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RESEARCH ARTICLE

EFFICIENT REGENERATION PROTOCOL FROM COTYLEDONARY PETIOLE EXPLANT OF *BRASSICA JUNCEA* VAR. NRCHB-101

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ABSTRACT

Establishment of a competent protocol for high frequency plant regeneration is done by analyzing a range of factors such as genotypes, combinations of plant growth regulator, types of explants, age of explants *etc.* in present study maximum shoot regeneration frequency observed was 56.5% for cotyledonary petiole on Murashige and Skoog (MS) medium supplemented with 2 mg/L BA (6-benzyladenine) and 0.2 mg/L NAA (1-naphthalene acetic acid). Out of all the explants reported from *Brassica* species cotyledonary explants generated the highest frequency in regeneration of shoots. The regenerated plantlets were acclimatized, hardened and transferred to field and grown to maturity in the greenhouse. All the regenerated plants obtained were fertile and morphologically indistinguishable with the source plants.

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INTRODUCTION

Brassica crops are the third vital source of vegetable oil globally, next to soybean and groundnut. Indian mustard (*Brassica juncea* L.) possesses drought tolerance, disease tolerance, tolerance to high temperature and is also involved in phytoremediation of heavy metals (Pandian *et al*., 2006, Clemente et al. 2005). *Brassica* species have been subjected to several genetic manipulations for crop improvement. Various plant tissues have been reported for shoot regeneration of *B. juncea viz.* shoot tips (Guruprasad *et al*., 2011), cotyledons (Chen *et al*., 2005), hypocotyls (Bano *et al*., 2010), petiole (Pua and Chi, 1993), leaf discs (Dutta *et al*., 2008; Bhuiyan *et al*., 2009), peduncle (Eapen and George; 1997), transverse thin cell layers (Bhuiyan *et al*., 2009), microspores (Prem *et al*., 2005), and protoplasts (Hu *et al*., 1999). Factors like genotype, explant age, source of explant, regeneration medium, growth regulators used and other physical conditions affects the regeneration of shoots from explants. *In vitro* culture of cotyledonary petiole and hypocotyls has been reported from almost all the species of *Brassica*.

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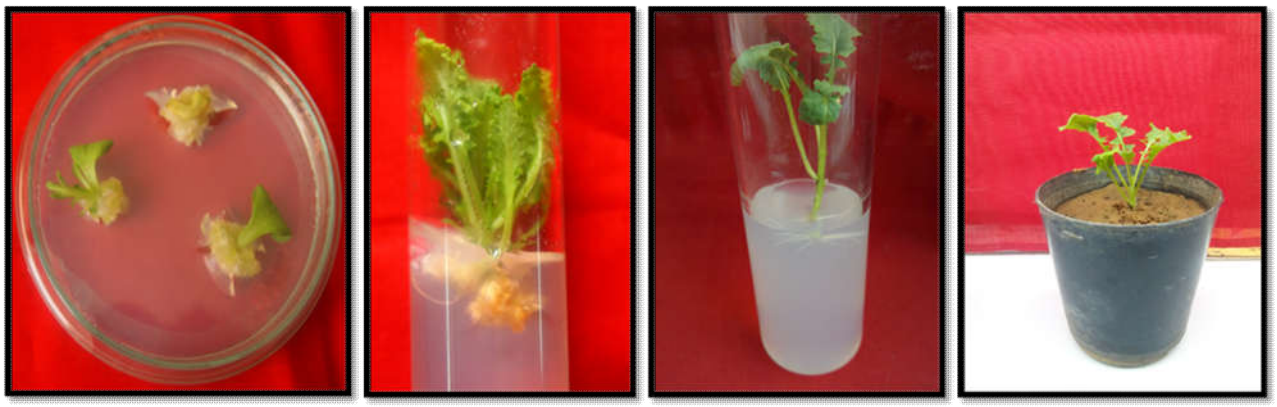
In the present communication, an account of a high efficiency plant regeneration protocol in *B. juncea* has been given by optimizing the above stated factors that can noticeably influence the efficiency of shoot regeneration.

MATERIALS AND METHODS

Plant material: *Brassica juncea* (L.) Czern. & Coss. variety NRCHB-101 was used to determine shoot regeneration. The seeds were obtained from National research centre on Rapeseed-Mustard (NRCRM), Bharatpur, Rajasthan.

Preparation of explants: The seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 3-4 min. and rinsed 3-4 times (for approx. 2-3 min) with distilled water. Water was drained completely and seeds were dried on sterile paper towel. Then the surface sterilized seeds were germinated aseptically on MS basal medium in Petri dishes (100 × 40 mm) (Murashige and Skoog, 1962) and were maintained at 25 ± 2 ºC, 60% relative humidity, 16 /8 hrs of photo/dark period along with cool fluorescent lights at an intensity of 30 µmol m^{-2} s^{-1}. Cotyledons with 2-3 mm petiolar region were excised from 5-days old seedlings.



(a) (b) (c) (d)

Figure 1: The regeneration process in cotyledonary petiole of *B. juncea* cv. NRCHB-101 (a) Callusing and shoot induction (b) Shoot elongation(c) Rooting (d) Hardening

Culture of explants: To opt for best possible shoot regeneration medium the explants (cotyledonary petiole) were cultured on MS medium supplemented with various combinations of BAP (1-5 mg/L) and NAA (0.1- 0.5 mg/L) along with 30 g/L sucrose . All media were adjusted to pH 5.7 by using 0.1 N NaOH, solidified by using agar (8g/L) and were autoclaved at 121^{o}C and 1 kg cm^{-2} for 15-18 min.

