nitrogen was left in the soil after harvest which could be available to the successive crop.

Although a major portion of the fixed nitrogen was recovered in different parts of the plant, and in the soil after harvest, about 40% of the fixed nitrogen could not be accounted for. In fact, there are several reports of significant loss of nitrogen from flooded rice soils. For instance, Datta *et al*[14] observed that 24% of ammonium sulphate was lost through denitrification mainly as N_2 gas. Further, according to Becking[6], in general, only 10–50% of the nitrogen in the plant was derived from fertilizer nitrogen and 50–90% of the applied fertilizer nitrogen was probably lost to the system. This loss in rice soils is considerable since in other aerable crop soils and soil types, nitrogen losses (by denitrification) as measured by tracer techniques range from 10 to 40%[5,15]. Factors and mechanisms of significant nitrogen loss from the flooded rice soil used in the present study are not clear. Nevertheless, it may be reasonable to presume denitrification, operating in such systems, as the major means of nitrogen loss. Thus, the present study demonstrates the transfer of considerable amounts of biologically fixed nitrogen from the soil to the rice plant.

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19 September 1987

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IN VITRO PLANTLET FROM INTERNODE AND INFLORESCENCE AXIS OF *BRASSICA OLERACEA* VAR. *BOTRYTIS*

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Brassica oleracea var *botrytis* is an important vegetable crop grown throughout the world. The cell and tissue culture techniques are employed in the improvement of various crop plants[1,2]. Attempts have been made to induce differentiation and success has been reported from the callus[3,4], suspension[4], leaf lemina, mid-rib[5] segments and curd[6,7] explants. However, there is no report of differentiation from the internode and inflorescence axis. In the present communication differentiation and plantlet formation in this variety have been discussed.

Seeds of *Brassica oleracea* var. *botrytis* procured from the Agricultural Research Station, Durgapura, Jaipur were grown in experimental beds. Vegetative internodes and inflorescence axis were collected before and after flowering respectively. After surface-sterilization with 0.1% mercuric chloride and thorough washings, small segments (10 mm long) of explants were cultured on MS medium[8] supplemented with Kn (kinetin), BAP (benzyl-aminopurine) with or without IBA (indole butyric acid) and NAA (naphthalene acetic acid). All the growth substances were added to the medium before autoclaving at 1.06 kg/cm^2 pressure for 15 min. All the cultures were incubated in a growth room at 26 ± 2°C under continuous light (3000 lux) and observations were recorded daily up to 30th day. Five replicates of each treatments were kept and all the experiments were repeated twice.

Kn and BAP (0.5–5 mg/l) induced callus from the cut ends of both the explants after one week of

Figure 1. Shoot buds differentiated from the inflorescence axis on MS + BAP (5 mg/l).

incubation. Unlike BAP, Kn induced more callus. In internodal segments the lower level (0.5 mg/l) of Kn and BAP induced 2–3 roots after 20 days, while higher concentrations (1–5 mg/l) of both Kn and BAP induced 2–5 shoots in 10–15 days of incubation, through hard and compact callus. In inflorescence axis 5–10 shoot buds differentiated in all the concentrations (figure 1). Best response was recorded on 5 mg/l of BAP and this medium was treated as induction medium. On addition of (1.5–3 mg/l) of IBA or NAA in induction medium highly profused callus was formed and shoot buds differentiated from it. The number of shoot buds per explants decreased with increasing level of an auxin. However there was no change in the percentage response except on 3 mg/l of NAA.

In the present study, the best response was observed on cytokinin alone as in leaf lemina[5], hypocotyl of *B. campestris*[9] and other species of

Table 1 *Response of differentiated shoot buds to different levels of NAA and/or IBA*

Medium MS + (mg/l)		Average no. of roots/shoots	Average size of 5 roots (cm)
IBA	0.5–1.0	Few	5.2
	3.0	20.6	10.6
	5.0	19.4	13.6
NAA	0.5–1.0	Numerous	Very small
	3.0	Numerous	1
	5.0	Numerous	1
NAA	2 + IBA 3	23.4	6.0
	2 + IBA 5	10.0	11.4

100% Rooting response was observed in all cases.

