



Effect of potassium and ammonium nitrate media on *in vitro* growth response of potato (*Solanum tuberosum* L.)

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Abstract

Different amount of KNO_3 and NH_4NO_3 in MS media were tested the morphogenic responses of shoot length, shoot fresh weight and multiplication rate of five potato cultivars. The growth response significantly varied with or without nitrate. The effect of NT1, NT2 and NT3 culture media on shoot growth was non-significant but their effect on shoot fresh weight was significant. Multiplication rate (a major attributing trait was not much affected either in low or high levels of nitrate media. High nitrate media yielded higher shoot fresh weight but not significantly improved shoot length and multiplication rate.

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Introduction

The investigation for a particular morphogenic response is of great relevance with the proper adjustment of media components. In general, MS (Murashige and Skoog, 1962) medium has been used for various *in vitro* growth purposes which were developed for tobacco pith callus. It was suggested that nutrient level in MS media sometimes higher than the required for optimal plant growth (Pierik, 1987). In most of the cases the prescribed MS media are used for various *in vitro* plant growth purposes but the actual elementary investigation is sometimes necessary for a particular developmental study. Nitrogen is one of the prime component in MS media and significantly influences the growth and morphogenesis of *in vitro* plant culture (Gamborg and Shyluk, 1970). With potato plantlets cultured *in vitro* Amirouche *et al.* (1985) showed that some cultivars grow at similar rates in Murashige and Skoog (1962) and Knop's media (Nozeram *et al.*, 1977). Similar observations have been made in wheat plants (Lewis and Chadwick, 1983) and in soybean cells (Gamborg and Shyluk, 1970). Different nitrogen sources (NO_3^- , NH_4^+ , glutamic acid and their combinations) influenced the growth and morphogenic responses (node number, shoot length, and stem, leaf and root dry weight) of three micropropagated potato cultivars (Spunta, Kennebec, Huinkul) were reported by Avila *et al.* (1994) and they found the influential role of nitrogen in the media as in agreement with Evans (1993).

The present investigation was carried out to determine the effect of key nitrate source (KNO_3 and NH_4NO_3) in MS basal media on micropropagation efficiency of five potato cultivars (Atlanta, Shepody, All Blue, Diamant and Shilbilaty).

Materials and methods

The nodal microcuttings of *in vitro* grown plantlets were prepared by single nodes and cultured in MS liquid media subjected to different levels of KNO_3 and

NH_4NO_3 (Table 1). The media contained 3% sucrose and the pH of the media were adjusted to 5.8 before autoclaving. The Experiment was repeated at least two times and each comprised four replicate flasks of ten plantlets of each cultivar arranged in a completely randomized design. The growth condition was maintained at $25 \pm 1^\circ\text{C}$ under 16/8 h d/n cycle with photon flux of $50 \text{ moles m}^{-2} \text{ s}^{-1} \text{ mol}$ for 3 weeks. Culture vessels were randomly placed every 1 week to ensure equal exposure to the light. After 3 weeks data were recorded on shoot length, shoot fresh weight and multiplication rate and were subjected to ANOVA and means were separated by Duncan's Multiple Range Test. The shoot multiplication rate was calculated by output of nodes divided by input of nodes as calculated by Hussey and Stacey (1981).

Table 1. Amount of nitrate contents used in MS culture media.

* Media	Nitrate content (mg/l)	
	KNO_3	NH_4NO_3
NT0	0	0
NT1	475	413
NT2	3800	3300
NT3 (Control)	1900	1650

*MS media having different levels of KNO_3 and NH_4NO_3 without changing other components.

Results and discussion

The amount of KNO_3 and NH_4NO_3 in MS media markedly affected the *in vitro* growth responses of potato cultivars especially with or without nitrate treatments. It was noticed that no significant differences were raised in shoot length with varied nitrate treatments but zero nitrate media differed significantly from the treated ones (Table 2). However, Shepody reached highest shoot length of 8.93 cm in NT1 (low nitrate) media followed by same cultivar at NT2 (high nitrate) media (Fig. A). It was observed that shoot fresh weight increased as nitrate content was increased in all varieties except Diamant (Fig.B).

