J. Phytol. Res. 4 (1) 1991

IN VITRO STUDIES ON GROWTH AND DIFFERENTIATION IN HYPOCOTYL CALLUS OF *BRASSICA CAMPESTRIS* cv *YELLOW SARSON*

SURENDRA SINGH, ANIL MATHUR and R.K. SHARMA

P.G. Dept. of Botany, M.S.J. Autonomous College, Bharatpur-321001, India.

Callus isolated from hypocotyl segments of *Brassica campestris* cv *yellow sarson*, on MS medium fortified with 0.1 mg/l of IAA and 0.5 mg/l of kinetin, was fragile and remained undifferentiated throughout the course of experiments. Callus showed typical sigmoid growth after sub culturing. On simple MS medium growth was negligible. Incorporation of auxins and cytokinins singly as well as in combinations, showed different effects on growth. When used singly, auxins induced rooting, while cytokinins induced shoot buds. Combinations of lower auxins and higher cytokinins evoked shoots while roots were recorded on lower cytokinins and higher auxins,

Keywords : *In vitro;* Differentiation; *Brassica campestris.*

Introduction

Technique of tissue culture provides a means of studying growth and differentiation of plant tissues under precisely controlled conditions. Clonal propagation of elite plants is the other aim achieved. *Brassica* Sps are economically important and are grown for vegetables and vegetable oil. This technique has also been employed in clonal propagation of *Brassica* species (Clare and Collins, 1974; Kartha, *et al.*, 1974; Bajaj and Nietsch, 1975; Pareek and Chandra, 1981; Dunwell, 1981), but most of the studies are on explant culture. In this communication an extensive study has been made on growth and differentiation in hypocotyl callus of *B. campestris* cv *yellow sarson* in continuation of the previous studies on this cultivar (Singh *et al.* 1981, 1985; Singh and Chandra 1984 a, 1984 b, 1986 a, and Singh and Mathur 1985, 1987; Singh. 1988).

Material and Methods

Seeds of *Brassica campestris* cv *yellow sarson* procured from I.A.R.I., New Delhi, were germinated aseptically. 10 mm long segments of hypocotyl were cultured on MS (Murashige and Skoog, 1962) medium having agar (0.8%), auxins and

cytokinins. Callus formation was recorded to be the best on the medium enriched with 0.5 mg/1 of K (Kinetin) and 0.1 mg/1 of IAA (Indole acetic acid). Fragile and undifferentiated callus was multiplied on the same medium by monthly subculturing. From this stock callus about 200 mg (Fresh weight) per flask was subcultured, when experiments on growth and differentiation were carried out. Different concentrations of K, BAP (Benzyl amino purine); IAA, IBA (Indole butyric acid), NAA (Naphthalene acetic acid), 2,4-D (2, 4 dichlorophenoxy acetic acid), singly as well as in combinations were tried. Five replicates of each treatment were kept and experiments were repeated, standard error of arithmatic mean was calculated. All cultures were incubated at room temperature and 3000 Lux illumination.

Result and Discussion

The growth of the stock callus was slow initially but improved after 10 days. Fast growth was recorded after 15th day of incubation with maximum growth on 30th day. Growth of stock callus shows typical sigmoid curve (Fig. 1).

(i) *Effect of medium with Auxins*: Growth of the callus on auxins showed a remarkable variation from auxin to auxin and concentration to concentration (Fig. 2). Growth increased with increasing level upto some extent but it declined, on further increasing the concentration of IAA, IBA, and NAA. 2,4-D was inhibitory for growth. At lower level (0.5-1.0 mg/l) none of the auxins, induced differentiation of any kind. But higher levels (3.0—5.0 mg/l) of IAA, IBA and NAA induced little or more roots. 2,4 D at any level failed in root induction. Higher levels of IBA, NAA and IAA induced roots in 100% cultures but there was remarkable difference in roots. IBA (5.0 mg/l) induced longer roots while similar concentration of IAA and NAA induced moderate roots, as also recorded in *B. oleracea* var *botrytis* (Singh and Mathur 1985, 1987).

