



**Figure 1 A-C.** *Najas marina* L. A & B—Transections of portion of anther. A. Meiocytes stained with aniline blue. Note the absence of callose about meiocytes ( $\times 450$ ). B. Mature pollen grains stained for insoluble polysaccharides with PAS reaction. Note the abundant PAS-positive starch grains and unstained nuclei ( $\times 450$ ). C. SEM photograph of mature pollen grain showing many evaginations on the entire surface formed due to the pressure exerted by densely accumulated starch grains within ( $\times 2000$ ).

requirements since the microsporogenesis proceeds normally even without callose deposition.

Lack of callose around meiocytes and their derivatives and its correlation with the absence of exine in microspores and mature pollen grains support the earlier hypotheses<sup>4,5</sup> that callose serves to protect enzyme systems responsible for exine deposition and provides templates for the future exine establishments.

The authors thank Dr P. N. Bhatt, M.S. University, Baroda, and Dr B. J. Apparao for valuable discussions. One of us (BKJ) is grateful to UGC, New Delhi for financial assistance.

26 November 1984

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#### IN VITRO PLANTLET FORMATION FROM COTYLEDONS, LEAF LAMINA AND MID-RIB OF CAULIFLOWER

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TISSUE culture techniques are used in clonal propagation<sup>1,2</sup> of elite genotypes. Propagation through explant culture has an advantage over callus and cell suspension cultures as cytological aberrations are



quite common in such cultures<sup>2</sup>. Cauliflower (*Brassica oleracea* L var *botrytis* L) is an important vegetable crop of India and attempts have been made to propagate it using callus<sup>3</sup> and curd explants<sup>4</sup>. In the present communication we report differentiation from three explants viz cotyledons, segments of leaf lamina and leaf mid-rib of *B. oleracea* L var *botrytis*. Although differentiation has been reported from different explants of *B. campestris*<sup>5-7</sup>, *B. juncea*<sup>8</sup>, *B. oleracea* var *capitata*<sup>9</sup> and *B. oleracea* var *medulosa*<sup>10</sup>, yet only a few reports are available on explant culture of cauliflower.

Seeds of cauliflower (procured from Agricultural Research Station, Durgapura, Jaipur) were grown in fields as well as in aseptic conditions in culture tubes on MS medium<sup>11</sup>. Cotyledons collected from a 15-day old aseptically grown seedlings were cultured without surface sterilization, while leaf-lamina (10 × 10 mm) and leaf mid-rib (10 mm long) were separated from each other after surface sterilization with 0.1% mercuric chloride solution. After thorough washing, all the explants were implanted on MS medium enriched with K (kinetin), BAP (benzyl aminopurine), IAA (indole-3-acetic-acid), IBA (indole butyric acid) and NAA ( $\alpha$ -naphthalene acetic acid). The culture conditions were similar to our previous studies<sup>6,7</sup>.

IAA/IBA/NAA 0.5–1.0 mg/l induced sparse callus from the cut surfaces of all the explants, while higher levels of these auxins induced rooting. IBA (5 mg/l) induced better rooting than any other auxin tested. Better response was shown by leaf-lamina segments, while only a few roots were induced from the leaf mid-rib segments.

K/BAP (0.5–5 mg/l) induced callus from all the explants in 7–10 days. At higher concentrations (3–5 mg/l), the callus induced, was more compact and granular and the growth was very slow, but at lower levels (0.5–1 mg/l) callus showed better growth and was friable. (K or BAP) (1–5 mg/l) induced 2–4 shoots per explant in 60–100% explants of cotyledons which originated directly as well as *via* callus, (figure 1) 5–10 shoots per explant in 60% cultures of leaf lamina (figure 2) and 15–20 shoots per explant in 100% cultures of leaf mid-rib segments (figure 3). In all the cases the maximum response was recorded on 5 mg/l of BAP. As in the present study, higher concentrations of cytokinins also induced shoot buds from the internodes of *B. campestris*<sup>7</sup> cotyledons and internodal segments of *B. juncea*<sup>8</sup>.

IAA, IBA and NAA (0.5–3 mg/l) when added in induction medium (IM, MS+BAP 5 mg/l) the percentage response decreased at their higher levels as in



Figures 1–4. 1–3. Differentiation of shoots from cotyledons (MS+kinetin 3 mg/l), leaf lamina; (MS+kinetin 5 mg/l) and mid-rib segments; (MS+kinetin 5 mg/l), respectively. 4. Plantlet developed from cotyledons on MS+BAP 5 mg/l+IBA 1 mg/l. Note development of roots also from the base of differentiated shoots.

cotyledon cultures of *B. juncea*<sup>8</sup>. In cotyledons, roots were also formed after shoot differentiation to give rise to plantlets (figure 4), as in hypocotyl segment cultures of *B. campestris*<sup>12</sup>.

