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The Clean Stock Program and The Shoot Tip

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The importance of growing plants from small shoot tips has been recognized (12). The major reason has been for elimination of virus from vegetatively propagated stock (1, 2, 3, 4, 5, 6). However, fungus spores on the surface of cuttings are not eliminated by conventional clean stock culturing techniques (1, 2, 3). Shoot tip techniques offer a means to eliminate inoculum carried on or in cuttings in addition to the potential elimination of viruses. The following experiments and observations report the progress of a program which will incorporate shoot tip techniques into the existing Colorado carnation clean stock program.

Methods and Materials

Shoot tips were removed from carnation cuttings and placed in sterile test tubes. The tubes contained 15 ml of autoclaved Gautheret's media (13) modified slightly as follows: ½ strength Knop solution with 1 mg/L naphthaleneacetic acid, 1 mg/L thiamine, 8 mg/L adenine, .5 ml/L Berthelot's solution and 40 g/L glucose. Paper wicks were used to support the tip within the tube (10) and low intensity fluorescent light (9), (200 fc), was supplied. The temperature of the room where the tips were grown was approximately 65°F.

Upon removing the tips from the tubes the small plants were set in pots of river sand in a shaded area of the greenhouse. When 3-4 inches tall the plants were transplanted to pots or benches of volcanic scoria. Cuttings were propagated under standard conditions under mist. In all comparison groups, cuttings and shoot tips were both derived from the same mother plant.

Cuttings and shoot tips used for experimentation were from carnation plants of the following varieties and selections: Red Sim, Gayety, and special selections R 8, R 10, WP 2, PS 2. In addition, observations of flower color and growth habit were made on 64 selected plants derived from shoot tips, including White Sim, Pink Sim, Gayety, Red Gayety, White Pikes Peak, Pink Mamie, Light Pink Littlefield, and Orchid Beauty.

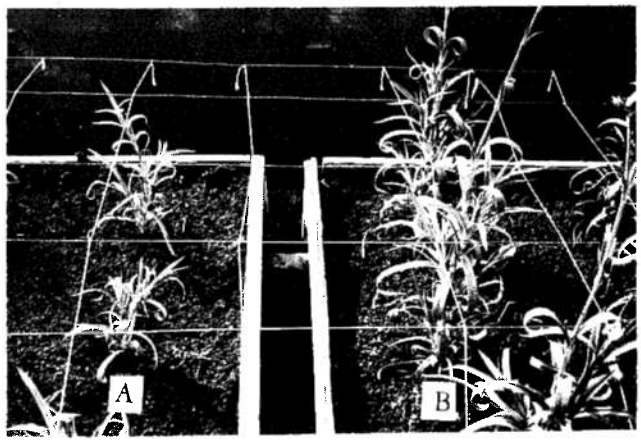

Figure 1. Conventional (A) and shoot-tip (B) plants during the first year of growth.

The 1 mm shoot tip, referred to below, includes the apical meristem and at least 1 pair of leaf primordia. The 200u shoot tips may include only the apical meristem.

Results

Sixteen shoot tips, 1 mm long, were taken from Red Sim and 4 from Gayety in July 1960. Six 200u tips were taken and and grown from Red Gayety. These plants reached the greenhouse benches in September 1960 in the case of the 1 mm Red Gayety and October 1960 for the 200u Red Gayety and Gayety.

Conventional cuttings from corresponding Red Gayety and Gayety plants were rooted and planted in September 1960 and grown in the benches with the plants derived from shoot tips.

During the first year, the shoot tip plants appeared much more vigorous than the conventional cuttings (Fig. 1). True comparison was difficult since the two groups of plants were grown under vastly different environmental conditions until all were placed into the greenhouse benches.

These plants were cut back in June of 1961 and cuttings taken in July. Fifty cuttings were propagated from the Red Gayety shoot tip plants and 50 from the conventional Red Gayety cutting plants. Only 9 cuttings from Gayety shoot tip and 9 conventional Gayety plants were propagated at this time. In addition, 5 cuttings were propagated from each of 4 plants grown from 1 mm shoot tips and their corresponding mother plants, selections R 10, R 8, WP 2, PS 2. The shoot tip mother plants of the special selections were grown in a different greenhouse and were at least 7 months younger than the corresponding conventional mother plant.