Highest shoot fresh weight (104.25 mg) was obtained in Shilbilaty followed by Shepody (97.5 mg) at NT2 media. The shoot multiplication rate was highest (6-8 fold) in Shepody at NT2 media followed by same cultivar (5-7 fold) at NT1 media. The multiplication rate higher in high nitrate media is in agreement with the results obtained by Evans (1993) with different potato genotypes.

Table 2. Mean performances of nitrate media treatments on shoot length and shoot fresh weight.

Treatments	Shoot length (cm)	Shoot fresh weight (mg)
NT0	1.84b	17.20c
NT1	5.95a	55.40b
NT2	6.21a	85.15a
NT3 (Control)	5.84a	60.85b

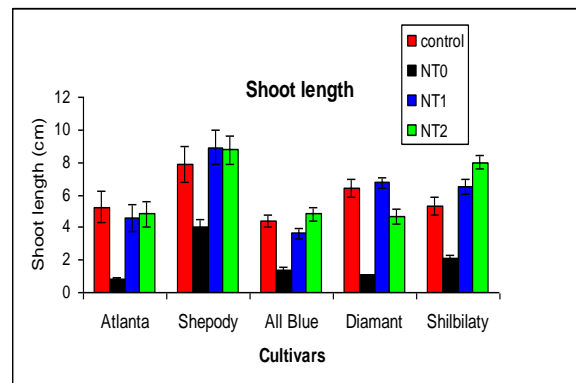
Means followed by the same letters are not significantly different ($P < 0.05$).

Table 3. Effect of nitrate treatments in MS media on multiplication rate after 3 weeks of culture.

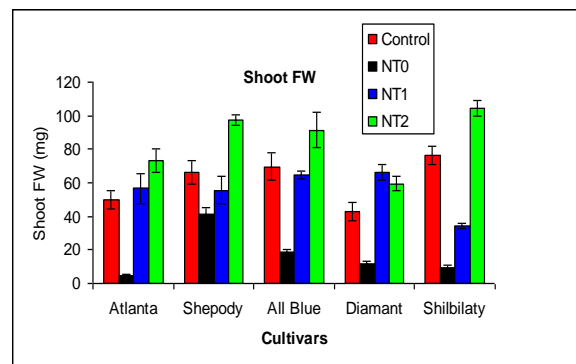
Treatments	Multiplication rate (fold)				
	Atlanta	Shepody	All Blue	Diamant	Shilbilaty
NT0	2-3	3-5	1-2	1-2	2-3
NT1	3-5	5-7	3-5	4-7	4-5
NT2	3-4	6-8	3-4	3-5	4-5
NT3 (Control)	3-6	3-5	2-3	4-6	3-5

The multiplication rate was also noted poor in zero nitrate media but the exception to this was Shepody where 3-5 fold multiplication was achieved (Table 3). It was also observed that internode length increased with the decreased of nitrate content and produced much reduced leaves (data not shown). Among the cultivars tested the best growth occurred in Shepody at all records. It was noted that low nitrate media (NT1) produced better shoot length in Shepody and Diamant whereas higher nitrate media (NT2) resulted maximum shoot fresh weight (Figs. A-B). The zero

nitrate media gave poor performances at all parameters and cultivars and the control responded moderately. This experiment with NT1, NT2 and NT3 media were comparable with respect to growth traits and demonstrated that the micropropagation efficiency did not much improve when the nitrate increased (from NT1 to NT3) in the medium. The results is in contrast with Pierik (1987) and Pennell (1987) who suggested that MS medium contained higher levels of salts, particularly nitrogen which were too high for optimal growth.



A



B

Fig. (A-B). Shoot length and shoot fresh weight obtained with four different nitrate media for five cultivars. Values are averages of four replicates. Bars at the top of each column represent standard errors.

The results suggest that it would be more cost-effective to use low level of nitrate in the media and the experiment may give a potential idea to find out the low nitrate salt potato micro-propagation methods

effective for some commercial cultivars. The research needs further refinement and exploration.

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