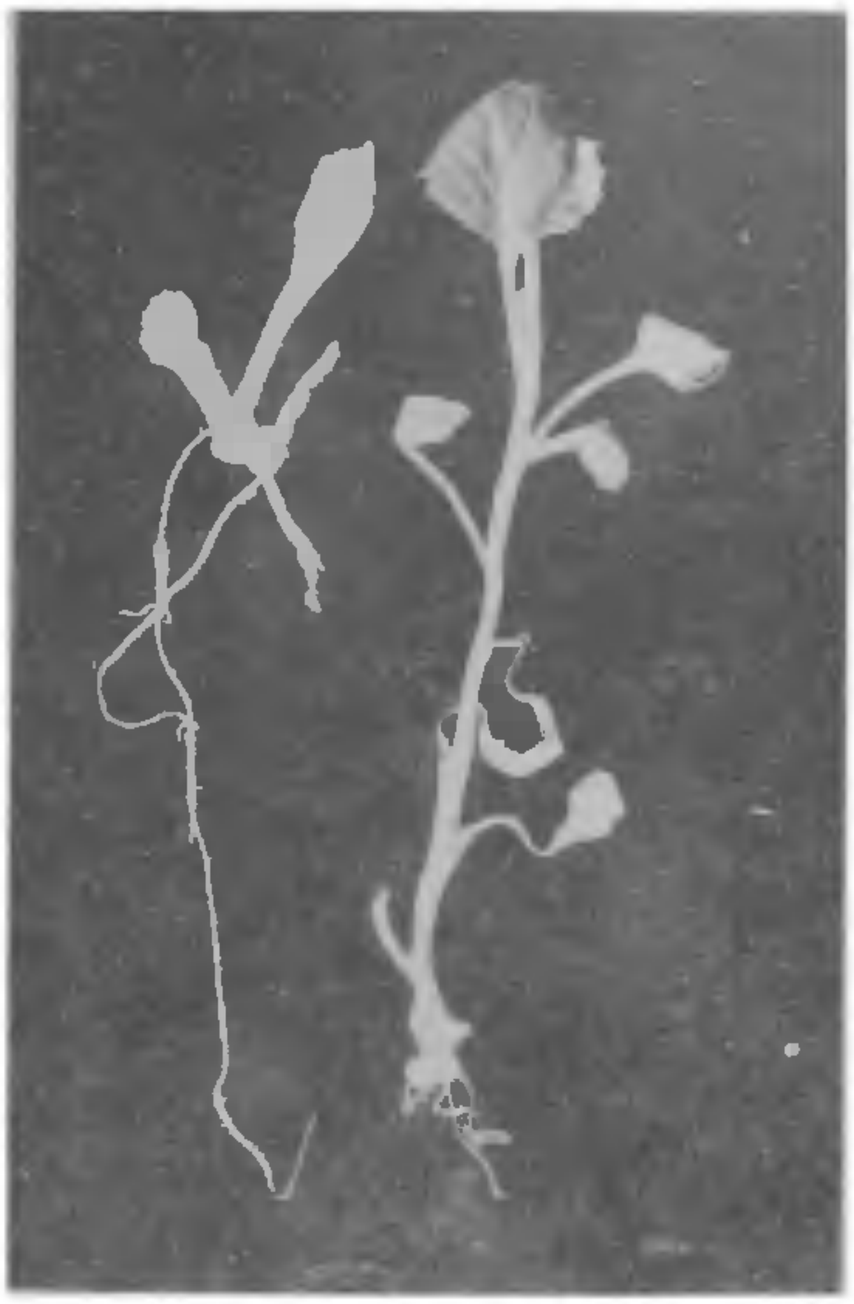

Figure 2. Plantlet developed from the differentiated shoots cultured on MS + IBA (3 mg/l).

Brassica[10,11]. This does not agree with the findings on curd explants of *B. oleracea* var. *botrytis*, in which better response was observed on auxin-cytokinin combinations.

It was observed that both the explants responded slightly differently which may be due to the change in physiological stage of explants (vegetative and reproductive). In curd[6] and leaf[5] explants several shoot buds per explant were differentiated while in the present study lesser number of shoot buds per explant was recorded but the shoots were stronger and healthier.

