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Effect of Genotype and Explant on Shoot Regeneration in *Brassica juncea*

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ABSTRACT

In order to utilize biotechnological approaches for the improvement of *B. juncea,* a high frequency reproducible protocol for complete plantlet regeneration is required. With this aim cotyledon and plumule explants derived from 5 day old seedlings of five different cultivars of B. *juncea* were cultured on MS medium supplemented with 1.0 mg/l BAP and 0.2 mg/l IAA. Varuna exhibited significantly higher frequency (85%) of shoot regeneration than other genotypes, whereas RH 30 exhibited more number of shoot regeneration per explant but the frequency of shoot regeneration in this variety was poor than that of Varuna. The explants, cotyledon and plumule from Varuna produced the highest frequency (75% and 95%) of shoot regeneration, whereas both the explants from RH 30 produced highest number of shoots per explant. It is clear that different genotypes and explants have different potential for frequency of shoot regeneration as well as number of shoot regeneration per explant.

Keywords: *Brassica juncea,* Genotype, explants, shoot regeneration

INTRODUCTION

Brassica juncea (L) Czern & Coss is one of the most important oil seed crop in the world. The main emphasis in mustard research programme is given to the development of high yielding, disease resistant varieties with improved oil quality, i.e. low erucic acid and linolenic acid content. Conventional breeding has achieved some initial gain in the improvement of yield and quality of mustard. But due to a lack of genetic variability, chances of further improvement through conventional breeding appears to be bleak (Rai, 1996). Recent approaches to induce genetic variability through induced mutagenesis have been found quite useful for qualitative and quantitative traits of economic importance. However extent of desirable variability is quite limited (Anuradha *et al.,* 1992). Thus, it is inevitable to utilize recent biotechnological approaches, *viz.,* somatic hybridization, cybridization (Kirti *et al.,* 1992a, Kirti *et al.,* 1992b; Prasad *et al.,* 2010) and genetic transformation (Barfield and Pau, 1991) to create genetic variability.

In order to utilize biotechnological approaches for the improvement of *B. juncea*, a high frequency reproducible protocol for complete plant regeneration is a prerequisite. Although, there are reports on regeneration of plantlets from somatic tissue, particularly cotyledons (George and Rai, 1980; Narasimhulu and Chopra, 1988), hypocotyls (Kirti and Chopra 1989a, Barfield and Pau, 1991) and protoplast (Kirti and Chopra, 1989b) of *Brassica* but most of the studies are limited to optimization of growth regulator required for regeneration. Therefore, an understanding effect of explants and genetic factors regulating morphogenesis may help in the development of regeneration protocol with wider applicability than physiological approach.

MATERIALS AND METHODS:

Sterilization of seed: The seeds of five cultivars, *viz.,* Varuna, Pusa Bold, RH30, RLM514 and RLM 619 of *B juncea* were surface sterilized by keeping the seeds in 1.0% (v/v) cetrimide solution for 5 minutes, followed by

transferring them to 0.1% (w/v) mercuric chloride solution for 10 minutes and quick dipping of seeds in 70% alcohol after $HgCl_2$ treatment for 20-30 second. The surface sterilized seeds were washed 5 times in sterilized distilled water to remove traces of $HgCl_2$ and alcohol, etc.

Seed germination: The surface sterilized seeds of five cultivars were transferred in culture tubes containing semi-solid half strength MS (Murashige and Skoog, 1962) basal medium. These cultures were kept in culture room maintained at 24±2 ^{0}C for seed germination under 25 $\mu Em^{-2}\ s^{-1}$ light intensity for 16/8 hr photoperiod.

Preparation of explants: Cotyledon and plumule explants were excised from 5 day old *in vitro* grown seedlings of five cultivars of *B. juncea.* These explants were transferred on regeneration medium containing 1mg/l BAP and 0.2 mg/l IAA.

Culture Conditions: Cultures were incubated at 24±2 ^{0}C under 16/8 hr white light from cool fluorescent tubes at unit of irradiance 25 $\mu Em^{-2}\ s^{-1}$ for shoot and root regeneration.

Statistical analysis of data:

Data were recorded as percentage of explants showing shoot regeneration and number of shoots regenerated per explant. The experiment was conducted according to a nested design. Each experiment had two replicates. The analysis of varience carried out to detect the significant differences among the treatment means (Steel and Torrie, 1980; Gomez and Gomeze, 1984). The experiment means were compared using DMRT.

RESULT AND DISCUSSION:

Both explants, *viz.,* plumule and cotyledon isolated from 5 day old *in vitro* grown seedlings of five cultivars, namely Varuna, Pusa Bold, RH 30, RLM 514 and RLM 619 of *B. juncea* were transferred on M S medium containing 1.0 mg/l BAP and 0.2 mg/l IAA respond differentially. The cotyledon explants exhibited expansion of its surface and formation of little amount of callus at their petiolar cut end after 8-10 days of culture. Small greenish nodular structures developed in calli after 8-10 days. These nodular structure on further development produced shoot buds and shoots. However, plumule explants show swelling on regeneration medium which later on resulted in multiple shoot regeneration without callus. Initially, the growth of first regenerated shoot was fast and later on subsequently arrested.

