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Effect of Nitric Oxide on Root and Shoot Development of *Citrus medica* Seedlings

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ABSTRACT

Vegetative propagation is most preferred method for propagation of citrus plants. *Citrus medica* seedlings are widely used as rootstocks. Better growth performance of a root system is important in a rootstock plant, to produce a better budded or grafted plant later. It is important to investigate how to improve the growth of root system while enhancing the other growth performance of citrus seedlings. In this study, effect of sodium nitroprusside (SNP) as a nitric oxide (NO) donor on root and shoot growth characteristics of *Citrus medica* seedlings planted in poly bag under net house condition was investigated two weeks, four weeks, and six weeks after applying chemical treatment. Four treatments of 25, 50 and 75 µM concentrations of SNP and control without SNP were applied on seedlings one week after transplanting in poly bag. Growth parameters of seedling height, shoot fresh weight, number of lateral roots, length of tap root, root dry weight, and number of leaves were observed at two weeks, four weeks, and six weeks after applying chemical treatments. The application of SNP greatly induced the root architecture and shoot characteristics of *Citrus medica* seedlings. Sodium nitroprusside concentration of 75 µM recorded the highest growth performances.

KEYWORDS: Growth, Nitric oxide, Roots, Shoots, Sodium nitroprusside

INTRODUCTION

Citrus fruits constitute several species of the genus citrus belongs to sub family Aurantiodeae and family Rutaceae. This family mainly comprises of sweet orange, sour orange, pummelo, mandarin, grape fruit, lime, lemon, and other orange varieties (Krueger and Robert, 2003). *Citrus medica* is commonly named as citron. It is mainly used in Ayurveda medicine and as rootstock which helps vegetative propagation of many plants belong to its own family, especially sweet orange. Citrus trees are propagated both by vegetative means and from seeds. The trees that produce by seedlings are not similar to parents in quality. Therefore, vegetative propagation is preferred because it ensures uniform quality, regular and early bearing.

Rootstock is the plant part which donates the root system for budded or grafted plant. Root system of rootstock plant plays a major role in the growth performance of a budded or grafted plant. Rootstock may be selected for traits such as resistance to drought, root pests and diseases, tree vigor, rooting depth, tolerance to water logging conditions, and adaptability to various soil and climatic conditions.

Roots are the life line of a plant which required for the acquisition of water and nutrients for responses to abiotic and biotic signals in the soil, and to anchor the plant in the ground and the storage organ in some species (Nibau *et al.*, 2008). The length of the primary root and density of lateral roots determine the architecture of the root system (Malamy and Benfy, 1997). Controlling plant root architecture is a fundamental part of plant development and evolution enabling a plant to respond to changing environmental conditions and allowing plants to survive in different ecological niches (Nibau *et al.*, 2008). In root architecture, lateral root formation and development is the major process in plant.

Nitric oxide (NO) is a bio active molecule that functions in numerous physiological processes in plant which can play a significant role in broad spectrum of plant development process that determine root architecture (Lanteri *et al.*, 2006). Nitric oxide promotes lateral root development, auxine depended process (Correa-Aragunde *et al.*, 2004; Lanteri *et al.*, 2006), adventitious root formation (Pagnussat *et al.*, 2002) as well as stimulated shoot development (Tan *et al.*, 2013) required for root organogenesis (Panngussat *et al.*, 2002), increased growth (Shao-hu *et al.*, 2007), and root hair formation (Lombardo *et al.*, 2006). Low concentration of Sodium Nitroprusside (SNP) promoted both the amounts and the elongation of lateral root but inhibited the elongation of lateral root and primary root at higher concentration (Geo and Yang, 2011).

Sodium nitroprusside (SNP) is widely used as NO donor which act as a signal molecule in plants responsible for the expression regulation of many antioxidant enzymes, due to its continuous long lasting NO production compared with others (Floryszak-Wieczorek *et al.*, 2006), relatively low cost and well-documented application (Zandonadi *et al.*,

2010) and also its high efficiency to release NO in plant cell (Kumar *et al.*, 2010).

