Development of interspecific hybrids of *Brassica juncea* and *Brassica alba* using in *vitro* culture for improving *Alternaria* blight resistance

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Abstract

White mustard (*Brassica alba*) is an important source for *Alternaria* blight resistance and can provide valuable source for transferring this trait in Indian mustard (*Brassica juncea* L. Czern & Coss). Unfortunately the traditional methods have failed to generate the interspecific hybrids which are vital for improving *Alternaria* blight resistance in Indian mustard. Efforts were made to rescue interspecific hybrids through *in vitro* culture. The interspecific crosses were made between two promising *Brassica juncea* cultivars viz. Varuna and RH 0345 and *Brassica alba* and immature siliqua containing developing seeds were collected at 20 Days after pollination (DAP). These were surface sterilized and developing seeds were cultured on selected Murashige and Skoog (1962) modified media. The hybrid seedlings were developed in MS medium without growth regulators and supplemented with 500 mg/l casein hydrolysate and MS medium supplemented with 2.5 mg/l kinetin. Both the media were found quite promising in rescuing interspecific hybrids. The plants exhibited morphological characteristics of both the parents in cultures. These plants are being maintained in vitro through nodal cultures. The plants will be evaluated under field conditions in the coming season for selection of desired hybrids for better *Alternaria* blight resistance and other agronomic characteristics.

Key words: Indian mustard (*Brassica juncea* Czern & Coss.), White mustard (*Brassica alba*), Interspecific hybridization, In vitro culture, *Alternaria* blight

Introduction

Brassica juncea (2n= 36) is the major oilseed crop of Northern India and contributes nearly 27% of the edible oilseed pool of the country. The productivity of *Brassica juncea* is affected by several biotic and abiotic factors. Fungal diseases are the main biotic stresses in oilseeds which result in considerable yield and economic losses in India as well as around the globe. *Alternaria* leaf blight caused by *Alternaria brassicae* and *A. brassicicola* is a serious disease of Indian mustard (*Brassica juncea*) and results in 30-50% yield losses alone in the country. The disease and pest resistance of *Brassica juncea* has to be substantially enhanced in order to stabilize production and realize the yield potential. Genetic variation for disease and pest resistance is not adequate. Therefore, Studies on the interspecific hybrids within Brassicaceae family should be undertaken to increase the diversity of economically important traits of oilseed rape such as *Alternaria blight* resistance. Focus on alien germplasm via interspecific and intergeneric hybridization, radiation or chemically induced mutations, identification and cloning of respective genes controlling disease resistance from wild germplasm into susceptible cultivars can substantially improve this important oilseed crop. Wide hybridization can be attempted using conventional breeding methods. A stable and valuable *Alternaria* resistance has been introduced by a backcross program using the hybrids *B. napus* × *Diplotaxis erucoides* (Klewer *et al*. 2002). Interspecific hybridization can be easily forced in the family of *Brassicaceae*, whereas under natural conditions the gene flow is very limited (Brown and Brown 1997). Sexual incompatibility barriers can be overcome by pollination of very young buds and subsequent ovule or embryo rescue techniques (Takeshita *et al*. 1980; Inomata 1985). However, wide variability among different combinations was observed in the ability of pollen to germinate on the stigma, the frequency of pistils showing pollen tubes, and the ovule fertilization efficiency (Brown and Brown 1996). There is no resistant source in *Brassica juncea* for *Alternaria* blight, however, *Brassica alba* is resistant. Conventional breeding methods have not been successful to recover interspecific hybrids. In vitro culture of ovules and embryo rescue through in vitro culture provide a good opportunity to recover interspecific and intergeneric hybrids (Siemens, 2002).

Materials and Methods

Three varieties/genotypes of *Brassica juncea* L. namely RH0345, Varuna and RH 8812 and *B. alba* were grown in the Experimental farm area of Oilseeds section, Department of plant Breeding, CCS Haryana agricultural university, Hisar. All flowers past anthesis were removed from three to five inflorescences of each maternal plant used in this study. Bud pollinations were accomplished by removing approximately one-eight to one-fourth of the top of the calyx without damaging the emerging stigma. Pollen was applied next day of emasculation to the exposed stigma from a dehisced anther of the selected donor parent. Swollen siliquae were excised between 5 to 20 days after pollination (DAP) and aseptically cultured in modified MS medium. Alternatively siliquae were brought to laboratory and surface sterilized with mercuric chloride (1% w/v) for 5-8 minutes followed by three washings with sterilized distilled water in laminar flow. Sterilized siliques were dissected aseptically exposing developing ovules. Developing ovules (both healthy and shriveled) were placed into 150 × 15 mm tubes

containing 20 ml of two modified Murashige and Skoog (1962) media given below-

1. Murashige and Skoog (1962) medium supplemented with 0.5 gL^{-1} casein hydrolysate
2. Murashige and Skoog (1962) medium supplemented with 2.5 mgL^{-1} Kinetin