Analysis of variance showed that the variety and explants within variety had significant effect on frequency of explants showing shoot regeneration and number of shoots regenerated per explant (Table 1).

Table 1: Analysis of variance for the effect of variety and explant on frequency of explants showing shoot regeneration and number of shoots regenerated/explant.

Source of variation	Degrees of freedom (df)	Mean squares	
		Frequency (%) of explants showing shoot regeneration	Number of shoots/ explant
Variety	4	632.5**	4.03**
Explant within variety	5	1270.0**	3.10**
Within Varuna	1	400.0**	0.81**
Within Pusa Bold	1	1600.0**	5.06**
Within RH 30	1	625.0**	4.0**
Within RLM 514	1	1225.0**	1.82**
Within RLM 619	1	2500.0**	3.80**
Error	10	30.0	0.046

***P<0.01*

Table 2: Comparison by DMRT among varieties of *B. juncea* for the frequency of explants showing shoot regeneration and number of shoots regenerated/explant. Each mean is based on two replicates, each replicate had 20 cultures.

Variety	Frequency (%) of explants showing shoot regeneration	Number of shoots/ explant
Varuna	85.0^b	4.45^b
Pusa Bold	75.0^b	4.72^b
RH 30	72.5^b	5.5^c
RLM 514	57.5^a	3.22^a
RLM 619	55.0^a	3.17^a

**Different letters in the superscript indicate significant difference between means (P<0.05).*

Table 3: Comparison by DMRT between cotyledon and plumule explants within varieties for the frequency of explants showing shoot regeneration and number of shoots regenerated/explant. Each mean is based on two replicates, each replicate had 10 cultures.

Variety	Frequency (%) of explants showing shoot regeneration		Number of shoots/ explant	
	Cotyledon	Plumule	Cotyledon	Plumule
Varuna	75^c	95^b	4.0^b	4.90^b
Pusa Bold	55^b	95^b	3.60^b	5.85^c
RH 30	60^b	85^{ab}	4.50^b	6.5^d
RLM 514	40^a	75^a	2.55^a	3.9^a
RLM 619	30^a	80^a	2.20^a	4.15^a

**Different letters in the superscript indicate significant difference between means (P<0.05).*

A comparison by DMRT revealed that response of each variety or genotype differed significantly with respect to the frequency and number of shoot regeneration. Varuna exhibited significantly higher frequency (85%) of shoot regeneration than other genotypes, whereas RH 30 exhibited more number (5.5) of shoots regeneration per explant but the frequency of regeneration in this variety was poor than that of Varuna. Pusa Bold exhibited more shoots per explant, while the other two genotypes were comparatively poorer than Varuna and Pusa Bold in shoot regeneration (Table 2). Variation in shoot regeneration due to genotypes has been reported in tissue culture of many species *viz., Vigna radiata* (Singh *et. al.,* 1986), *Brassica species* (Murata and Orton, 1987) and *Nicotiana tobacum* (Ogura and Tsuiji, 1977). Frankenberges *et. al.,* (1981) concluded that some recessive genes were associated with shoot regeneration in tomato. However, additive gene action for shoot regeneration was reported in cauliflower (Buiatti *et. al.,* 1974).

A comparison by DMRT for the effect of different explants (cotyledon and plumule) within variety revealed that both explants behaved differentially in different varieties for frequency of shoot regeneration and number of shoot regenerated per explant. Cotyledon and plumule of Varuna produced the highest frequency of shoot regeneration *viz.,* 75% and 95% respectively, whereas both explants (cotyledon and plumule) of RH 30 produced highest number of shoots per explant *i.e.,* 4.5 and 6.5, respectively (Table 3). Differential behavior of explants with respect to shoot regeneration has been reported in *B. juncea* (Tyagi and Rangaswami 1997), *Vigna radiata* (Singh *et al.,* 1986). Same explants obtained from different varieties respond differentially. The varying responses of same explants of different variety were reported in *B. juncea* (Kirti and Chopra, 1989b, Pental *et al.,* 1993). From the above result, it is clear that different varieties and explants have different potential for frequency of shoot regeneration as well as number of shoot regeneration per explant.

In order to obtain complete plantlets, shoots of 1.5-2.0 cm were excised aseptically and transferred on M S medium containing 0.2 mg/l IBA. Healthy plantlets with long roots were obtained at this concentration. Root regeneration also occurred on auxin free medium but the frequency and number of roots per shoot were lower than those on IBA containing medium.

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