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# PLANTLET FORMATION FROM CALLI DERIVED FROM HYPOCOTYL AND COTYLEDON OF BLACK MUSTARD (*BRASSICA NIGRA* L.)

**MANEESHA SINGH and Y. K. BANSAL**
Department of Biosciences, R. D. University, Jabalpur 482001, India.

The present communication deals with the *in vitro* studies on black mustard (*Brassica nigra* L.) with a view to assess the shoot formation and subsequent plantlet regeneration from calli induced on media supplemented with various PGRs. Optimal multiple shoot formation occurred from hypocotyl and cotyledon derived callus implanted on MS media fortified with 2, 4-D + BAP and IBA + BAP.

**Key words:** Black mustard; *Brassica nigra*; Plantlet; Tissue culture.

## Introduction

Black mustard *(Brassica nigra L.)* is one of the important species of oil seed Brassicas, which is extensively cultivated throughout India. It is notable for its seeds which possess several important properties such as stimulant, stomachic, medicinal and are used as condiment[1].

In recent years major efforts in *Brassica* research have focussed on utilization of tissue culture technology for crop improvement[2]. Efficient and regeneration is an essential prerequisite for exploiting *in virro* techniques for crop improvement[3]. In *B. nigra* direct plantlet regeneration has been obtained[4]. Besides, *in vitro* studies have also been made using anther[5], callus[6] and protoplast[7]. The present investigation has been undertaken to study the differentiation of multiple shoots and plantlets from calli emerged from hypocotyl and cotyledon explants through a simple and rapid protocol.

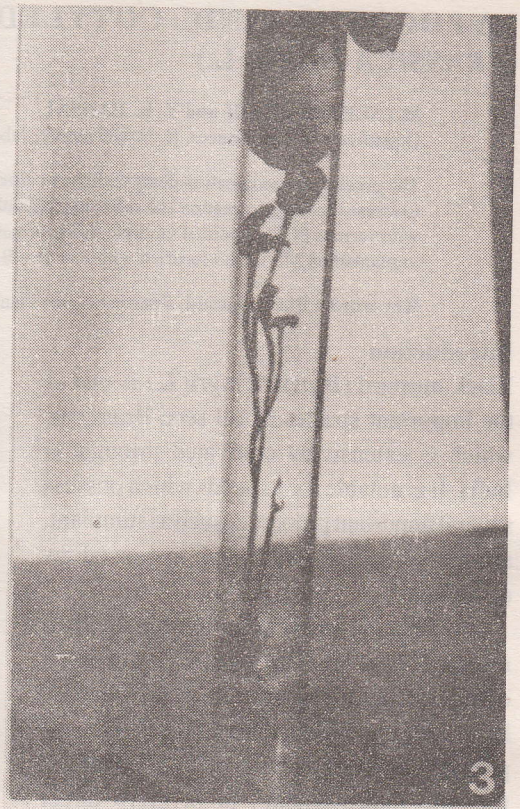
## Meterial and Methods

Seeds of *B. nigra* var. laha procured from JNKVV, Jabalpur, were surface sterilized serially in 1% (v/v) teepol (2min), 70% (v/v) ethanol (2 min) and 0.1% (w/v) aqueous murcuric chloride (10min) with intermittent rinses in double distilled water. Seeds were germinated in conical flasks containing moist sterile cotton. Explants were made in the form of hypocotyl (1.0cm) and cotyledon (1.0cm$^2$) from six days old seedlings and inoculated aseptically on MS medium[8] containing growth regulators viz. Indole-3 butyric acid (IBA) and 2,4- Dichlorophenoxyacetic acid (2,4-D) in different concentrations alone as well as in combination with cytokinin 6-Benzylamino purine (BAP). Cultures were incubated at 25 $\pm$2°C temperature, 65 $\pm$ 10% relative humidity and 1500 lux light intensity from cool white fluorescent tubes with 16 h photoperiod. All treatments contained 20 replicates and each treatment was repeated thrice. The explants were subcultured onto fresh medium every four weeks and responses were scored every third day and recorded as percentage average.

## Results and Discussion

*Callus formation from explants* : Out of the two seedling explants, cotyledon was found to be the more efficient in producing rapidly growing callus. MS medium with 2, 4-D at high concentration (1.0 and 5.0mgl$^{-1}$) was found to be the best for callus initiation from both cotyledon (100%) as well as hypocotyl (85%). This is in contrast to an earlier report where only a low callusing in this species was reported on MS + 2, 4-D. Within one week of incubation, explants enlarged significantly and produced copious callus at their cut ends





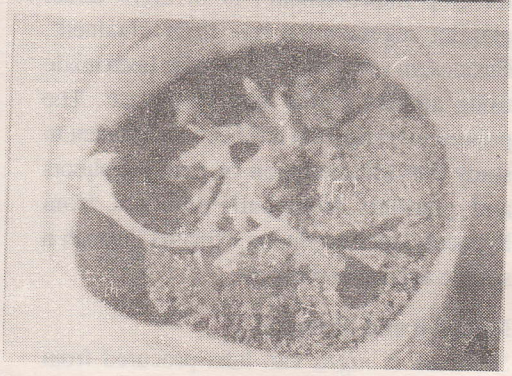



Fig. 1. Shoot bud initiation from callus (hypocoty1) obtained on MS + 2, 4-D (1.0 $mgl^{-1}$) after 5 weeks of culture.

Fig. 2. Multiple shoot formation from hypocotyl callus on MS + IBA (1.0 $mgl^{-1}$) + BAP (1.0 $mgl^{-1}$) after 8 weeks.

