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In Vitro Propagation of Broccoli from Stem, Leaf, and Leaf Rib Explants[1]

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Abstract. **Leaf explants of broccoli [*Brassica oleracea* L. (Italica group)] produced either callus, roots, shoots, or both on Murishige and Skoog media (M&S) media with 4.0 to 6.0 mg/liter kinetin and from 8.0 to 9.0 mg/liter indoleacetic acid (IAA). Sections of leaf rib developed callus and then multiple shoots and roots on media with 8.0 to 10.00 mg/liter kinetin and 9.0 to 10.0 mg/liter IAA. Stem explants formed very little callus, but differentiated roots and shoots with 3.0 to 20 mg/liter kinetin and 9.0 to 10.0 mg/liter IAA. Successful transplanting to soil was achieved with all explants.**

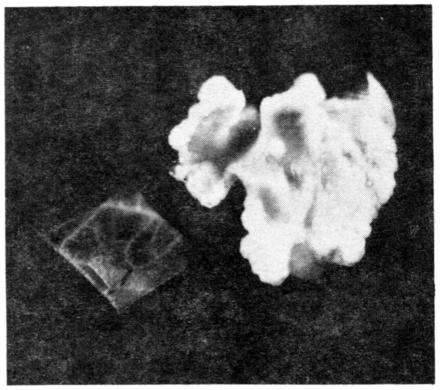

Fig. 1. Fresh broccoli leaf explant (left) and a similar explant after callus has developed.

Various cruciferous plants, including kale (3, 4), Brussels sprouts (2), cabbage (8), and cauliflower (5, 7, 9) have been propagated through tissue culture. Anderson and Carstens (1) reported that flower buds tissue of broccoli differentiated into roots and shoots but not leaf or stem tissue. Flower buds are produced late in life cycle in limited numbers, and hence restrict the rate of propagation. Our objective was to develop a method for propagating broccoli plants from leaf and stem tissue.

Leaves and stems of 'Green Comet' hybrid broccoli taken from both vegetative and flowering field-grown plants and from young vegetative plants raised in growth chambers were surface sterilized for 5 min with a solution of 2.75% sodium hypochlorite plus 0.5% Tween 20. After a sterile distilled water rinse, plant parts were placed for 2 min in a 70% ethanol solution containing 0.5% Tween 20. Leaf explants varied in size from about 2 mm^2 to 1 cm^2. Rib explants 3 mm long were taken from the region between the petiole and the leaf blade. Stems were cut into 1 cm sections with each then cut longitudinally and each half placed cut surface down on M&S (6) medium with 3 g/liter sucrose and 6 g/liter agar, pH adjusted to 5.7, and then placed in controlled temp rooms at either 21° or 30°C illuminated with Gro-lux lamps at 6 and 6.4 klx for a 16 hr photoperiod.

Explants from each tissue (rib, stem, leaf) were tested on 160 different combinations of kinetin (1-10 mg/liter) and IAA (1-16 mg/liter), as shown in Table 1. Once the general range for root and shoot formation was identified, further tests were run within that range to determine optimal concn.

The developmental state of the donor plant at the time explants were taken did not influence the rate of differentiation. Explants from field-grown plants from June through Nov. included plants in the vegetative and flowering stages and explants from vegetative plants grown in growth chambers all readily produced roots and shoots.

Leaf explants usually developed callus around the cut edges prior to the formation of roots and shoots (Fig. 1). Roots were generally formed before shoots. Occasionally, roots and shoots formed directly without any callus (Fig. 2). The optimal medium for leaf explants contained from 3.0 to 6.0 mg/liter kinetin and 8.0 to 9.0 mg/liter IAA (Table 1). Roots and shoots differentiated by the end of 5 weeks at 21°C and within 3.5 weeks at 30°C.

Leaf ribs were placed on media with the basal end in the agar. Profuse callus production occurred at the basal end, forming a ring around the explant. From 1 to 8 shoots and numerous roots developed on each explant. The time required for root and shoot development at each temp was the same as that for leaf explants. The optimal medium contained 8.0 to 10.0 mg/liter

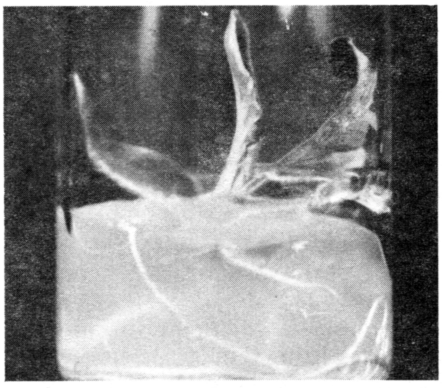

Fig. 2. Leaf expant with shoot and roots but little callus.

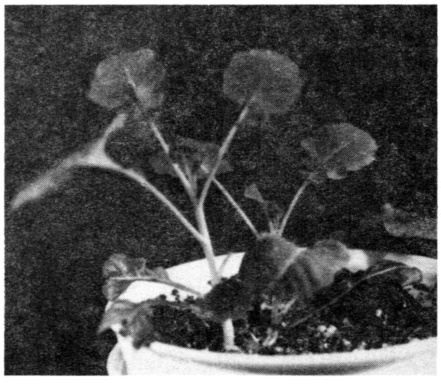

Fig. 3. Plants grown from leaf explants.

