EFFECT OF DIFFERENT DOSES OF GA_3 AND NITROGEN ON SEEDLING GROWTH OF TRIFOLIATE ORANGE [*Poncirus Trifoliata* (L.) Raf.] UNDER OPEN AND PLASTIC TUNNEL CONDITION

B. Chalise*, R.L. Shrestha*, K.P. Paudyal** and H.P. Subedi*
*National Citrus Research Program, Paripatle, Dhankuta
**Horticulture Research Division, Khumaltar, Lalitpur

ABSTRACT

A field experiment was conducted at National Citrus Research Program, Paripatle, Dhankuta during 1st November, 2004 to 1st September, 2005 to determine the effect of different doses of GA_3 and nitrogen under open and plastic tunnel condition on growth of trifoliate orange seedling. The experiment was laid out in two factorial split-plot design consisting three replications of each treatment. The open and plastic tunnel was considered as the main plot factor (Factor-A) and the combination of different doses of gibberellic acid (GA_3) and nitrogen as sub plot factor (Factor-B). The treatments were applied in seedlings two months after germination. Seedlings were planted at 15×10 cm spacing in 90 cm×80 cm sized experimental plots supplied initially with 5 kg FYM per plot containing 48 plants. White transparent plastic sheets were used for covering the plant in the form of tunnel. GA_3 was applied as foliar spray at one month intervals while nitrogen was applied through urea as soil application at two split doses in 1st April and 1st May. Ten sample plants were selected for the study from each bed. From the study, it was found that the growth of seedling of trifoliate orange was highly accelerated under the plastic house condition as compared to the open condition. The height of seedling (47.75 cm), number of leaf per plant (25.27) and size of seedling diameter (5.193 mm) were found highest at 300 days after sowing under plastic tunnel condition. Similarly, the highest growth of seedling height (48.81 cm), number of leaf per plant (27.20) and seedling diameter (5.383 mm) were observed in 120 ppm GA_3 and 400 kg nitrogen/ha combination at similar age of the seedling.

Key words: *Poncirus trifoliata*, Plastic tunnel, GA_3, nitrogen and accelerated growth

INTRODUCTION

Citrus is the most important fruit crop of mid-hill region of Nepal. APP (1995) has identified citrus as the number one priority crop for mid-hill region. Citrus is commercially cultivated in 42 mid-hill districts (Regmi *et al.*, 2009). Mandarins, sweet oranges, acid limes and lemons are the major species of citrus cultivated in the country. Acid limes are being cultivated successfully in the Terai region of Nepal. There are numbers of problems in the citriculture of Nepal. Decline of the citrus orchard is the major problem faced by the citrus growers from east to far-west of the country. The reasons for citrus orchard decline includes huanglongbing disease, citrus tristeza virus, phytophthora root rot, powdery mildew, gummosis, citrus nematodes scale insects, lack of irrigation soil nutritional factors *etc* (NCRP, 2005). Some of the factors associated with citrus decline due to some diseases and pests can be avoided using disease and pest resistance rootstocks. Trifoliate orange is the most commonly and widely used rootstock for citrus in Nepal, because it is compatible with all species. The different cultivars of trifoliate orange exhibit a great cold hardiness (up to -15°C). This cold hardiness is partially conferred to the scion.

It remarkably improves the cold resistance of canopy. Therefore, this rootstock is recommended for areas with cold winters. Trifoliate orange rootstock also improves the fruit quality especially by increasing the amount of soluble sugar content (minimal gain of 1°Brix). Trifoliate rootstock induces a compact canopy adapted to higher density planting and secures good returns. It develops a vigorous root system, pivotal and lateral, thus less sensitive to transplantation shocks and damping off than others. This rootstock is tolerant of *Phytophthora* gummosis, cachexia-xyloporosis and nematodes especially the *Tylenchulus semipenetrans*. It is resistant to the citrus tristeza virus disease also (Aubert and Vullin, 1998). After visiting Nepal, Roistacher (1997) reported that trifoliate orange is the primary rootstock that would suit very well for many areas of the country and do best with heavier soil of Nepal. Though it has several desirable qualities, the production of seedling takes longer time increasing the cost of production of sapling in nursery. Therefore, this study was conducted to develop the technology of trifoliate orange seedling rootstock production suitable for grafting scion within a year.