Differentiated shoots taken from 20–25 days of incubation were cultured in liquid MS medium supplemented with 0.5–5 mg/l of IBA and NAA, singly as well as in combination and better results were obtained on their higher levels (table 1). NAA alone induced numerous but feeble roots as also in *B. campestris*[11], while IBA induced lesser number but longer roots (figure 2). Best response was also observed on combination of NAA (2 mg/l) and IBA (3 mg/l) in earlier studies[11,12].

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EFFECT OF GA_{4+7} ON GERMINATION AND EARLY SEEDLING GROWTH OF MAIZE UNDER WATER STRESS

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IMPROVING seed performance of plants under stressing regimes is of increasing economic importance. Recent research interest in pre-sowing seed treatments for improving field emergence under stress has shown considerable benefits[1,2]. The effects of water stress on germination and seedling growth of maize have been reported[3,4] but studies pertaining to stress alleviation by seed pre-treatments are scarce. Poor seed performance under water stress might be associated with alteration in the endogenous levels of phytohormones and it is thus probable that an exogenous supplementation might help in the alleviation. In the present communication, the effects of GA_{4+7} on the germination and early seedling growth of maize were investigated under simulated water stress conditions.

Seeds of *Zea mays* L. cv. Partap were obtained from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana. Seeds were germinated in petri dishes (9 cm) over two layers of filter paper moistened with 5 ml of water or test solution. Solutions of polyethylene glycol 6000 (PEG) at -0.3 and -0.6 MPa were used[5]. Solutions of GA_{4+7} (25, 50, 75 and 100 ppm) were prepared. Seeds were pre-soaked for 24 h in water and various concentrations of GA_{4+7} at 30 ± 1°C. Seeds were incubated in dark for germination at the same temperature. A seed was credited with germination when its radicle protrudes about 2 mm. Germination counts were taken daily. The coefficient of rate of germination (CRG) was calculated as $[100\ \Sigma N/\Sigma(DN)]$ where, D is the number of days counted from the beginning of germination test, and N is the number of seeds which germinate on day D. Higher the CRG value, greater is the rate of germination. Observations on primary root length, shoot length and seedling dry weight (roots + shoot) were recorded after 5 days. The data were statistically computed using analysis of variance.

The rate of germination of stressed seeds was markedly lowered compared with the unstressed ones (table 1). The decrease was greater at

Table 1 *Effect of seed pre-soaking on germination and early seedling growth of maize under water stress*

Pre-soaking treatment	Coefficient of rate of germination at osmotic potential (MPa)			Primary root length (mm) at osmotic potential (MPa)			Shoot length (mm) at osmotic potential (MPa)			Seedling dry weight (roots + shoot) (mg) at osmotic potential (MPa)		
	0	−0.3	−0.6	0	−0.3	−0.6	0	−0.3	−0.6	0	−0.3	−0.6
None	45.5a	34.3a	30.8a	107.2a	50.4a	31.1a	82.8a	5 6a	4.1a	59.5a	43.6a	37.7a
Water	79.4b	61.0b	49.1b	134.5b	69.0b	38.9b	91.7b	33.3b	7.8b	62.6b	52.0b	40.6b
GA_{4+7} 25 ppm	79.4b	67.0c	63.8c	156.7c	65.6b	40.1b	122.2c	30.7b	10.0c	68.4bc	52.7b	45.6c
GA_{4+7} 50 ppm	80.0b	69.3c	62.3c	155.6c	66.2b	43.6b	122.8c	31.7b	11.2c	72.2c	52.5b	47.1c
GA_{4+7} 75 ppm	81.0b	68.4c	63.4c	155.2c	79.2c	62.8c	121.8c	32.3b	12.3c	72.8c	53.2b	50.5d
GA_{4+7} 100 ppm	76.5b	70.0c	62.2c	168.9c	76.5c	63.2c	133.3c	31.7b	16.0d	86.7d	53.6b	51.1d

Mean values in a column with similar suffixes do not differ significantly at $P = 0.05$.