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STANDARDISATION OF MEDIUM FOR MICROPROPAGATION OF RECALCITRANT POTATO (*SOLANUM TUBEROSUM* L.) CULTIVAR 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 cultivar Kufri Jyoti using nodal explants with different concentrations and combinations of NH_4NO_3, GA_3 and NAA. Virus-indexed nodal cuttings established in full-strength Murashige and Skoog (MS) 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.

KEYWORDS: Ammonium nitrate, growth regulators, micropropagation, recalcitrant

INTRODUCTION

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*., 2014). The rapid spread of pests and diseases and need for clean and quality planting material has stimulated it's production through aseptic micropropagation techniques (Saraswathi *et al*., 2014). The major disadvantage of *in vitro* micropropagation in potato is that some of the cultivars require variety specific protocols for its successful mass multiplication (Venkatasalam *et al*., 2010). Central Potato Research Institute released about four dozen potato cultivars suitable for different agro-climatic zones among them Kufri Jyoti is most important cultivar and occupies the major potato producing area both in hills as well as in plains because of its wide adaptability due to its early bulking nature. However, based on the performance on the standard MS medium, the cultivar Kufri Jyoti has been classified as recalcitrant since the microplants during *in vitro* multiplication shows very slow growth, clumping of internodes, yellowing of basal leaves and premature senescence (Venkatasalam *et al*., 2011).

Major factors limiting the rate of multiplication are overall short height of the plantlets and crowding of the nodes (Miller *et al.*, 1985). Nitrogen is essential macronutrient needed by plant and incorporated into the medium in various forms like ammonium nitrate, potassium nitrate and a small portion is

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also contributed by amino acids. No inhibitory effects were observed when ammonium and nitrate ions are supplied simultaneously (Pia Walch- Lin *et al*., 2000). Sotiropoulos *et al.* (2005) proposed that the effective N uptake of *in vitro* plantlets depends on a balance between both nitrate and ammonium ions. Hence, from the past decade ammonium nitrate has been used as sole source of nitrogen both in field as well as in micropropagation.

The role of balanced plant hormone is also important for maintaining the growth and physiology of potato microplants. These are applied in micromolar concentration, but it is very much effective in regulating physiology of plants including height, leaf expansion, stem elongation and fresh weight. Plant hormones like gibberellins and auxins are known to affect the plant growth. NAA (α-naphthalene acetic acid) is a plant hormone in the auxin family and it is an ingredient in many commercial post-harvest horticultural products as well as rooting agent (Dimitrios *et al*., 2008). Development of long root is of particular significance for explants prior as well as after transplantation. Long roots helps to extract the nutrients and also allow to anchorage which may increase transplant establishment.

On the other hand, another prominent phytohormone, Gibberellic acid (GA_3), has the potential regulatory effect on growth and flowering process. In addition, GA_3 application increased petiole length, leaf area and delayed petal abscission and senescence (Khan and Chaudhry, 2006). Gibberellin increases the shoot height of potato by causing elongation of internode (Lovell and Booth, 1967). Thorpe and Meier (1973) confirmed the inhibition of shoot initial formation by gibberellic acid however, subsequent development was not repressed. Since, plant hormones are known to control development of potato plants and they can be measured or manipulated. 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 cultivar Kufri Jyoti. During investigation, special attention was paid on parameters such as microplant height, number of nodes and inter-nodal length in order to increase the *in vitro* multiplication rate.

MATERIALS AND METHODS

The study was carried out at 3˚ 06′ N, 77˚ 10′ E and 2160 m (latitude, longitude, msl) at Central Potato Research Institute, Shimla during 2008-2011 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 (1962) 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) as well as NAA (0.0, 0.05 and 0.1 µM). Different concentrations and combinations of ammonium nitrate, GA_3 and NAA were used for the study [N_1: NH_4NO_3 20.63 mM; N_2: NH_4NO_3 25.79 mM; N_3: NH_4NO_3 30.94 mM; N_4: NH_4NO_3 36.11 mM; N_5: NH_4NO_3 41.25 mM; 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]. Therefore, a total of twenty-five different combinations of ammonium nitrate with growth regulators were used for the investigation. The said concentrations and combinations inthis study

was decided based on the results obtained from the preliminary study that has been carried out in our laboratory. The experiment was carried out in a factorial (5×5) completely randomized design 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 hour photoperiod (irradiance of 60 µmol m^{-2} s^{-1}) at temperature of 22±1°C. After twenty eight days of culturing, observations were recorded on morphological parameters such as micro-plant height (cm); number of green leaves, nodes and roots; inter-nodal and root length (cm); fresh as well as dry mass (mg) as described by Venkatasalam *et al.* (2011).