MATERIALS AND METHODS

The study was conducted at National Citrus Research Program, Paripatle, and Dhankuta during 1st November, 2004 to 1st September, 2005. The seeds were sown at the spacing 5×7 cm spacing in well prepared seedbed supplied initially with 5 kg FYM per plot in 1st November, 2004. After sowing, seeds were covered with white transparent plastic sheet. The irrigation was given at the interval of seven days. The germination of seed was completed at about three months after sowing and the treatments were applied in seedling at 2 months after the germination. The spacing was maintained by uprooting and planting the seedling at the same bed. The seedlings were planted at 15×10 cm spacing in 90cm × 80 cm sized experimental plots. The experiment was laid out in two factorial split plot design consisting three replications of each treatment. Each experimental unit contained 48 plants. The spacing between replications and plots was 50 cm and 25 cm respectively. The open and plastic tunnel was considered as the main plot factor (Factor-A) and the combination of different doses of GA_3 and nitrogen as sub plot factor (Factor-B). White transparent plastic sheets were used for covering the plant in the form of tunnel. The treatments were allotted randomly in the experimental plots. GA_3 was applied as foliar spray at one month intervals while nitrogen was applied through urea as soil application at two split doses one in 1st April and the next in 1st May. Ten sample plants were selected randomly for the study from each plot. The recorded data were summarized, arranged in MS-Excel and analyzed by MSTAT-C package. The means were separated by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Growth of seedling height

The effect of growing condition *i.e.* open and plastic condition was found highly significant at 180 and 240 days after sowing of the seed and found significant at 300 days after sowing. At 180 days after sowing highest seedling height (25.48 cm) was recorded in seedling grown under plastic tunnel. Similarly, at 240 and 300 days after sowing plastic tunnel grown seedling exhibited 44.34 cm and 47.75 cm seedling height (Table 1).

The effect of GA_3 and nitrogen was found highly significant from 180 days to 300 days after sowing. Among the different combination of GA_3 and nitrogen, the highest growth (25.05 cm) was recorded in 120 ppm GA_3 + 400 kg N/ha at 180 days after sowing, 120 ppm GA_3 + 300 Kg N/ha at 240 days after sowing and again 120 ppm GA_3 + 400 kg N/ha at 300 days after sowing (Table 1).

Among the interaction of growing condition, GA_3 and nitrogen combination, the highest growth of seedling height was observed in 80 ppm GA_3 + 200 kg nitrogen at 180 days after sowing, while at 240 and 300 days after sowing 120 ppm GA_3 + 400 kg N/ha gave the maximum seedling height under plastic tunnel

Table 1. Effect of different doses of GA_3 and nitrogen under open and plastic tunnel condition on growth of trifoliate orange seedling height at NCRP, Paripatle, Dhankuta, 2005

Treatments	Seedling height (cm)		
	180 DAS	240 DAS	300 DAS
Growing conditions			
Open	19.62^{b}	34.06^{b}	41.84^{b}
Plastic tunnel	25.48^{a}	44.34^{a}	47.75^{a}
CV (%)	8.45	4.04	6.57
F value	141.7807**	631.3602**	60.3840*
CD ($P \leq 0.05$)	2.119	1.761	3.272
SEm±	0.3482	0.2894	0.5378
Doses of GA_3 and nitrogen			
0 ppm GA_3+200 Kg N/ha	23.83^{bc}	40.05^{cd}	43.27^{bc}
0 ppm GA_3+300 Kg N/ha	21.67^{d}	38.58^{de}	45.20^{b}
0 ppm GA_3+400 Kg N/ha	20.38^{ef}	36.21^{ef}	40.20^{d}
80 ppm GA_3+200 Kg N/ha	23.15^{c}	36.14^{ef}	44.83^{bc}
80 ppm GA_3+300 Kg N/ha	21.75^{d}	35.46^{f}	42.35^{cd}
80 ppm GA_3+400 Kg N/ha	24.38^{ab}	39.72^{cd}	45.43^{b}
120 ppm GA_3+200 Kg N/ha	21.13^{de}	41.53^{bc}	44.72^{bc}
120 ppm GA_3+300 Kg N/ha	24.08^{bc}	45.28^{a}	48.29^{a}
120 ppm GA_3+400 Kg N/ha	25.05^{a}	42.98^{ab}	48.81^{a}
Control	20.08^{f}	36.07^{ef}	44.88^{bc}
CV (%)	3.46	5.30	4.39
F value	30.8188**	15.4984**	10.0415**
CD ($P \leq 0.05$)	0.9138	2.431	2.305
SEm±	0.3186	0.8476	0.8035