All cuttings were grown for 2 months in volcanic scoria. At this time their general growth was compared by direct observation and by harvesting the above ground portion of the plant and recording the fresh and oven dry weight of each group of plants.

These results (Table 1) indicate only one consistent difference between the plants derived from shoot tip plants and the corresponding conventional plants. The percentage of dry matter was greater, with only one exception, in the shoot tip plants. This increase in dry matter is felt to be a consistent and significant trend.

The cuttings from the shoot tip plants from the selections R 10, W 2, and PS 2 had greater wet and dry weights than the cuttings grown in the greenhouse, but some of this may be only a reflection of their different environmental backgrounds.

Table 1. A comparison of the growth of cuttings derived from shoot tip mother plants and cuttings derived from conventionally propagated mother plants.

Variety or selection	No. of plants compared	Fresh weight in grams		Dry weight in grams		% of dry matter	
		Shoot tip	Conventional	Shoot tip	Conventional	Shoot tip	Conventional
Red Gayety	25	2839	2845	434	401	15.3	14.1
Red Gayety	25	2508	2287	398	341	15.9	14.9
200u Red Gayety	5	573	649	87	90	15.2	13.8
Gayety	9	805	924	138	133	17.1	15.3
Selections:							
R 8	5	421	506	66	70	15.7	13.8
R 10	5	504	101	73	55	14.5	13.8
WP 2	5	417	274	62	41	14.9	15.0
PS 2	5	554	422	178	60	16.0	14.2

General observation of 90 additional shoot tip plants

Approximately 50% of the shoot tips taken from the mother plants survived. This was, however, subject to great variation largely dependent on the environment within the test tube.

It requires 9-10 months to produce a flowering plant from a shoot tip. Timing can be broken down as follows:

1. 3-4 weeks are required in the test tube for callus formation and root development.
2. 6-12 weeks are required before transplanting the tip from the tube.
3. The plant produces the first flower and cuttings in about 7 months after transplanting from the tube.

The 90 plants derived from shoot tips have been carefully observed for at least 9 months. Of these plants 4 were observed to sport or mutate. These were commonly occurring sports and it is recognized that these observations of gross morphology and flower color do not adequately test more subtle changes.

No bacterial or fungal carnation diseases have occurred in the 90 shoot tip plants or in the 150 cuttings taken from shoot tip plants. Several plants derived from cuttings which were used as comparison plants have shown infection with Fusarium roseum f. cerealis (Cke.) Snyder and Hansen.

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Discussion

Comparison of cuttings taken from shoot tip plants with cuttings taken from conventionally propagated plants suggests a desirable increase in the percentage of dry matter as a result of the shoot tip techniques. The comparison of the cuttings grown together in the greenhouse suggest that this difference is not environmental. The increase in percentage of dry matter may be the result of decreased virus content, though no direct evidence for this was observed. Those selections with different environmental backgrounds which were compared followed this established trend and further suggest a non-environmental response.

The general observations suggest that the mutation rates increase as a result of the shoot tip technique. However, these mutations are not so frequent as to eliminate the practical application of the procedure. The mutation rate does indicate that CAREFUL and COMPLETE selection is required before any shoot tip derived cuttings are released to the grower as mother stock.

The carry over of inoculum of F. roseum f. cerealis is eliminated from the original shoot-tip plant. The shoot tip plant presumptively is free of fungal pathogens and has been demonstrated free of some virus pathogens (11). Some evidence indicates that inoculum is not carried on the cuttings taken from the shoot-tip plant. If practices of sanitation, isolation, and protection are carefully followed, the elimination of primary carry-over inoculum can only serve to lower the inoculum now found in the clean stock. Thus, it is possible that, if the limits of practical application allow, a nearly perfect clean stock may be developed.

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