrations of ammonium nitrate, GA_3 and NAA on microplant height and number of leaves on Kufri Jyoti.

Conc. of NH_4NO_3 (mM)	Microplant height (cm)						No. of leaves per plantlet					
	G_0	G_1	G_2	G_3	G_4	Mean	G_0	G_1	G_2	G_3	G_4	Mean
N_1: 20.63 (MS)	2.74	4.25	5.27	5.05	6.20	4.70	2.5	3.0	3.9	2.8	2.3	2.9
N_2: 25.79	3.46	4.10	5.99	5.94	7.66	5.43	3.1	3.5	3.8	3.6	4.9	3.8
N_3: 30.94	3.97	5.60	4.80	5.27	6.76	5.28	3.5	3.6	3.3	3.8	4.1	3.7
N_4: 36.11	2.80	4.30	4.21	4.16	5.38	4.17	2.9	3.5	3.3	3.9	4.0	3.5
N_5: 41.25	1.90	2.21	3.29	4.07	4.23	3.14	1.5	2.4	2.4	2.2	3.0	2.3
Mean	2.97	4.09	4.71	4.90	6.05		2.7	3.2	3.3	3.3	3.7	
Factor	N	G	NG				Factor	N	G	NG		
$CD_{(0.05)}$	0.61**	0.61**	1.36**				$CD_{(0.05)}$	0.57**	0.57**	1.27**		

as dry mass was significantly influenced by different concentrations of NH_4NO_3, growth regulators and their interaction. Among different concentrations, NH_4NO_3, 25.7 mM significantly increased the micro-plant height (5.43 cm) and the same concentration also recorded maximum root length (8.92 cm) (Fig. 1; Table 1-3).

Slightly longer inter-nodal length (1.16 cm), maximum number of roots (4.8), fresh and dry mass of microplants was recorded in the media supplemented with 30.94 mM NH_4NO_3. Further increase of NH_4NO_3 rather abolished the pattern this may be due to the progressive reduction in nitrate reductase activity at high N concentration (Jang *et al.,* 4). Movahedi *et al*. (6) and Rai *et al*. (10) also reported similar results in potato during micropropagation. Addition of nitrogen to sea grasses generally causes an increase in leaf and/or shoot growth but may have no or negative effect on root production (Peralta *et al*., 8). Both positive and negative effects of N application have also been reported by Belanger *et al*. (2). Further support for these results comes from mesocosm experiments by Peralta *et al*. (8).

Among different concentration and combination of growth regulators, the medium supplemented with GA_3 (0.58 µM) + NAA (0.1 µM) significantly increased the microplant height (6.05 cm), number of leaves (3.7), nodes (4.6), inter-nodal length (1.20 cm), number of roots (6.0), root length (9.0 cm) and fresh as well as dry mass. All the test concentrations of growth regulators significantly increased the root length in comparison to the medium without growth regulator (4.7 cm) and the maximum root length (8.43 cm) was recorded in medium containing GA_3 (0.58 µM) + NAA (0.05 µM), which was at par with the GA_3 (0.58 µM) + NAA (0.1 µM) (Fig. 1; Table 1-3).

Our results revealed that different concentrations of GA_3 and NAA in combination synergistically influenced the morphological characters. Increase in the concentration of GA_3 and NAA tends to increase height; number of leaves and nodes; internodal and root length; fresh and dry mass of potato plantlets. This may be due to high concentration of GA_3; which increases the hydrolysis of starch and sucrose into fructose and glucose (Khan and Chaudhry, 5). Badoni and Chauhan (1) obtained better results for shoot

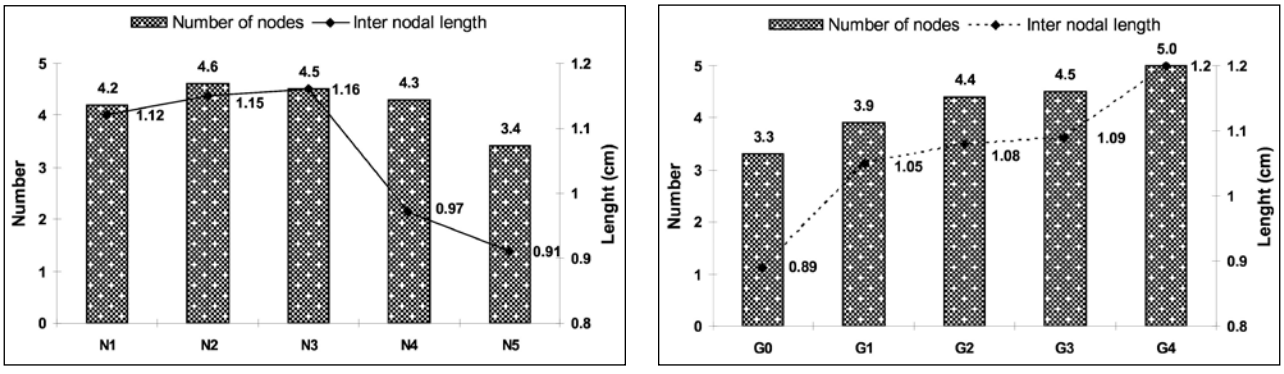

Fig. 1. Effect of different concentrations of ammonium nitrate and growth regulators on number of nodes and inter-nodal length of Kufri Jyoti micro-plants.