SEm± = Standard error of mean difference, CV = Coefficient of variation, CD ($P \leq 0.05$) = Critical difference at probability value 0.05, Treatment means followed by common letter(s) within a column are not significantly different at 5% by DMRT, DAS=Days after sowing

The physiological activities of a living being at higher temperature are also higher (Hartmann *et al.*, 2007). As compared to the open condition, the temperature inside the tunnel becomes almost 1.5 to double (Chalise, 2010) which accelerated the photosynthesis process resulting into the fast growth of the seedling. The diffused light from the transparent plastic may have higher photosynthetic efficiency. Another cause of faster growth may be due to the effect of endogenous hormone on the growth of plant. At higher temperature the efficiency of plant hormones also increased which caused elongated internodes of trifoliate seedling. The more growth of height may be due to the apical dominance resulted by the auxins whose activities are again higher at higher temperature. Nitrogen is the primary nutrient for the plant which is required for the production of many cellular compounds. This is the important element of the chloroplast. Higher nitrogen results more green leaves and there would be more photosynthesis in the leaves. Higher food production and utilization accelerates the growth of the plant. Similarly, at lower level of endogenous hormone, external application of it can enhance the growth. The externally applied GA_3 showed higher growth of the trifoliate orange seedling.

Number of leaves per plant

The effect of growing condition on number of leaves per plant was found significant at 180 days after sowing, highly significant at 240 days after sowing and significant at 300 days after sowing. At 180 days after sowing plant grown under plastic tunnel gave 17.54 leaves per plant, 23.07 at 240 days after sowing and 25.27 leaves at 300 days after sowing (Table 2).

The effect of GA_3 and nitrogen on leaf number of trifoliate orange seedling was found highly significant at 180, 240 and 300 days after sowing. At 180 days, the highest number of leaves was produced by 120 ppm GA_3 + 300 kg N/ha which was statistically at par with 120 ppm GA_3 + 400 kg N/ha, at 240 days after sowing highest number of leaf (23.68) was produced by 120 ppm GA_3 + 300 kg N/ha and was at par statistically with 120 ppm GA_3 + 400 kg N/ha. Similarly, at 300 days after sowing, highest number of leaves (27.20) was given by 120 ppm GA_3 + 400 kg N/ha, this was statistically at par with control (Table 2).

Table 2. Effect of different doses of GA_3 and nitrogen under open and plastic tunnel condition on trifoliate orange leaf number at NCRP, Paripatle, Dhankuta, 2005

Treatments	Number of leaf per plant		
	180 DAS	240 DAS	300 DAS
Growing condition			
Open	15.94^{b}	19.10^{b}	23.16^{b}
Plastic tunnel	17.54^{a}	23.07^{a}	25.27^{a}
CV (%)	7.88	4.00	4.06
F value	21.9687*	332.5863**	69.2394*
CD (P≤0.05)	1.466	0.9381	1.091
SEm±	0.2409	0.1542	0.1794
Doses of GA_3 and nitrogen			
0 ppm GA_3+200 Kg N/ha	15.12^{e}	20.62^{bc}	24.55^{c}
0 ppm GA_3+300 Kg N/ha	15.93^{de}	20.12^{cd}	23.63^{cd}
0 ppm GA_3+400 Kg N/ha	15.50^{e}	19.43^{d}	21.93^{e}
80 ppm GA_3+200 Kg N/ha	16.55^{cd}	20.23^{bcd}	22.08^{e}
80 ppm GA_3+300 Kg N/ha	16.12^{de}	20.40^{bcd}	22.78^{de}
80 ppm GA_3+400 Kg N/ha	17.45^{bc}	21.32^{b}	23.72^{cd}
120 ppm GA_3+200 Kg N/ha	16.72^{cd}	21.23^{bc}	24.27^{c}
120 ppm GA_3+300 Kg N/ha	18.60^{a}	23.68^{a}	25.90^{b}
120 ppm GA_3+400 Kg N/ha	18.17^{ab}	23.15^{a}	27.20^{a}
Control	17.27^{bc}	20.68^{bc}	26.12^{ab}
CV (%)	4.92	4.17	4.34
F value	11.3226**	14.0233**	16.8019**
CD (P≤0.05)	0.9649	1.030	1.231
SEm±	0.3364	0.3592	0.4293