Samaradiwakara and Jayasekera (2015) have reported, 50 µM SNP is a suitable level for *Citrus medica* seedlings and it could give higher root and shoot characteristics.

In this study, NO was supplied externally to the plants and the rooting behavior was checked under four different concentration levels. The objective of the present study was to investigate the optimum level of NO concentration on best performance of root and shoot characteristics of *Citrus medica* seedlings as rootstocks.

MATERIALS AND METHODS

Experimental Site

This study was carried out in a net house at the Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Makandura. Experimental site was situated in the low country intermediate zone (IL_{1a}), Experiment was carried out from February to May 2016.

Layout of Experiment

Two hundred and eighty eight (288) *Citrus medica* seedlings were used for this experiment. There were four treatments and four replicates. Treatments were arranged in randomized complete block design (RCBD). Each treatment had 18 plants per replicate planted in black poly bags.

Growing of Citrus medica Seedlings

The seeds of *Citrus medica* collected from a farmer in Alawwa area. Well ripened fruits were selected and seeds were carefully extracted. The mucigeneous layer around the seeds was washed and seed coat was removed. Seeds were sown in a germination bed. The seeds were allowed to germinate and continuous watering was done twice a day. After one month, uniformly germinated seedlings were selected and transplanted at the rate of one seedling per poly bag (black, 20×45 cm) filled with top soil, compost and sand on 1:1:1 ratio. Watering was done regularly when needed.

Treatment of SNP

Four different concentrations of sodium nitroprusside dihydrate; (Analar NORMAPUR), 25, 50, and 75 µM, were compared with the control devoid of SNP. Above concentration solutions were prepared in laboratory. The treatments were applied one week after transplanting at the rate of 50 mL per seedling twice a day continuously for five days. The control was provided with same amount of distilled water without SNP.

Measurements of Growth Parameters

Characters of growth and root architecture of seedlings were measured in three stages, two weeks, four weeks and six weeks after application of treatments. For each stage four plants were selected randomly from each treatment for the purpose of data recording.

Seedling Height (cm)

Height of the seedling was measured from base of the stem to the tip of the stem.

Number of Leaves

The number of the leaves in the seedling was counted.

Shoot Fresh Weight (g)

Fresh weight of the aerial part of the plants was measured.

Number of Lateral Roots

The number of lateral roots on tap root was counted.

Length of Taproot (cm)

The length of tap root was recorded from end of the root tip up to the base of the stem.

Root Dry Weight (g)

Root dry weight was measured after oven drying the roots at 105 °C for 24 hours.

Statistical Analysis

Statistical analysis was done by using SAS software (version 9.2) package.

RESULTS AND DISCUSSION

Growth Parameters

Application of SNP significantly increased the root and shoot characteristics of *Citrus medica* seedlings over the control, after two weeks, four weeks, and six weeks. Seventy five micro molar concentration of SNP was more effective than other concentrations and maximum growth parameters were recorded.

In two week old *citrus* seedlings, highest amount of root dry weight, number of lateral roots were recorded in 75 µM concentration of SNP. Control, 25 and 50 µM concentration of SNP did not show any significant difference for root dry weight, and number of lateral roots (Table 1). Concentration 75 µM recorded the highest value while control recorded the lowest value which was not significantly different for seedling height, shoot fresh weight, and length of tap root (Table 1).

All the concentrations of SNP have significantly increased the shoot and root characteristics of four week old citrus seedlings over the control, and 75 µM concentration recorded higher values for all the growth parameters measured (Table 2). In four week old seedlings, shoot fresh weight, length of tap root, root dry weight, number of lateral roots were highest in 75 µM concentration of SNP, while 25, 50, and 75 µM concentrations of SNP recorded the maximum seedling height (Table 2). There was no significant difference among three treatments of control, 25, and 50 µM concentrations of SNP for length of tap root. The concentration of SNP 25 µM and control did not show significant difference for number of lateral roots (Table 2).

