



In vitro propagation of *Brassica nigra* L. by using seeds

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Abstract

The major outcome of plant tissue culture is the regeneration ability of plants *In vitro* which is based on the principle of totipotency. The growth ability of plants is established by using organic media components which is supplemented with hormones and vitamins as growth regulators of varied concentrations. The work mainly talks on the study of different concentrations of plant growth regulators used for the induction of callus formation and the proliferation of roots and shoots in different media compositions. In *Brassica nigra* L. the direct development of shoots and roots without formation of callus was seen, which is due to the usage of seeds. The seeds were cultured in MS medium with different concentrations and combination of Hormones. The media prepared was MS1, MS2 and MS3 with different concentrations of growth regulators like auxin (NAA) and (BAP) which differed in three series of media. The Auxin and Cytokinin concentrations in MS2 media was kept more which was 5mg/L when compared to MS1 and MS3, so the growth rate in MS2 was more with the interval of time, so in the study it represented Murashige and Skoog is one of the most preferable media for the growth of mustard plant *In vitro*. The results obtained according to the time interval is presented in this paper.

Keywords: mustard, *In vitro* regeneration, seed, growth regulator

Introduction

Brassica nigra which is commonly called as a black mustard commonly consumed as spice and oil majorly in India. About 13.2% of the world's edible oil supply comes from this crop. This spice was seen during Sanskrit records which is back to about 3000 BC (Mehra *et al*, 1968) and considered as one of the domesticated crops. The word Mustard is derived from the Latin word Mustum. At first Romans started to use mustard as a condiment, and then started to use in Europe countries for seasoning dishes. Apart from being used as a condiment it has also got medicinal applications mainly for the remedy of Scorpion bites. *Brassica nigra* is endemic to the southern Mediterranean region. This crop leads to the evolution of several species within the genus Brassica.

It is an annual herbaceous plant which attains a height of about 1m. The main taxonomic feature of *Brassica* genus where the shape of the flower is cruciform with four petals. In recent years the oil seeds of *Brassica* species cultivation have increased to the greater level and have become the second largest contributor to the world's supply of vegetable oil (Zhai *et al*, 2001). Due to the growing world population and increasing industrialization the demand for edible oil and biofuels is increasing, thus cultivation of oil seed crops has gained great importance (Indrajit *et al*, 2008). *In vitro* regeneration and transformation have height prospects to fulfill breeding needs as conventional methods took abundant space when compared to the micro propagation. The study talks about the regeneration of seeds *invitro* but organogenesis has been achieved in a variety of explants. The *In vitro* culture of mustard seeds is inducted by organic supplements and by the usage of great promoters. Murashige and Skoog medium of varied concentrations is used for the growth of plants *In Vitro*. The major hormones used as the growth regulators are auxin (IAA) and cytokinin (BAP), coconut water is used as the organic supplement. Cotyledon explants produce the highest shoot regeneration frequency from four-day old germinated seedlings in comparison with non-germinated seeds (Suman Kashyap *et al*, 2019). The three media of varied concentration were MS1, MS2 and MS3; the concentrations of growth promoters in all the three Medias were interchanged and looked for the growth results. The auxin and cytokinin concentration in MS1, MS2, MS3 were 2 mg/L, 5 mg/L and 1 mg/L. In MS3 the concentration of the growth promoters is very less when compared to the other media concentrations. Amino acid Lysine in all three media concentration was 2 mg/L, 5 mg/L and 1 mg/L. Coconut water which is used as an organic supplement in all three Medias, which is 100 mg/L.

The pH in all three Medias was maintained about 6.5 to 7. As the concentration of growth promoters in MS2 is more compare to MS1 and MS3 the shoot and root formation was rapid and elongation was seen with the increasing in time interval. The development of seeds in prescribed conditions which was direct type of growth without the formation of callus. In the present research paper, the micro propagation of mustard seeds was used and the results obtained are presented.