Fig. 3. Complete plantlet formation on 2, 4-D (0.1 $mgl^{-1}$) + BAP (1.0 $mgl^{-1}$) from cotyledon callus after 7 weeks.

Fig. 4. Vigorously growing plantlet (15 weeks) after transfer to paper cup.

**Table 1.** Frequencies (%) of hypocotyl (H) and cotyledon (C) derived calli exhibiting shoot and plantlet regeneration.

(after 16 weeks of incubation)

| Sr. No. | Growth media ($mgl^{-1}$) | Explant | Shoot differentiation | Plantlet regeneration | No. of shoot bud per explant | Height of plantlet |
|---|---|---|---|---|---|---|
| 1. | Control | H | 20 | - | - | - |
| | (Basal MS) | C | 10 | 8 | 1 | 2.0 |
| 2. | 2,4-D 0.1 | C | 21 | 10.5 | 1 | 1.8 |
| 3. | 2, 4-D 1.0 | C | 50.0 | 18.6 | 1 | 1.9 |
| 4. | IBA 1.0 | C | 4.0 | 3.8 | 1 | 1.1 |
| 5. | IBA 5.0 | C | 4.4 | 4.0 | 1 | 0.8 |
| 6. | 2,4-D + BAP | H | 31.5 | 10.8 | 1 | 1.8 |
| | 0.1 + 1.0 | C | 46.6 | 35.0 | 1 | 2.2 |
| 7. | 0.1 + 5.0 | H | 28.8 | 9.6 | 1 | 3.1 |
| | | C | 44.4 | 31.3 | 1 | 2.8 |
| 8. | 1.0 + 1.0 | H | 13.3 | 5.6 | 2 | 3.4 |
| | | C | 35.5 | 13.8 | 3 | 3.2 |
| 9. | 1.0 + 5.0 | H | 12.5 | 2.8 | 2 | 3.8 |
| | | C | 32.8 | 10.5 | 5 | 4.1 |
| 10. | IBA + BAP | | | | | |
| | 0.1 + 0.1 | C | 20.0 | 20.0 | 1 | 3.3 |
| 11. | 0.1 + 1.0 | C | 33.3 | 30.0 | 2 | 3.4 |
| 12. | 0.1 + 5.0 | C | 20.0 | 12.2 | 1 | 2.6 |
| 13. | 0.1 + 10.0 | C | 34.0 | 20.5 | 1 | 1.9 |
| 14. | 1.0 +0.1 | C | 14.9 | 14.9 | 2 | 5.1 |
| 15. | 1.0 + 1.0 | H | 12.5 | 8.7 | 4 | 6.2 |
| | | C | 15.5 | 15.0 | 5 | 8.0 |
| 16. | 1.0 + 5.0 | H | 14.0 | 13.5 | 1 | 4.8 |
| | | C | 20.8 | 20.0 | 1 | 3.8 |
| 17. | 1.0 + 10.0 | H | 13.3 | 12.8 | 1 | 2.1 |
| | | C | 20.0 | 19.0 | 1 | 2.5 |

on 2, 4-D and IBA alone as well as in combination with BAP.

All the concentration of IBA supported callus formation. In general, the callus was compact and creamy similar to the one reported in *B. juncea*[3] and the entire explant generated a lump of white compact callus within 2 weeks.

*Plant regeneration* : The first shoot buds appeared on the callus surface after 6-7 weeks of culture (Fig. 1) (Table 1). Both cotyledon and hypocotyl calli exhibited efficient shoot regeneration frequencies of 46.6 and 31.5% respectively in a combination

of 2, 4-D (0.1 $mgl^{-1}$) and BAP (1.0 $mgl^{-1}$), proving that both the explants are amenable for indirect shooting. High morphogenic potential of both cotyledon[9-12] ; as well as hypocotyl[2,4] has been reported in different *Brassica* species in the past.

2,4-D and IBA at their low concentration (0.1 $mgl^{-1}$) in conjunction with high (1.0 and 5.0 $mgl^{-1}$) concentrations of BAP produced efficient shoots as well as plantlets. 2,4-D which has generally been reported to be antagonistic to shoot differentiation[13,14] exhibited a tendency for callusing as well as plantlet regeneration in the present study when used singly or in combination with BAP.

The number of shoots per regenerating callus varied considerably ranging from one to many in combined treatments from both the type of calli (Fig.2). These buds differentiated slowly in the beginning (upto first subculture) and were transfered to basal MS when about 5-6 mm long. Such buds differentiated relatively faster subsequently producing shoots with normal leaves. Shoots attained heights of 6-8 cm within 12-15 weeks (Fig-3). Shoots often showed callus at their base. Similar callus formation along with *in vitro* differentiated shoot buds has observed in *Glycine hispida*[15].

These shoots appeared normal although the leaves were slightly smaller than those of plant grown from seed. Once differentiated, the shoot buds elongated on the basal media and were transferred to various media to induce rooting. Rooting of the *in vitro* differentiated shoots occurred on MS medium containing 1.0 $mgl^{-1}$ IBA. Such suitability of IBA for root induction has been reported in *Prosopis cineraria*[16].

The requirement of different levels of auxin alone or auxin and cytokinin combination to obtain regeneration is probably due to differential endogenous levels of plant growth regulators[17].

Of the 35% shoots turned into plantlet only a low number (5%) plants survived (Fig.4) due mainly to the constant presence of callus throughout the length of root, resulting in inadequate root growth.

Our results showed that vigorous multiple shoot bud and plantlet formation is possible in *B. nigra* from calli. The fact that the callus derived plantlets resembled the normal seedlings in leaf shape and branching pattern etc. emphasizes that the regenerants may be stable in nature.

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