The cotyledonary petioles were placed upright with petiolar region in contact with the medium and were incubated in the same conditions as described above. Shoot regeneration analysis was carried out with three replicates for each treatment ten explants in each Petri dish (90 × 15 mm). After two weeks total number of explants having shoot buds was scored and numbers of shoots induced per explant were also counted. Shoot buds regenerated were sub-cultured on MS medium supplemented with 0.2 mg/L BAP in conical flasks (100 ml) for elongation of shoot. 2-3 cm long shootlets were further transferred to MS medium supplemented with different concentration of IBA (0.25-1.0 mg/L) for root initiation in the regenerated shootlets.

Acclimatization and Hardening: Regenerated plantlets with well-developed root system were transferred to the plastic pots containing autoclaved garden soil, farmyard soil and sand in the ratio of 2:1:1. These plantlets were irrigated with 1/8 MS basal salt solution and are covered with porous polyethylenes to maintain high humidity. 7-10 days old regenerated plantlets were transplanted from culture room conditions to field for further growth and development.

RESULTS AND DISCUSSION

In the present study effect of different growth regulators on shoot regeneration were observed in *B. juncea* var. NRCHB-101. The protocol established is functional for proficient micropropagation of *B juncea* var. NRCHB-101 to recover huge number of vigorous transformed plants during genetic transformation studies of *Brassica juncea*. In this experiment to set up regeneration protocol from cotyledonary petioles of *B. juncea* var. NRCHB-101, callus was induced in the explants in all the combinations of BAP and NAA used (Fig. 1a). Maximum callusing and shoot regeneration was observed on MS medium supplemented with BAP 2 mg/L and NAA 0.2 mg/L (Table 1).

Table 1. Influence of different combinations of phytohormone on plant regeneration from cotyledonary petiole of *Brassica juncea*

BAP (mg/L)	NAA (mg/L)	Callusing/explants Mean±SD	No. of Shoots/callus Mean±SD
1	0.1	6.9±0.25	1.9±0.15
2	0.2	9.7±0.03	4.9±0.11
3	0.3	5.5±0.87	1.5±0.25
4	0.4	8.7±0.55	2.2±0.55
5	0.5	3±0.30	1.2±1.09

Table 2. Effect of various concentrations of IBA on root formation in shoots of *Brassica juncea*

Auxin (mg/L)	Root/explants Mean±SD
IBA	
0	1.8±0.49
0.25	5.7±0.25
0.5	9.3±0.57
0.75	3.9±0.11
1.0	2.0±0.15

This was followed by medium containing 4 mg/L BAP and 0.4 mg/L NAA. However medium containing 4 mg/L BAP and 0.4 mg/L NAA exhibited low shoot regeneration response as compared to medium containing 2 mg/L BAP and 0.2 mg/L NAA medium (Table 1). It was also witnessed that lowering the concentration of BAP increased the shoot regeneration after callusing (Fig. 1b). Callusing and shooting was initiated in about 8-10 days and 12-14 days from inoculation of explants respectively (Fig. 1a). Maximum number of shoots per callus/explants was recorded to be 4.9 shoots Table 1). Though rooting was observed in explants on media containing no growth regulator but this response was very slow in regenerated shootlets. Medium supplemented with different combinations of IBA (0.25-1.0 mg/L) was found to be helpful for rooting. Maximum rooting was observed in media containing 0.25 mg/L IBA (Table 2).

Root formation was observed in about 15-18 days after inoculation of shootlets. (Fig. 1c). Plantlet of *B. juncea* var. NRCHB-101 with well developed root system were obtained after 8-10 weeks after inoculation of explants on different media (Fig. 1d). In the present study efficient shoot regeneration was witnessed on MS medium containing BAP 2 mg/L, NAA 0.2 mg/L in the absence of $AgNO_3$. While in another study on *B.oleracea* (Munshi *et al*., 2007) reported maximum shoots were regenerated on MS medium containing 2 mg/L BAP and 0.1 mg/L NAA.

Bangash *et al.* (2013) recorded highest shoot regeneration in medium having 3 mg/L BAP and 0.5 mg/L NAA in presence of 20 µM $AgNO_3$. One of the study reported by Guruprasad *et al*. (2011) showed similar results in shoot tip explants with maximum shoot regeneration on MS medium containing BAP 2 mg/L, NAA 0.2 mg/L. Similarly Mollika *et al*. (2011) reported BAP 2 mg/L, NAA 0.2 mg/L and and 0.5 mg/L Kinetin to be the best phytohormones combination for shoot regeneration in *B. juncea*. In *B. juncea*, stem segments cultured on medium containing combination of 2 mg/L BAP and 0.2 mg/L IAA was reported to regenerate shoots at 100% frequency (Sharma *et al.,* 2004). In present study induction of root and growth was witnessed on MS medium containing IBA 0.5 mg/L. Similar to our study root induction was observed on MS medium containing IBA 0.5 mg/L in *B. juncea* (Singh *et al.* 2009). While in another study, Ahmad *et al.* (1994) reported best rooting on half strength MS medium containing 0.5 mg/L IAA and Guruprasad *et al*. (2011) reported maximum rooting in shootlets on MS medium containing 1 mg/L NAA.

Conclusion

Thus from above conducted experiment the regeneration protocol so developed for *B juncea* var. NRCHB-101 has been proved to be more efficient and productive which will be helpful for numerous genetic transformation investigations to be done to increase the yield of economically important varieties of *Brassica*.

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