Materials and methods

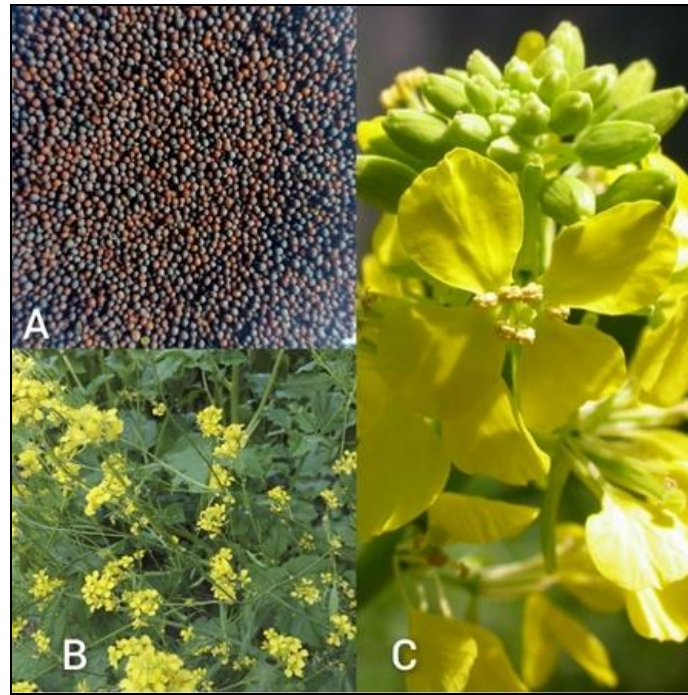


Fig 1: *Brassica nigra* L.A. Seed B. Habit C. Flowers.

The seeds of mustard (*Brassicainigra*) that belongs to the family Brassicaceae, which is used for the propagation of Plants *invitro* obtained from seed center of Bangalore and used in the laboratory of tissue culture in the college. In first part of the experiment surface sterilization of the explants was carried out and it is the initial stage for the sterilization of seed. The seeds of mustard were washed in the running water to remove all the debris which it contained with the help of 10% Teepol solution for about 15 minutes, after 15 minutes the continuous washing of the seeds carried out to remove all the foam which was accumulated, then the seed were transferred to culture bottle containing double distilled water. After the process of sterilization, the seeds were cultured for germination on MS media which was the most preferred media for the growth of mustard seeds *invitro*. The media used for the propagation of plants *invitro* was Murashige and Skoog media of varied concentrations to see the growth in different concentrations of media named as MS1, MS2 and MS3. This medium is supplemented with sucrose (30.0 g/L) which is common for all the three media as the energy supplement and it is common for all the three media. The concentration of auxin and cytokinin which differed in all the three media, Auxin(2, 4-D, alpha naphthalene acetic acid) and cytokinin (benzyl amino purine and kinetin) are used as the growth regulators. In MS1 the concentration of auxin and cytokinin is 2mg/L, MS2 5mg/L and in MS3 its 1ng/L. In different media the concentration of amino acid lysine was different as 2mg/L in MS1, 5mg/L in MS2, and 1 mg/L in MS3. When compared to other two media the concentration of MS3 was less and the results were recorded. The organic supplement for the media used was coconut water which is 100mL/L which kept constant for in all the three media for the experiment. The pH was adjusted at the ray of 6.5-7, then the addition of agar as the solidifying agent for the prepared solution. The media prepared poured on cultures bottles which was autoclaved further sterilized by maintaining temperature of about 121°C and pressure at 15 Psi for an hour, then the inoculation of the seeds was carried out in the Laminar Air flow Chamber. Under the Laminar air flow chamber the seeds from double distilled water is transferred to 0.1% Mercuric chloride to remove all the excess foam, this was carried out for the duration of 15 minutes with constant shaking. After 15 minutes of washing time the seeds were transferred to autoclave distilled water for less than a minute, this is carried out with a series of autoclaved distilled water. The seeds are transferred to hydrogen peroxide solution for less than a minute and kept in double distilled water, then inoculated in the series of media concentration. The inoculated culture bottles were incubated at 23°C under 16 hours of light and 8 hours of dark condition with the humidity of 80% for the successful growth of the plant. The growth of the seed was observed regularly for the initiation of root and shoot according to the time interval. The growth data was recorded on daily basis.

Result

In vitro propagation of cotyledonary explants of *Brassicainigra*. L was inoculated on M.S media of various concentrations of BAP and IBA. After sterilization of mustard seeds, they were cultured on MS medium for germination. Murashige & Skoog, 1962 of 3 various concentrations of IBA and BAP were used. Explants were swollen after 3-4 days of culture and appearance of shoot buds was noticed within a week, from the total of 3 combinations of BAP and IBA tested, MS medium with 5mg/L IBA and 5mg/It BA showed highest shoot regeneration, nearly (84.55%) of shoot regeneration frequency was achieved using cotyledon explants. MS

medium with 2mg/ It IBA and 2mg/L BAP showed (79.3 %) of shoot regeneration and MS medium with 1mg/ It IBA and 1mg /L BAP showed(50.5 %)of shoot regeneration frequency, hairy development of root was also observed within 12 days of culture. Callus was not seen in cotyledon explants. Progressive enhancement in germination of seeds was seen when seeds were treated with MS2 when compared to MS1 and MS3 with respect to time. The present study indicated it is possible to induce shoot regeneration directly on MS1, MS2, and MS3 but percentage of shoot regeneration showed variation.