(ii) *Effect of cytokinin used singly*: On the addition of K or BAP in MS medium, growth of the callus decreased continuously with increasing levels from 0.5-5.0 mg/l (Figs. 2 and 3 first vertical row). None of the cytokinin at 0.5 mg/l level induced shoot buds, while few shoot buds per culture (in 20% cultures) were recorded on 1.0 mg/l of both K and BAP. The reponse increased both in terms of percentage and number of shoot buds per culture on increasing levels with maximum response on 5.0 mg/l of BAP. On 5.0 mg/l of K shoot buds were recorded in 100% culture but number of shoot buds was lesser, as also reported in explant and callus cultures of *B. juncea* (Pareek and Chandra, 1981), *B. campestris* (Singh

Fig. 1 Showing growth of hypocotyl callus of *Brasssica campestris* cv *yellow sarson* on MS medium + Kinetin (0.5 mg/l) + IAA (0.1 mg/l). →

FIG 1

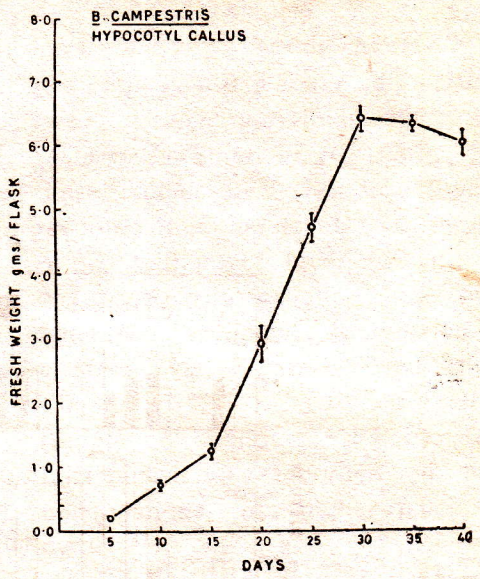

Fig. 2 Showing effects of various concentrations of Kinetin used singly and in combination with auxins (IAA, IBA, NAA, 2,4 - D) ncorporated in MS medium, on fresh weight yield and differentiation of shoots and roots in hypocotyl callus of *Brassica campestris* cv *yellow sarson.*