Data analyses

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 differences (LSD) at 0.05 level of probability.

RESULTS AND DISCUSSION

Microplant height

The microplant height was significantly influenced by different concentrations of NH_4NO_3, growth regulators and their interaction. Among different concentrations of NH_4NO_3 25.7 mM significantly increased the microplant height (5.43 cm) as compared to standard MS medium (4.70 cm). Increasing the concentration beyond 30.94 mM significantly reduced the microplant height and the minimum (3.14 cm) was observed at 41.25 mM. Among different concentrations and combinations of growth regulators, GA_3 (0.58 µM) + NAA (0.1 µM) significantly increased the microplant height (6.05 cm) as compared to without growth hormone (2.97 cm). In interaction, the media containing NH_4NO_3 (25.79 mM) supplemented with GA_3 (0.58 µM) and NAA (0.1 µM) significantly increased the microplant height (7.66 cm) as compared to standard MS medium (2.74 cm) however, it was found to be at par (6.76 cm) with NH_4NO_3 (30.94 mM) + GA_3 (0.58 µM) + NAA (0.1 µM) (**Table 1**).

Number of leaves

The number of leaves was significantly higher (3.8) in media contaning 25.79 mM NH_4NO_3 in comparison to standard MS medium (2.9). Among different concentrations and combinations of growth regulators, GA_3 (0.58 µM) + NAA (0.1 µM) significantly increased the number of leaves (3.7) as compared to standard MS medium (2.7) however, it was found to be at par with

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

NH_4NO_3 (mM)	Microplant height (cm) Number 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**		

all other combinations. In interaction, the media containing NH_4NO_3 (25.79 mM) supplemented with GA_3 (0.58 µM) and NAA (0.1 µM) recorded significantly higher number of leaves (4.9) as compared to standard MS (3.5) which was found to be at par with many other treatment combinations of ammonium nitrate and growth regulators **(Table 1)**.

Most widely used MS media in plant tissue culture was largely based on empirical modifications of a few basic formulations however, optimum requirement of mineral nutrients vary between plant genotypes. Results revealed that elevated NH_4NO_3 significantly affected the morphological parameters of microplants in potato like height and number of leaves. However, increase in the nitrogen regime tends to produce taller plants having more number of nodes; longer roots and internodes; as well as fresh and dry weight upto the critical level (25.79 mM) but significantly at par with standard MS medium. Further increase in NH_4NO_3 concentration rather abolished the pattern. This may be due to the progressive reduction in nitrate reductase activity at high N concentration (Jang *et al.*, 2008).

Number of nodes

Medium containing NH_4NO_3 (25.79 mM) registered maximum number of nodes (4.6) however, it was found to be at par with standard MS medium (4.2) **(Fig. 1)**. Among different concentrations and combinations of growth regulators, medium supplemented with GA_3 (0.58 µM) + NAA (0.1 µM) registered significantly maximum number of nodes (5.0) as compared to standard MS medium (3.3) **(Fig. 2)**.