Table 1: Table showing Growth of plant at different time point.

Media	Week	15 days	25 days	30 days
MS 1	0.4 cm	1.2 cm	2.5 cm	3.2 cm
MS 2	0.6 cm	1.7cm	2.7 cm	3.3 cm
MS 3	0.5 cm	1.4 cm	2.3 cm	2.9 cm

Once roots emerged from the plants, the plants were covered by plastic bags with holes for 5-6 days to maintain humidity, and then were transferred to the green house, and irrigation was done on alternate days with normal tap water, after 28- 30 days plants were produced showed no difference from seed derived control plants.

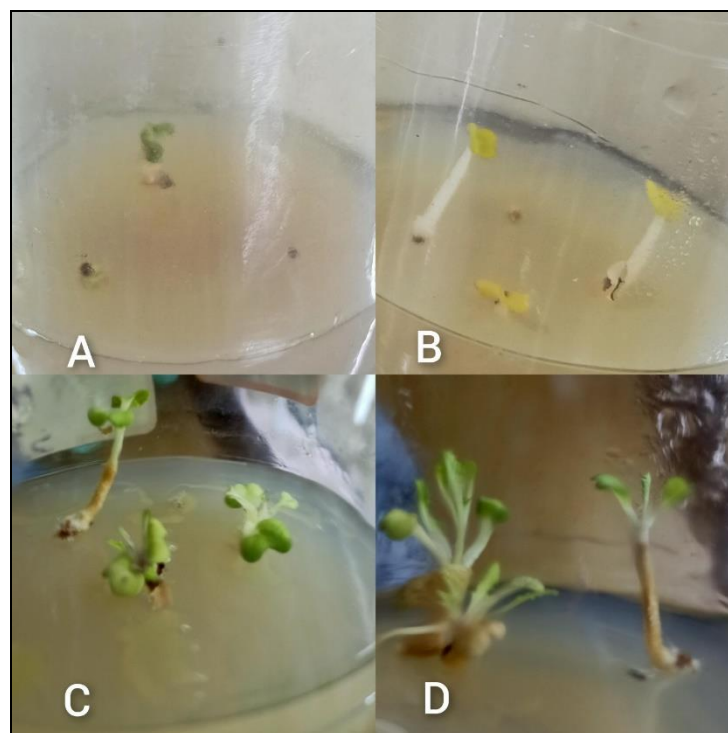


Fig 2: A) Growth seen after a week of inoculation. B) Growth seen after 15 days of inoculation. C) Growth seen after 25 days of inoculation. D) Growth seen after 35 days of inoculation.

Discussion

Mustard seeds were cultured on Murashige and Skoog medium supplemented with different concentrations of hormones. After 5-6 days on MS media generation of shoot buds was seen. Callus induction was not seen. MS media supplemented with IBA 5mg/l and BA 5mg/l showed best results within a week for shoot initiation. MS medium supplemented with NAA 2.0mg/l and BA 0.5 mg/l shown shoot initiation after 6days (Basaket *al*, 2012). Root initiation was reduced on interaction of MS 1, IBA 2mg/l and BA 2mg/l. Maximum of root initiation was seen in MS 2, IBA 5mg/l and BA 5mg/l. Root initiation was less on interaction with MS, 0.5mg/l NAA and was maximum in MS, 1mg/l NAA (Basak *et al*, 2012) [5]. Similar kind of results was obtained by Chen *et al* (2005) [2] and also by Kharb and Chowdury (1995) [1]. The plants had developed both roots and shoots without the requirements of separate rooting and shooting media as it is done in traditional tissue culture methods. Difference in concentration of growth hormones in the MS media has led to difference in shoot regeneration frequency in each medium, Initiation and formation of organ of plant cell involves dedifferentiation and redifferentiation of plant cells, these two processes are determined by exogenous plant growth hormones in the medium to some extent, it also depends on endogenous plant hormones The maximum number of shoots was observed in MS 2 medium and lowest number was observed in MS3. The highest number of shoot was observed in MS medium supplemented with IBA 5mg/l and BA 5mg/l. The number of shoots increased with increase in IBA and BA concentrations. Production of more plant were obtained in MS 2 medium with IBA 5mg/l and BA 5mg/l. Complete plants was obtained when shoots were rooted in MS medium with NAA 1 mg/l (George and

Rao, 1980). It was observed that *Brassicainigra*. L was best genotype for shoot and root induction under *invitro* condition. Therefore, further micro propagation might be successful with *Brassica nigra*. L variety.

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