SEm± = Standard error of mean difference, CV = Coefficient of variation, CD (P≤0.05) = Critical difference at probability value 0.05, Treatment means followed by common letter(s) within a column are not significantly different at 5% by DMRT, DAS=Days after sowing

The interaction of growing condition and different combination of GA_3 and nitrogen was found statistically highly significant at 180 days to 300 days after sowing for number of leaf per plant. At 180 days after sowing, highest leaf number (19.70) per plant was recorded in 120 ppm GA_3 + 300 kg N/ha; at 240 days after sowing highest leaf number (26.57) was recorded in 120 ppm GA_3 + 400 kg N/ha; at 300 days highest leaf number (29.20) was observed in 120 ppm GA_3 + 400 kg N/ha under plastic tunnel.

The number of leaves per plant was found somewhat similar in most of the treatments. Higher the internodal distance lower will be the number of leaves. Therefore, control produced 26.12 leaves which is statistically at par with 120 ppm GA_3 + 400 kg N/ha which produced 27.20 leaves per plant.

Collar diameter of seedling

The effect of open and plastic tunnel condition of growing on size of trifoliate orange seedling diameter was found significant at 180 days and highly significant at 240 and 300 days after sowing. At 180 days after sowing, the plants grown under plastic tunnel exhibit greater diameter (2.737 mm) as compared to open condition. At 240 days after sowing seedling grown under plastic tunnel gave 4.003 mm diameter and again at 300 days after sowing plants under the tunnel produced 5.193 mm collar diameter (Table 3).

Table 3. Effect of different doses of GA_3 and nitrogen under open and plastic tunnel condition on trifoliate orange diameter growth at NCRP, Paripatle, Dhankuta, 2005

Treatments	Seedling diameter (mm)		
	180 DAS	240 DAS	300 DAS
Growing condition			
Open	2.580^{b}	3.517^{b}	4.643^{b}
Plastic tunnel	2.737^{a}	4.003^{a}	5.193^{a}
CV (%)	2.88	3.94	4.16
F value	59.7027*	160.2709**	108.0355**
CD (P≤0.05)	0.08605	0.1648	0.2277
SEm±	0.01414	0.02708	0.03742
Doses of GA_3 and nitrogen			
0 ppm GA_3+200 Kg N/ha	2.650^{bc}	3.967^{a}	5.000^{c}
0 ppm GA_3+300 Kg N/ha	2.583^{bc}	3.850^{abc}	5.217^{b}
0 ppm GA_3+400 Kg N/ha	2.583^{bc}	3.583^{d}	4.750^{de}
80 ppm GA_3+200 Kg N/ha	2.700^{ab}	3.717^{bcd}	4.533^{f}
80 ppm GA_3+300 Kg N/ha	2.533^{c}	3.650^{cd}	4.733^{e}
80 ppm GA_3+400 Kg N/ha	2.550^{c}	3.683^{bcd}	4.700^{e}
120 ppm GA_3+200 Kg N/ha	2.650^{bc}	3.750^{bcd}	4.917^{c}
120 ppm GA_3+300 Kg N/ha	2.817^{a}	3.867^{ab}	5.050^{c}
120 ppm GA_3+400 Kg N/ha	2.800^{a}	3.867^{ab}	5.383^{a}
Control	2.717^{ab}	3.667^{bcd}	4.900^{cd}
CV (%)	3.93	4.10	2.73
F value	5.4507**	3.7414**	21.6896**
CD (P≤0.05)	0.1228	0.1814	0.1571
SEm±	0.04282	0.06325	0.05477

SEm± = Standard error of mean difference, CV = Coefficient of variation, CD (P≤0.05) = Critical difference at probability value 0.05, Treatment means followed by common letter(s) within a column are not significantly different at 5% by DMRT, DAS=Days after sowing

The effect combination of different doses of GA_3 and nitrogen on size of seedling diameter was found highly significant at 180, 240 and 300 days after sowing. At 180 days, the highest seedling diameter (2.817 mm) was produced by 120 ppm GA_3 + 300 kg N/ha. At 240 days after sowing, maximum size of diameter (3.967 mm) was given by 0 ppm GA_3 + 200 kg N/ha. Similarly, at 300 days after sowing, highest size of seedling diameter (5.383 mm) was given by 120 ppm GA_3 + 400 kg N/ha (Table 3).