Table 1. Effect of 160 combinations of kinetin and IAA on broccoli leaf explants.

Kinetin	IAA (mg/liter)															
(mg/liter)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	C	C	O	R	R	O	O	R	RS	R	R	O	O	C	O	O
2	R	O	R	R	R	O	R	R	R	R	O	S	R	C	O	O
3	O	R	O	O	R	O	O	RS	RS	R	O	O	R	O	O	O
4	R	R	O	O	R	R	O	RS	RS	O	O	O	O	C	C	O
5	O	R	O	O	R	O	O	RS	RS	O	O	O	O	O	O	O
6	O	O	O	O	O	R	O	RS	R	R	O	O	O	O	O	O
7	O	O	O	O	O	R	O	O	O	O	O	O	O	O	O	O
8	O	R	O	O	R	R	O	O	O	O	O	O	O	O	O	O
9	O	O	O	O	O	O	O	O	O	R	O	O	O	O	O	O
10	O	O	O	O	R	O	O	O	O	O	O	O	O	O	O	O

O = No Response; C = Callus; R = Roots; S = Shoots.

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kinetin and 9.0 to 10.0 mg/liter IAA.

Stem explants developed on media with 3.0 to 20 mg/liter kinetin and 9.0 to 10.0 mg/liter IAA formed little or no callus and differenetiated roots and shoots in 4 weeks at 30°C or 5 weeks at 21°C. Stem explants were not well suited for *in vitro* propagation because many of the stem explants did not differentiate roots or shoots and contamination was a problem. Further plants from stem explants were of lower quality than leaf or leaf rib explants.

When plants from any of the 3 tissues were 3 to 4 cm high, they were transferred to pots of soil. Initially transplants were protected from dehydration by covering the pots with inverted beakers which were gradually lifted over a period of days, allowing the plant to adjust to lower humidity (Fig. 3).

The procedures reported here are less complex than previously reported by Anderson and Carstens (1) in that no particular preconditioning of plants was necessary and both shoots and roots formed on one medium.

The divergence of our results from Anderson and Carstens (1) who failed to achieve shoot from leaf or stem explants may be due to the fact that we worked with a hybrid culture while they worked with inbreds. Differences in clones and lines remain to be investigated but our studies suggest large-scale cloning of broccoli can be achieved with leaf tissue and proper attention to auxin-cytokinin ratios.

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Modification of Critical Freezing Temperatures in Fruit Buds by Elevated Tissue Water Content[1]

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Abstract. **Laboratory studies on freezing fruit buds of peach (*Prunus persica* (L.) Batsch cvs. Elberta and Golden Queen), apricot (*Prunus armeniaca* L. cv. Moorpark), grape (*Vitis Labrusca* cv. Concord) and apple (*Malus domestica* Borkh cv. Topred Delicious) from Prosser, Washington, USA, and Auckland, New Zealand, showed that wet fruit buds were more susceptible to low temp injury than dry buds. As buds dried, freezing damage decreased. It is suggested that where overtree sprinkling is used, care must be taken to ensure that fruit buds are dry before freezing temperatures occur.**

Orchards and vineyards are increasingly being irrigated from sprinklers installed above the trees or vines. Plantings may be irrigated during the frosty time of the year. Increased frost injury may be observed on the wet buds, flowers or shoots.

Overtree sprinkling of fruit trees during warm periods in late winter and early spring has recently been shown to cool fruit buds, thereby delaying bud development and reducing the likelihood of freeze injury (1, 2). However, in several cases where this technique has been used, more bud damage was observed in sprinkled plots than in control plots after the occurrence of damaging freezes (4, 12). In addition, peach fruit buds from sprinkled trees were found to be less cold hardy than those from unsprinkled trees (4). Wet flowers and fruit have been reported to be less cold resistant than when dry (3, 5, 6, 11). Increased injury to wet buds may be expected from lower temp caused by evaporative cooling. Wet bulb temp was not low enough to account for the injury observed.

Since it seemed likely that the water content of fruit buds would be increased by sprinkling, we examined the possibility that water content of buds could be correlated with increased susceptibility to freezing. Laboratory experiments were carried out at Prosser, Wash. in the northern hemisphere springs of 1976 and 1977, and at Auckland, New Zealand, in the southern hemisphere spring of 1976.

Prosser experiments. One-year-old shoots of 'Elberta' peach were collected at 3 dates during dormancy and 4 during spring development in 1976. In 1977 it was 7 and 3 dates, respectively. Five bundles of 5 randomly selected shoots were assigned to each of three 24-hr treatments:

(a) for the control treatment, shoots were kept dry at 21°C;
(b) shoots were treated as for (a) but given a single, momentary water dip immediately prior to the freezing test; and
(c) shoots were given a single, momentary water dip followed by exposure to intermittent mist in a mist chamber at 21°.

In 1977, freezing experiments were also performed on grape shoots and post petal-fall apple fruits. There were 3 treatments: dry shoots; dry shoots dipped into water momentarily before the freezing test; and wet shoots removed from vines or trees immediately after the cessation of irrigation by sprinkling.

After treatment, shoots were placed

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