Table 2. Effect of different concentrations of ammonium nitrate, GA_3 and NAA on number of roots and root length on Kufri Jyoti.

NH_4NO_3 (mM)	No. of roots per plantlet						Root length (cm)					
	G_0	G_1	G_2	G_3	G_4	Mean	G_0	G_1	G_2	G_3	G_4	Mean
N_1: 20.63 (MS)	2.4	3.5	6.5	4.4	6.1	4.6	6.11	8.31	7.79	6.98	8.79	7.60
N_2: 25.79	2.9	4.3	2.1	5.3	5.8	4.5	6.09	8.13	8.85	11.98	9.54	8.92
N_3: 30.94	3.4	4.2	3.9	3.0	9.5	4.8	6.84	9.14	4.91	9.44	9.69	8.00
N_4: 36.11	4.3	3.1	3.7	4.8	3.0	3.8	4.36	7.15	8.46	7.66	7.90	7.11
N_5: 41.25	0.4	2.8	2.4	4.9	5.6	2.6	0.20	1.70	4.11	6.08	4.65	3.35
Mean	2.7	3.6	4.1	3.9	6.0		4.72	6.89	6.82	8.43	8.11	
Factor	N	G	NG				Factor	N	G	NG		
$CD_{(0.05)}$	1.49*	1.49**	NS				$CD_{(0.05)}$	1.77**	1.77**	3.95*		

Table 3. Effect of different concentrations of ammonium nitrate, GA_3 and NAA on microplant fresh and dry weight of Kufri Jyoti.

NH_4NO_3 (mM)	Fresh weight (mg)						Dry weight (mg)					
	G_0	G_1	G_2	G_3	G_4	Mean	G_0	G_1	G_2	G_3	G_4	Mean
N_1: 20.63 (MS)	238.31	298.13	457.86	429.44	462.75	377.30	24.93	35.87	49.96	53.79	57.12	44.33
N_2: 25.79	242.80	372.07	510.14	446.06	455.60	405.33	24.85	45.94	56.70	51.10	58.34	47.19
N_3: 30.94	350.49	510.34	386.57	418.80	474.09	428.06	38.77	65.85	42.43	48.40	52.70	49.63
N_4: 36.11	180.32	456.11	409.38	363.62	456.02	373.13	22.07	52.96	44.18	44.93	54.26	43.68
N_5: 41.25	219.38	192.57	322.12	361.27	281.72	275.41	21.84	28.01	36.40	45.35	34.35	33.19
Mean	246.26	365.84	417.21	403.84	426.07		26.49	45.53	45.93	48.71	51.35	
Factor	N	G	NG				Factor	N	G	NG		
$CD_{(0.05)}$	56.68**	56.68**	126.75*				$CD_{(0.05)}$	6.69**	6.69**	14.95*		

*Significant at ($p \leq 0.05$); ** Significant at ($p \leq 0.01$); G_0: Without growth regulator; G_1: GA_3 0.29 µM + NAA 0.05 µM; G_2: GA_3 0.29 µM + NAA 0.1µM; G_3: GA_3 0.58 µM + NAA 0.05 µM; G_4: GA_3 0.58 µM + NAA 0.1 µM.

regeneration and multiplication of potato on the media supplemented with GA_3 and NAA.

In the interaction, medium containing NH_4NO_3 (25.79 mM) + GA_3 (0.58 µM) + NAA (0.1 µM) significantly increased the microplant height (7.66 cm) and number of leaves (4.9) as compared to standard MS medium. Whereas, media containing 30.94 mM NH_4NO_3 supplemented with GA_3 (0.58 µM) and NAA (0.1 µM) recorded significantly maximum root length (9.69 cm), however, which was found to be at par with many other interactions. The same combination also recorded significantly maximum fresh and dry weight as compared to standard MS medium, however, which was found to be at par with many other interactions (Tables 1-3).

Slightly higher concentration of nitrogen (25.79 mM) is must to increase the morphological parameters in the cv. Kufri Jyoti under *in vitro* conditions than the recommended concentration in the MS medium (20.63 mM). The nitrogen source used can markedly influence growth and morphogenesis (Wilson and Bennett, 12). We found slow growth of microplantlets on the medium without growth regulators and the value of all the morphological characters was lower when compared with other combinations of growth regulators. Rapid multiplication can be obtained on the media supplemented with GA_3. A very high concentration of GA_3 (4.5 mg l^{-1}) gave better results in *in vitro* grown potato plant (Rabbani *et al*., 9).

It is concluded that to improve *in vitro* response of Kufri Jyoti, the ammonium nitrate concentration in the MS medium needs to be enhanced from 20.63 to 25.79 mM supplemented with growth regulators (GA_3 0.58 µM + NAA 0.1 µM). However, its effect on *ex-vitro* conditions needs further investigation on mini-tuber production behaviour and phenotypic characters of plants.

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