The interaction of growing condition and different combination of GA_3 and nitrogen was found statistically highly significant at 180 days to 300 days after sowing for seedling diameter. At 180 days after sowing, highest seedling diameter (2.933 mm) was recorded in 120 ppm GA_3 + 300 kg N/ha; at 240 days after sowing highest size of diameter of seedling (4.33 mm) was recorded in 0 ppm GA_3 + 200 kg N/ha; at 300 days highest diameter size (5.60 mm) was observed in 120 ppm GA_3 + 400 kg N/ha under plastic tunnel condition. Higher growth of trifoliate seedling diameter under plastic tunnel condition may be due to the higher food accumulation resulted by the higher photosynthetic rate under plastic tunnel. Diffused light through the transparent white plastic cover may have the higher photosynthetic efficiency. At higher temperature, the nutrient uptake efficiency of the root may be higher. The efficiency of the leaf in producing photosynthate may be aided further by the higher doses of GA and nitrogen.

CONCLUSION

The trifoliate orange is the most widely and popularly used seedling as rootstock for citrus propagation. It has several desirable qualities like cold resistance, resistant to *Phytophthora* gummosis, resistant to the citrus nematodes and to some extent tolerant to the citrus tristeza virus. Though, the production of the seedling is very difficult and time taking and costly. The growth of trifoliate orange is very sluggish in open field condition and difficult to achieve the graftable size within a year after sowing of seed. From the study it was found that trifoliate orange growth could be accelerated by sowing the seed in November and covering them with transparent plastic sheet until germination and then making transparent plastic tunnel over the bed and applying 120 ppm GA_3 + 400 kg N/ha to the plant. The height of seedling (47.75 cm), number of leaf per plant (25.27) and size of seedling diameter (5.193 mm) was found highest at 300 days after sowing under plastic tunnel condition. Similarly, the highest growth of seedling height (48.81 cm), number of leaf per plant (27.20) and seedling diameter (5.383 mm) was observed at similar age of seedling in 120 ppm GA_3 + 400 kg nitrogen/ha combination.

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REFERENCES

APP. 1995. Nepal agriculture perspective plan, final report. Agricultural Projects Services Center, Kathmandu, Nepal and John Mellor Associates, Inc., Washington DC, USA. 47p.

Aubert, B. and G. Vullin. 1998. Citrus nurseries and planting techniques. GTZ and CIRAD. Montellier Cedex 1, France. 183p.

Chalise, B. 2010. Effect of grafting dates and methods on success and growth of mandarin (*Citrus reticulata* Blanco) sapling. M.Sc. Thesis. Tribhuvan University, IAAS, Rampur, Chitwan, Nepal. 133p.

Hartmann, H. T., D. E. Kester, F. T. Davies, Jr. and R. L. Geneve. 2007. Plant Propagation Principles and Practices. 7th ed. Prentice Hall Pvt. Ltd., New Delhi, India. 880 p.

MOAC. 2009. Statistical information on Nepalese agriculture 2008/2009. Ministry of Agriculture and Co-operatives, Agri-Business Promotion and Statistics Division. Singha Durbar, Kathmandu, Nepal. 153p.

NCRP. 2005. Annual report 2004/2005. National Citrus Research Program, Paripatle, Dhankuta Nepal. 40 p.

Regmi, C., I. P. Kafle, K. P. Paudyal, R. P. Devkota, G. Aryal and G. Awasthi. 2009. Screen house system to produce quality planting materials of Citrus in Banepa. *In*: Proceedings of the 5th National Seminar on Horticulture, Kathmandu, Nepal, June 9-10, 2008. pp. 89-92.