Inter-nodal length

Slightly longer inter-nodal length (1.16 cm) was recorded in the media supplemented with 30.94 mM NH_4NO_3 however, it was at par with standard MS medium (20.63 mM) as well as 25.79 mM NH_4NO_3. The higher concentration above 30.94 mM was toxic to inter-nodal length (**Fig. 1**). Nitrogen levels greatly influenced shoot morphology (Evans, 1993) and the development of potato stem sections during the micropropagation. In potato, Rai *et al*. (2002) reported similar results with respect to plant height and stem number. Addition of nitrogen to seagrasses generally causes an increase in leaf and/or shoot growth but may have no or negative effect on root

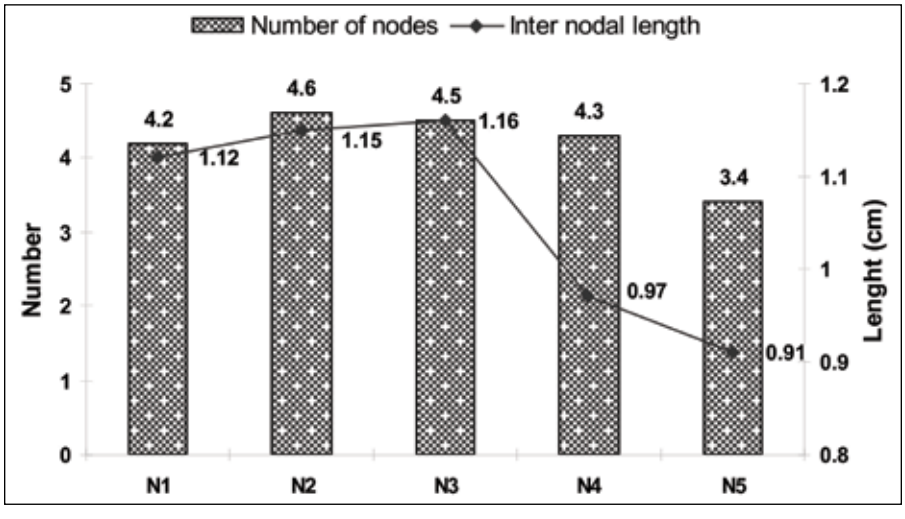

Fig. 1. Effect of different concentrations of ammonium nitrate on number of nodes and inter-nodal length of Kufri Jyoti microplants.

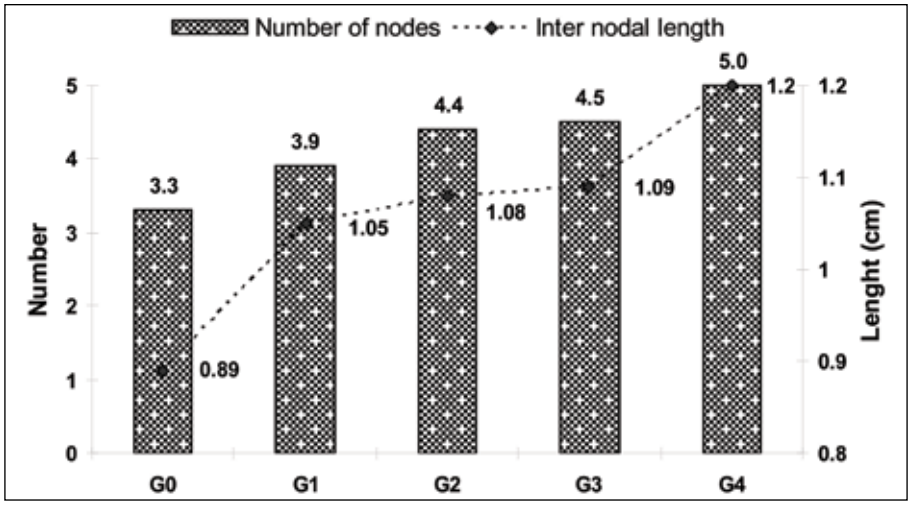

Fig. 2. Effect of different concentrations of GA_3 and NAA on number of nodes and inter-nodal length of Kufri Jyoti microplants.

production (Peralta *et al*., 2003). Both positive and negative effects of N application have also been reported by Belanger *et al*. (2002).

Among different concentration and combination of growth regulators, the medium supplemented with GA_3 (0.58 µM) + NAA (0.1 µM) registered significantly higher inter-nodal length (1.20 cm) as compared to standard MS medium (0.89 cm) and was found to be at par with all other concentration and combinations (**Fig. 2**).