In six week old seedlings, 75 µM concentration of SNP recorded the highest values and control has recorded the lowest values for all growth parameters. It was evident that treatments showed growth increment with the increase in concentration of the treatments. There was no significant difference among four treatments for length of tap root. Control, 25 and 50 µM concentration of SNP did not show significant difference for seedling height and shoot fresh weight (Table 3).

When considering number of leaves after transplanting, 75 µM concentration of SNP recorded the highest value while control recorded the lowest value which were significantly different (Table 4).

Table 4. Mean number of leaves after transplanting

Treatment	Number of leaves
T_1	5.27
T_2	5.94
T_3	5.88
T_4	6.52

(T_1- control, T_2- 25 µM, T_3- 50 µM, T_4- 75 µM)

Sodium nitroprusside was used as NO donor, SNP treated seedlings have growth increment of all shoot and root characteristics compared to the control (Figures 1, 2, 3, 4 and 5).

When considering root architecture, higher number of lateral roots, root dry weight and root elongation were recorded and that would ensure plant has established well in the soil. Higher number of leaves effected to influence the photosynthesis in treated seedlings than control and it helped in plant development. Sodium nitroprusside treated seedlings recorded rapid growth, thus contributing to a better rootstock when it is used for budded or grafted plant.

Table 1. Mean growth parameters of seedlings after two weeks

Treatment	Seedling height (cm)	Shoot fresh weight (g)	Length of tap root (cm)	Root dry weight (g)	Number of lateral roots
T_1	8.34	0.1290	13.01	0.0143^b	20.88^b
T_2	8.45	0.1300	13.28	0.0153^b	21.31^b
T_3	8.04	0.1248	12.89	0.0164^b	21.44^b
T_4	9.36	0.1474	15.48	0.0233^a	27.19^a
CV	*16.61*	*20.89*	*21.57*	*33.15*	*30.26*

T_1- Control, T_2- 25 µM, T_3- 50 µM, T_4- 75 µM. Means followed by same letter in each column are not significantly different at 0.05 level

Table 2. Mean growth parameters of seedlings after four weeks

Treatment	Seedling height (cm)	Shoot fresh weight (g)	Length of tap root (cm)	Root dry weight (g)	Number of lateral roots
T_1	8.99^b	0.210^c	19.28^b	0.0265^c	29.0^c
T_2	10.23^a	0.3071^{ab}	19.60^b	0.0362^{ab}	31.81^{bc}
T_3	10.23^a	0.2896^b	20.38^b	0.0355^b	34.63^b
T_4	10.40^a	0.3224^a	22.33^a	0.0418^a	45.44^a
CV	*10.62*	*10.16*	*13.31*	*22.41*	*18.77*

T_1- Control, T_2- 25 µM, T_3- 50 µM, T_4- 75 µM. Means followed by same letter in each column are not significantly different at 0.05 level

Table 3. Mean growth parameters of seedlings after six weeks

Treatment	Seedling height (cm)	Shoot fresh weight (g)	Length of tap root (cm)	Root dry weight (g)	Number of lateral roots
T_1	9.31^b	0.4159^b	20.20	0.0265^c	36.19^b
T_2	10.55^b	0.5277^b	19.92	0.0398^b	43.94^{ab}
T_3	9.81^b	0.5095^b	20.74	0.0329^{bc}	37.69^b
T_4	13.01^a	0.7662^a	22.76	0.0523^a	51.94^a
CV	*18.81*	*30.77*	*19.26*	*37.30*	*28.64*

T_1- Control, T_2- 25 µM, T_3- 50 µM, T_4- 75 µM. Means followed by same letter in each column are not significantly different at 0.05 level

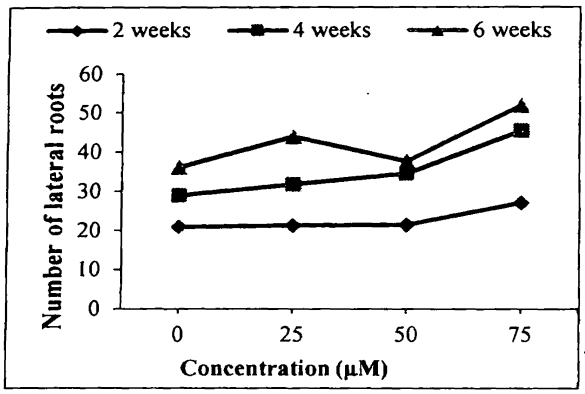

Figure 1. Number of lateral roots recorded for four treatments during three stages

