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# Regeneration in *Brassica Juncea L.Crez.* (Indian Mustard)

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**Abstract:** An attempt to regeneration of the Indian mustard (*Brassica juncea L.Crez.*) in which hypocotyls and cotyledon are used. A quality seed are standardized by surface sterilization using 1 to 2% NaCl solution for 10 min. Sterilized seed are transfer to nutrient medium (MS media ,Cytokinin ), for germination in presence of aseptic condition. We get 5 to 12 cm seedlings in bottle under controlled condition. Select the healthy seedling and separate hypocotyls and cotyledon under laminar air flow (LAR). Again the piece of hypocotyls and cotyledons are transferred to cytokinin known 0.2% medium derived from the MS(Murashige and Skoog) medium for regeneration. And put into controlled condition temperature 18 to 28 degree celcius in suitable light. After 8 day explants proliferated into mass of callus. This indicate the regeneration capacity of *Brassica juncea* species crop and variation of regeneration varies from hypocotyls to cotyledons. Which further use for genetic study , genetic transformation and crop improvement purpose.

**Keywords:** Regeneration, totipotancy, Embryogenesis.Sterilization.proliferation.MS medium. Transformation.

**Abbreviations:** MS media- murashige and Skoog (1962), NaCl-Sodium chloride, ABA-Abcsic acid, NAA-1-Napthalene acetic acid, PGR-Plant growth regulator, BAP-6-benzylaminopurine, LAR-Laminar air flow.

## I. INTRODUCTION

*Brassica juncea* belongs to the family Brassicaceae (Cruciferae) is an amphidiploids species with the genome (AA=20) of *B.compestris* L. and genome (BB=16) of the *B.nigra* L. Koch. (AABB=36) <sup>1</sup>. In India, the mustard is most important oilseed crop after groundnut accounting around 25% of total oil seed production. Indian mustard (Rai) cultivation has occupied about 85 to 90% of total area under cultivation of rapeseed and mustard <sup>2</sup>.Curently India account for about 12 to 13% of world oilseed area, 6 to 7 % of world oilseed output, 6 to 7 % of world oil meal production. In India rapeseed – mustard produced in area 21.1% and production 24.2% in among all the oilseed crops.<sup>3</sup>. The origin of *B.juncea* conflicting middle east seems to be the place of origin since the putative parents specie ,*B.nigra* and *B.compestris* would have been crossed (Olsson 1960, Mizushima and Tsunoda 1967).<sup>4</sup>. Rapeseed– mustard group of crop is grown in more than 70 countries globally on an area 31.68 mha with a production of 59.07 mton of seed and a productivity of 1,864 kg per ha. (FAO statistics ,2010) In India area (mha 6.51, 2010) Production (mton 7.67 , 2010) and productivity (kg/ha 1,179, 2010) <sup>5</sup>.

Oil content in brown sarson is 43%. Protein % in cake 38.52 to 42.2 % ,Ash % in cake 6.0 to 7.8 % . Iodine value 100.2 to 101.5 . Its oil generally rich in erucic acid ( 37.9 to 57%), it contan linolenic acid ( 4.7 to 13 %), the other important fatty acid present are linoleic acid (14% ) and oleic acid ( 13% ), Carbohydrate present in *B. juncea* are : total carbohydrate -(36.7%), free sugar (12.6%), Polysaccharide (2.2% ) , Composition of sugar as percentage are Stachyose 2.1%, Raffinose 1.2%, Melibiose 0.68%,Sucrose1.68%, Galactose 1.80%, Glucose 0.96%, fructose 2.10%,and other compound Pectic substances 6.5% and Cellulose 2.8%.<sup>6</sup>.

## II. MATERIALS AND METHODS

### A. Sterilization of Seed Surface

The seed material of *B.juncea* taken from the lab of proprietor (Allahabad Advance Agri Solution, Allahabad), 10gm. The first step to standardized our seed by surface sterilization. The seed must be surface sterilized to eliminate bacterial and fungal spore present on their surface. We achieved surface sterilization by washing them seed with distilled water properly for 20 min, and then with 1 to 2 % solution of sodium or calcium hypochloride for 10 min, then 0.1% of solution of mercuric chloride for-2 min, then the seed is rinsed several time with sterilized water to remove the disinfestant.<sup>7</sup>. This and the subsequent handling of explants or cultured cells and organ has to be done under aseptic condition, i.e in an environment free from bacteria and fungal spores through laminar air flow <sup>8</sup>.

