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### Authors

Regmi, C.  
Shrestha, S.

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# Modification of Shoot Tip Grafting Technique for Increasing Efficiency of Successful Grafts

C. Regmi and S. Shrestha

**ABSTRACT.** The shoot tip grafting (STG) technique has been applied in different countries for supplying disease-free citrus. The objectives of these studies were to modify the existing techniques to obtain more successful grafts. The combination of three conditions: a) exposure of seedlings to room light conditions, 12 hr daily for 3-9 days prior to grafting, b) benzyl aminopurine (BAP) treatment of the grafts at 0.5 mg/liter Murashige and Skoog (MS) medium for shoot development of the scion and c) transfer of successful grafts to the control MS medium (without any growth hormone) for 10-15 days prior to transferring to soil, have increased the number of successful grafts of one mandarin and one sweet orange variety grafted to rough lemon rootstock.

The climatic conditions in Nepal are quite favorable for citriculture. Citrus is grown in over 13,000 ha with an annual production of 78,639 metric tons. About 0.3 million citrus saplings are used by growers to plant 1,000 ha each year (6). Most are supplied by private nurseries in the country which produce seedlings as well as budded nursery trees. The abundance of virus and virus-like diseases such as greening, tristeza, xyloporosis, etc. and the lack of a proper mechanism for compulsory indexing of mother plants are accelerating the spread of many diseases in new areas (3, 4). Elimination of virus and virus-like diseases by shoot tip grafting (STG) and a development of systems for supplying disease-free citrus has been applied in different countries worldwide. In these studies, research on adaptation and modification of STG technique was carried out to obtain more successful grafts.

Rootstocks used for STG were local rough lemon. Scions selected were local Dhankuta mandarin and a local Sindhuli sweet orange. The rootstock and scion preparation, care of grafted plants and transfer to soil were that of Navarro (2) with modifications as follows:

*Exposure of the rootstock to light:*  
a) Complete darkness as per Navarro (2); (b) Twelve days in darkness followed by 3-9 days exposure to ordinary roomlight 12hr daily, prior to grafting.

*Growth regulator used in the MS medium:* a) As per formula of Navarro (2); (b) Use of BAP at 0.5 mg/l of MS medium.

*Transplanting of the STG plants to soil:* a) direct transfer of grafts to soil from the BAP medium; b) Transfer of the successful grafts to control MS medium for 10-15 days before transplanting to soil.

A triangular incision was used for grafting. Soil media used consisted of mixture of 1:1 peat and silt supplemented with micro and macro nutrients as per Roistacher (5). Soil mix was steam sterilized before use.

The STG technique (2) has been considered a great asset for the elimination of virus and virus-like diseases of citrus. However, only 35% of successful grafts were reported. Different researchers have been trying to modify it to increase the percentage of successful grafts. Triatminingsih *et al.* (7) obtained a 24% increase in the frequency of successful grafts over untreated controls by the use of BAP at 0.5 mg/l. They also evaluated the effect of the incision shape on the frequency of successful grafts and found triangular cuts to be the best. We therefore also tested this concentration of BAP and the triangular cut incision. Similarly, Chatisathian and Tontyaporn (1) exposed rootstocks to light to increase frequency of successful grafts. By exposing seedlings for up to 16 hr daily

of 750 lux they obtained 45% successful grafts. In our experiment we exposed seedlings to ordinary room light for 12 hr daily for 3 to 9 days prior to grafting and increased successful grafts by 16.8% (Table 1) over the method recommended by Navarro (2). Similarly, by adding BAP at 0.5 mg/liter increased grafting success by 18% (Table 2). We also had more success in transplanting when the growing grafted seedlings were first transferred to control MS medium for 10 to 15 days prior to transplanting to soil (Table 3).

Thus, the combination of these three methods have considerably improved graft and transplant success (Table 1, 2, and 3). These experiments indicate that we have been able to adapt the STG technique with some modifications. Successful STG plants are being indexed to assure that they are free of viruses and viroids. Finally, the technique will be recommended to "National Citrus Development Program" for further implementation.

TABLE 1  
EFFECT OF LIGHT EXPOSURE ON FREQUENCY OF SUCCESSFUL GRAFTS

Treatments	No. of plants treated	No. of successful grafts in vitro	Percent
1. Without exposure of seedling to light	144	2	1.4
2. 12 days dark + 3-5 days exposure to light	89	15	16.8
3. 12 days dark + 6-9 days exposure to light	64	10	15.6

TABLE 2  
EFFECT OF ADDITION OF BAP HORMONE TO THE MS MEDIUM ON SUCCESS OF SHOOT TIP GRAFTS

Treatments	No. of plants treated	No. of successful grafts in vitro	Percent
1. Without any growth hormone	172	2	1.2
2. BAP 0.5 mg/l.	133	24	18.0

TABLE 3  
RESULTS OF TRANSPLANTING TO SOIL AFTER STG

Treatments	No. of plants treated	No. of successful transplants to soil	Percent
1. Direct transfer of successful grafts from medium with BAP to soil	18	0	0
2. Transfer to control MS medium before transplanting to soil for 10-15 days	8	8	100

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