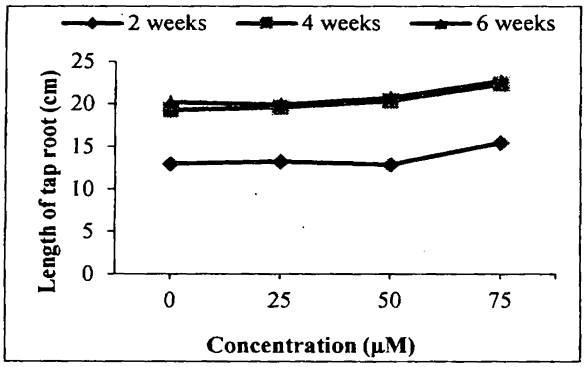

Figure 2. Length of tap roots recorded for four treatments during three stages

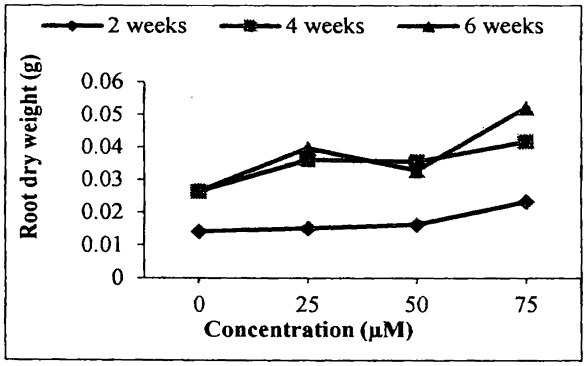

Figure 3. Root dry weight recorded for four treatments during three stages

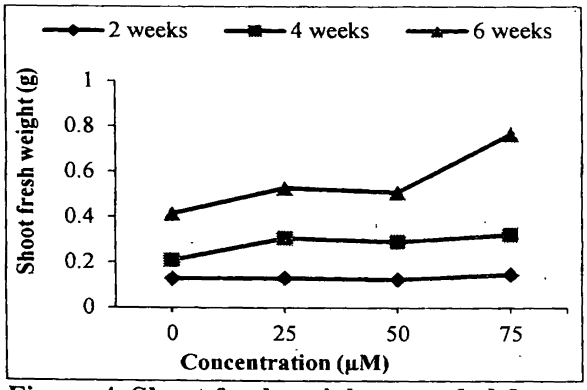

Figure 4. Shoot fresh weight recorded for four treatments during three stages

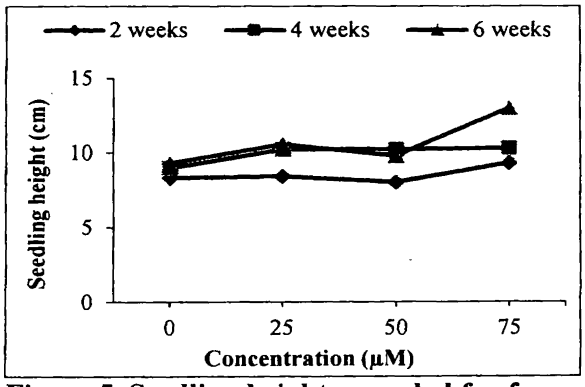

Figure 5. Seedling height recorded for four treatments during three stages

CONCLUSIONS

Sodium nitroprusside treatments have improved the root and shoot characteristics of the *Citrus medica* seedlings compared to control. Higher mean values of all growth parameters were recorded in 75 µM concentration level during three stages. But some growth parameters did not show significant differences between control and the treatments. Finally, 75 µM concentration of SNP observed the highest growth performance and can be concluded that it is the best concentration level for improved growth of *Citrus medica* seedlings for a better rootstock plant.

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