Differentiated shoots were rooted when cultured on filter paper bridges in liquid MS medium supplemented with 3–5 mg/l of IBA and/or NAA. Best results were obtained in a mixture of IBA and NAA.

Thanks are due to CSIR, New Delhi, for financial assistance.



18 October 1984; Revised 19 January 1985

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## EFFECTS ON GROWTH AND PHOTOSYNTHESIS OF *VIGNA RADIATA* CV PUSA BAISAKHI IN ENVIRONMENT CONTAINING SO<sub>2</sub> AND/OR NO<sub>2</sub>

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SULPHUR dioxide and nitrogen dioxide are the two most widespread air pollutants of industrialized countries, as combustion of fossil fuels generates SO<sub>2</sub> and NO<sub>2</sub>. Considerable information is now available on the effects of SO<sub>2</sub> on leguminous crops of the temperate region. Even low concentrations of SO<sub>2</sub> have been found to reduce photosynthesis<sup>1-3</sup>, translocation<sup>4</sup>, and biomass production<sup>5</sup> in beans. In ambient environment SO<sub>2</sub> and NO<sub>2</sub> are present together. Recent studies involving mixtures of these two pollutants have shown synergistic, additive or antagonistic effects on the growth and physiological processes of various crops<sup>6</sup>.

Since there is no report on the effect of pollutant mixture on legumes of the tropics, the present study

was initiated so as (i) to compare the effects of SO<sub>2</sub> and NO<sub>2</sub> both individually and in the mixture on the growth, and (ii) to evaluate the effects of SO<sub>2</sub> and/or NO<sub>2</sub> on the photosynthesis of young plants of mung bean (*Vigna radiata*).

Seeds of *V. radiata* (L) Wilczek CV Pusa baisakhi were grown in 10 cm plastic pots containing John Innes No. II potting compost. Seedlings were raised in a heated green house. Two days after germination, 40 seedlings of fairly uniform size were selected and 10 seedlings were randomly allocated to each of the four treatment chambers of the type described by Whitmore<sup>7</sup>. Three of these chambers were supplied with filtered air containing SO<sub>2</sub> alone, NO<sub>2</sub> alone, and mixture of SO<sub>2</sub> and NO<sub>2</sub>. The concentration of gases in these chambers was 266 µg<sup>-3</sup> SO<sub>2</sub>, 191 µg<sup>-3</sup> NO<sub>2</sub> and 266 µg<sup>-3</sup> SO<sub>2</sub> + 191 µg<sup>-3</sup> NO<sub>2</sub> respectively. The fourth chamber with only charcoal filtered air served as control. Concentration of pollutants within these chambers was regularly monitored using a Meloy SA 285 flame photometric analyser for SO<sub>2</sub> and a Meloy NA 520 chemiluminizer for NO<sub>2</sub>. The day length was 14 hr and the temperature was maintained at 23°C during the day and 17°C during the night. Illumination was from a horizontally fitted metal halide lamp providing a photon flux density of 350 µEm<sup>-2</sup> sec<sup>-1</sup>.

Fumigation continued for three weeks. Thereafter net photosynthesis (*P<sub>n</sub>*) of plants from all treatments was measured in clean air using an infrared gas analyser operating on a differential mode. These plants were then separated into root and shoot fractions for dry weight measurements.

In another experiment three-week-old plants were exposed to air containing 106.4, 159.6, 212.8 and 226 µgm<sup>-3</sup> SO<sub>2</sub> for measuring *P<sub>n</sub>* following the technique of Black and Unsworth<sup>1</sup>.

Table 1 shows the effect of the two pollutants on the shoot/root dry weight fractions when applied singly and in combination. While the yield reduction was insignificant in NO<sub>2</sub>, it was significantly affected by

Table 1 Shoot and root dry weights of *V. radiata* after 3 weeks exposure to 191 µg<sup>-3</sup> NO<sub>2</sub> or/and 266 µg<sup>-3</sup> SO<sub>2</sub>.

Treatment	Shoot wt (g)	Root wt (g)
Control	0.2788	0.0275
NO <sub>2</sub>	0.2603	0.0211
SO <sub>2</sub>	0.1918 <sup>a</sup>	0.0251
SO <sub>2</sub> + NO <sub>2</sub>	0.2486 <sup>b</sup>	0.0257

Significance of difference from control. <sup>a</sup> *P* < 0.001, <sup>b</sup> *P* < 0.05.