### B. *M.S Media and Inoculation*

For inoculation of seed in a MS media, we have already prepared MS media stock solution by following the composition of MS tissue culture medium (Murashige and skoog, 1962, *Physiol plant* 15:473- 497). Take MS media in each sterilized cotton bed prepared bottle (bottle are sterilized by autoclaving at 121 degree celcius at 15 psi for 15 min. The pH is adjusted within a range of 5.6 to 5.8.<sup>9</sup>. Now inoculated the surface sterilized seed into MS medium under the aseptic condition and bottle closed by paraffin tape.

### C. *Callus Formation and Shoot Regeneration*

The inoculated seed are maintained under a controlled environment, particularly in term of temperature and light. The temperature varies from 18 to 28 degree celcius. light is essential for this. the culture room or the incubator should be kept as clean as possible it minimized contamination.

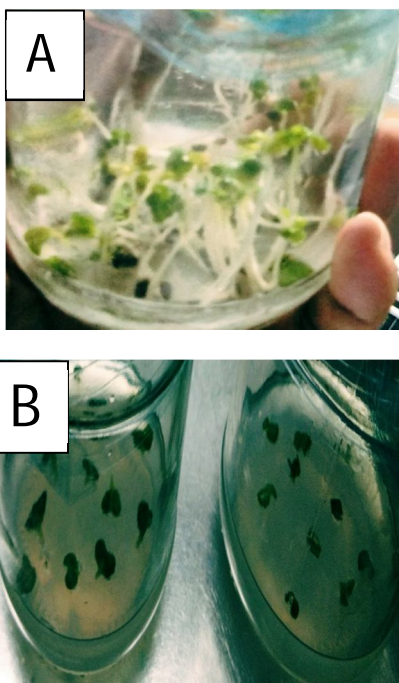


Fig. (A) Germinated seed under controlled condition (B) Sub culturing of seedling in form of cotyledon and hypocotyl under suitable medium.

### D. *Sub Culturing*

After a period of time 8 day the germinated seed take out and transfer to fresh basal MS medium which contain the concentration of 2,4-D (1-5mg/l), and sucrose (0.5 – 3%). Only cutting hypocotyl and cotyledon are used for sub culturing. The embryogenic calli, obtained from hypocotyl and cotyledon, were refered as HEC (Hypocotyl-derived embryogenic calli) and CEC (cotyledon-derived embryogenic calli), respectively.<sup>7</sup> In general callus culture are sub cultured every 4 to 6 week.

## III. RESULT AND DISCUSSION

The Indian mustard *B.juncea* seed start germination after inoculation in the MS medium. The matured seedling with two leaf stage were arisen at 8 to 10 days after germinating of the seed. In this attempt the hypocotyls and cotyledon of the germinating seed used for the sub culturing. After the regeneration we get normal diploid  $2n$  plantlet from hypocotyls and from cotyledon. The present study reveled that MS medium supplemented with 0.5 to 2.5 mg /litre of BAP along with 0.5 to 1.0 mg/l of NAA gives good response to maximum callus induction and shoot regeneration. Genetic modification can be done in the enzyme which involve in phytohormone production pathway, which can change the architecture of plant, as earlier genetic modification in gibberlin oxidase has been done to change plant architecture. Finally it was concluded that the regeneration protocal develop in the present investigation is reliable and it would be effectively utilized for the genetic transformation of Brassica species using different genetic trait of crop improvement.<sup>10</sup>

#### IV. CONCLUSION

After performing this experiment it may be concluded that the regeneration protocol developed in the present investigation for Brassica juncea is reliable and can be effectively utilized for genetic transformation.

#### V. ACKNOWLEDGEMENT

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