Fig. 2 ↓

FIG. 2

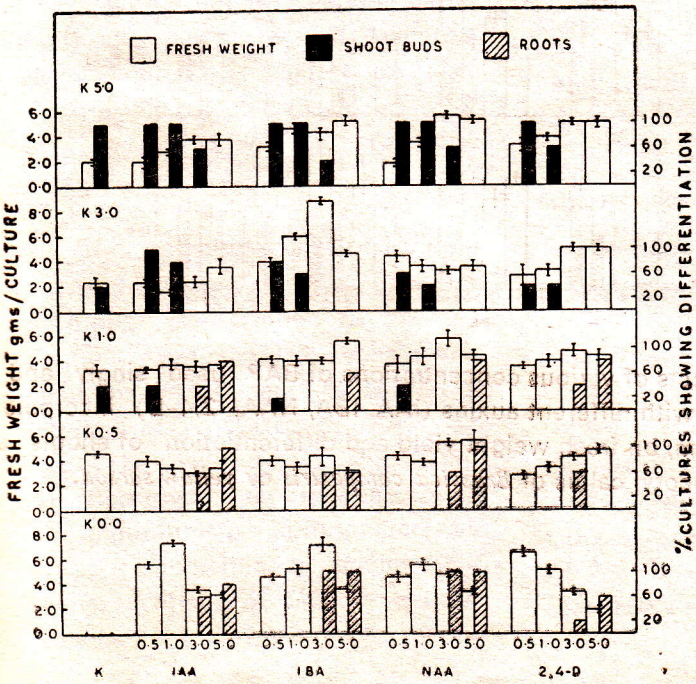

FIG. 3

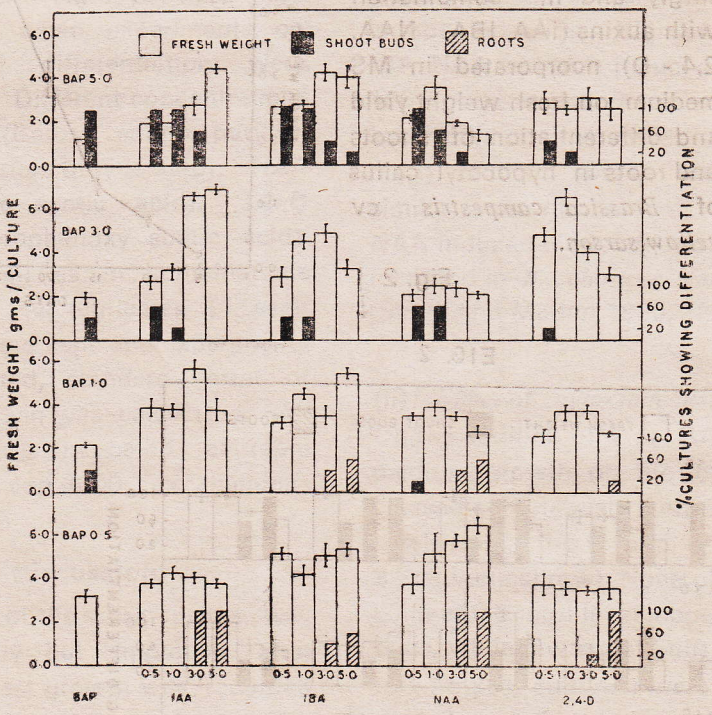

Fig. 3 Showing effects of various concentrations of BAP used singly and in combination with different auxins (IAA, IBA, NAA, 2,4-D) incorporated in MS medium, on fresh weight yield and differentiation of shoots and roots in hypocotyl callus of *Brassica campestris* cv *yellow sarson*.

and Chandra 1984 b, 1986 a, Singh *et al*, 1985) and *B. oleracea* var *botrytis* (Singh and Chandra, 1986 b).

(iii) *Synergestic effect of auxins and cytokinins* : Growth of the callus of combinations of auxins and K, and auxins and BAP has been shown in Figs. 2 and 3 respectively. There was s ⊕ t pattern of growth like stem callus of this variety (Singh *et al*, 1985), *B. oleracea* var *botrytis* (Singh and Chandra 1986 b). Growth showed more variation on the combinations of BAP and auxins in comparison to K and auxins. Maximum growth was recorded on the medium having 3.0 mg/l each of K and IBA.

Rhizogenesis was recorded at lower level of K/BAP (0.5 mg/l) combined with higher levels (3 0–5.0 mg/l) of auxins, however response was variable (Figs. 2 and 3) as also in the callus cultures of *B. oleracea* var *botrytis* (Singh and Chandra, 1986 b)

In the combinations of 1.0 mg/l of K and 0 5 mg/l of IAA, IBA or NAA only few shoots, in 20–40% cultures, were recorded, while combinations of 1.0 mg/1 of BAP with 0.5 mg/l of IAA, IBA or NAA did not evoke any response (Figs 2 and 3). Response in terms of percentage and shoot buds per culture increased in the combination of auxins with higher level of K or BAP, in general, response of differentiation varied from combination to combination. Noteworthy feature was that combinations of 2,4-D with K or BAP evoked poor response. 2,4-D may have inhibitory effect on shoot differentiation as in *B. oleracea* var *medullosa* (Lustinec and Horak, 1970), *B. campestris* (Singh *et al.*, 1985). In these studies best response was recorded on higher level (5 0 mg/l) of BAP with lower level (0.5 mg/l) of IAA, such observation has also been made in callus cultures of *B. oleracea* var *botrytis* (Singh and Chandra, 1986 b) *B campestris* (Singh and Chandra, 1984 b). No rhizogenesis was recorded on the combinations of higher level of cytokinin with any level of auxin as also in *B. oleracea* var *botrytis* (Singh and Chandra, 1986 b) or any other explant of this variety studied so far Singh *et al.*, 1981, 1985, (Singh and Chandra 1984 a,b, 1986 a).

Accepted August, 1991

References

Bajaj Y P S and Nietsch J P 1975, *J. Exp, Bot*, 26 883.

Clare M V and Collins H A 1974, *Ann. Bot.*, 38 1067

Dunwell K M 1981, *J Exp. Bot.* 32 789

Kartha K K, Gamborg O L and Constabel F 1974, *Physiol Plant.* 31 1217

Lustinek J and Horak J 1970, *Experientia* 26 919

Murashige T and Skoog F 1962, *Physiol. Plant.* 15 473

Pareek L K and Chandra N 1981, *Indian J. Exp. Biol.* **19** 874

Singh S 1988, *Curr. Sci.* **57** 730

Singh S, Banu S, Pareek L K and Chandra N 1981, *Indian J. Exp. Biol.* **19** 650

Singh S and Chandra N 1984 a, *Curr. Sci.* **53** 379

Singh S and Chandra N 1984 b, *Plant Cell Reports* **3** 1

Singh S, Garg K and Chandra N 1985, *Acta Bot. Indica* **13** 45

Singh S and Chandra N 1986a, *Beitr. Biol. pflanzen*, **60** 185

Singh S and Chandra N 1986 b, *Beitr, Biol. pflanzen.* **60** 191

Singh S and Mathur Anil 1985, *Curr. Sci.* **54** 391

Singh S and Mathur Anil 1987, *Acta Bot. Indica.* **15** 98