Our results revealed that different concentrations of GA_3 and NAA in combination synergically influenced the morphological characters. Increase in the concentration of GA_3 and NAA tends to increase height, number of leaves, number of nodes, internodal and root length; fresh and dry weight of plantlets of potato. This may be due to application of high concentration of GA_3 which increases the hydrolysis of starch and sucrose into fructose and glucose (Khan and Chaudhry, 2006). Badoni and Chauhan (2009) obtained better results for shoot regeneration and multiplication of potato cv. Kufri Himalini on the media. supplemented with GA_3 and NAA. Increase in number of nodes of potato has also been observed by Miller *et al.* (1985), when higher concentration of GA_3 and NAA (1.0 mg/l) was supplemented with vitamins. Hassan *et al*. (1990) were of the opinion that to increase node production higher concentration of GA_3 should be supplemented with other phytohormones (like BAP and NAA) and vitamins. In addition to GA_3, NAA also plays a little role in shoot elongation

Number of roots and root length

Higher number of roots was observed in the microplantlets grown on the medium containing 30.94 mM NH_4NO_3 (4.8) which was however, found to be at par with standard MS medium (4.6). Among different concentrations and combinations of growth regulators, the medium supplemented with GA_3 (0.58 µM) + NAA (0.1 µM) registered maximum number of roots (6.0) as compared to standard MS medium (2.7). Maximum root length (8.92 cm) was registered on the medium containing 25.79 mM NH_4NO_3 although it was at par with standard MS medium (7.60 cm) as well as 30.97 mM NH_4NO_3 (8.00 cm).

The increase in morphological parameters upto 25.79 mM levels shows slightly higher nitrogen requirement of this particular cultivar under *in vitro* conditions than the

recommended concentration in the MS media (20.63 mM). The nitrogen source used can markedly influence growth and morphogenesis (Wilson and Bennett, 2008). Further support for these results comes from mesocosm experiments by Peralta *et al*. (2003) where *Zostera marina* grown without fertilizer additions had significantly longer roots with root hairs.

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). As regards the interaction, the media containing 30.94 mM NH_4NO_3 supplemented with GA_3 (0.58 µM) and NAA (0.1 µM) recorded significantly higher root length (9.69 cm) which was however, found to be at par with many other interactions **(Table 2)**.

In our study, we found slow growth of micro plantlets on the medium without growth regulators and the value of all the morphological characters was lower when compared with other combinations of growth regulators. Prolonged proliferation stage on the media without growth regulators have also been reported by Gopal *et al.* (1980), Aburkhes *et al*. (1984) and Rosell *et al.* (1987). However, higher concentration of NAA inhibit root and shoot growth (Pennazio and Vecchiati, 1976). Rapid multiplication can be obtained on the media supplemented with GA_3 (Roest and Bokelmann, 1976; Muller and Lipschutz, 1984). A very high concentration of GA_3 (4.5 mg/l) gave better results in *in vitro* grown potato plant (Rabbani *et al*., 2001).

Fresh weight and dry weight

The fresh and dry weight was maximum in media containing 30.94 mM NH_4NO_3 however, which was at par with standard MS medium. Among different concentrations and combinations of growth regulators, GA_3 (0.58 µM) + NAA (0.1 µM) significantly increased the fresh and dry weight as compared to standard MS medium and was at par with lower concentrations of GA_3 and NAA. In the interaction, the media containing 30.94 mM NH_4NO_3 supplemented with GA_3 (0.29 µM) + NAA (0.05 µM) 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 (**Table 3**).

It is concluded that to improve *in vitro* response of Kufri Jyoti, the ammonium nitrate concentration in the MS medium needs to

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)	Number 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*		

*Significant at ($p \leq 0.05$); **Significant at ($p \leq 0.01$).

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

Concentration of 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 ($p \leq 0.01$).

Fig. 3. Comparative response of Kufri Jyoti on standard MS and modified medium (Enhanced level of ammonium nitrate (25.79 mM) supplemented with GA_3 0.58 µM and NAA 0.1 µM).

be enhanced from 20.63 mM to 25.79 mM supplemented with growth regulators (GA_3 0.58 µM + NAA 0.1 µM) **(Fig. 3)**. However, its effect on *ex-vitro* conditions needs further investigation on mini-tuber production behaviour and phenotypic characters of plants.

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