All the cultures were incubated at 26°C under illumination providing photoperiod of 16 hr. light and 8 hrs. of dark. After 4 weeks, germinated ovules were transferred to culture 150 ml flasks containing MS media supplemented with 2.5 mgL^{-1} kinetin and MS medium without growth regulators for shoot initiation and elongation. Regenerated plants were rooted in medium containing MS medium supplemented with 0.1 mgL^{-1} auxin (NAA) for further development. Later these plants were taken out of culture vessels and washed gently to remove agar and transferred to small plastic pots containing sterilized sand-soil mixture. Initially the plants were coved with polythene bags to retain moisture. Well established plants were moved to greenhouse.

Results

The cultured ovaries started growing and were found swollen within a week of culture Fig.1). Callus induction was observed at the excised end of the ovary. Further, the recovery of seeds was very poor. In general, the seed was poorly developed and shriveled. The recovered seeds do not germinate and no inter-specific hybrids were obtained. Mohapatra and Bajaj (1987) also reported poor success of ovary culture and reported only 16.6% germination of hybrid seeds. The ovule culture of Inter-specific hybrids gave promising results. The cultured ovules started growing readily and germinated to form shoots (Fig. 2). We did not observe any callus formation. The plants were recovered in all the three crosses attempted and gave variable success. Both the media supported ovule development and growth. The ovules excised from less than 7 DAP siliquae did not form plants, however later stages (10-15 DAP) gave good success. The Developed hybrid plants were also successfully cloned by node culture and maintained in laboratory (Fig.3). The plants were transferred to soil conditions in greenhouse and exhibited the morphological characters of both the species. The plants are under evaluation in this season for disease reaction.

Discussion

The ovule culture was developed to recover inter-specific crosses involving *Brassica juncea* and *B.alba* which may be very important for breeding programs aimed at introducing useful genes, e.g., resistance genes for *Alternaria* into *B. juncea* crop plants. Starzycki *et al*. (1999) used embryo rescue method to produce hybrids between *B. oleracea* var. gemmifera *(D.C.), B. oleracea* var. acephala, *B. oleracea* var. acephala subvar. Lacinista and *B. napus* to obtain yellow seeded genotypes. The objectives of this study were twofold. The first was to develop an ovule culture protocol specific to *Brassica juncea* local genotypes for the regeneration of immature ovules from interspecific hybrid crosses and, if necessary, from subsequent backcross generations. Further it could be used to restore fertility in the male sterile hybrid intermediaries by inducing amphidiploidy with *in vitro* colchicine treatments. Dierig *et al*. (2001) used colchicine treatment to overcome male sterility by inducing amphidiploidy. They submerged meristems of recently initiated shoot explants completely in a MS shoot regeneration media containing 0.1% colchicine for 48 hr. This process was repeated on the F_1 interspecific hybrids, utilizing primarily a *L. fendleri*, high oil germplasm line, WCL-LY2, as the paternal donor, to produce several BC_1 and one BC_2 backcross generation.

Table 1

Interspefic crosses	MS Medium +CH 500 mg/l		MS Medium + 2.5 mg/l Kinetin	
Brassica juncea L. genotypesX *B.alba*	Number of ovules cultured	Percent response	Number of ovules cultured	Percent response
RH0345	54	55.5	62	67.7
Varuna	38	52.6	55	63.6
RH8812	48	45.8	50	44.0

Conclusions

In summary, intra- and intergeneric hybrids point to value of introgression from wild relatives but also to the bottle-neck of the hybridization approach. Genetic stability of the resistance traits in the genetical background of *B. juncea* within the subsequent backcross generations is the crucial characteristic for an introgression of new genes in the gene pool of oilseed Brassica.

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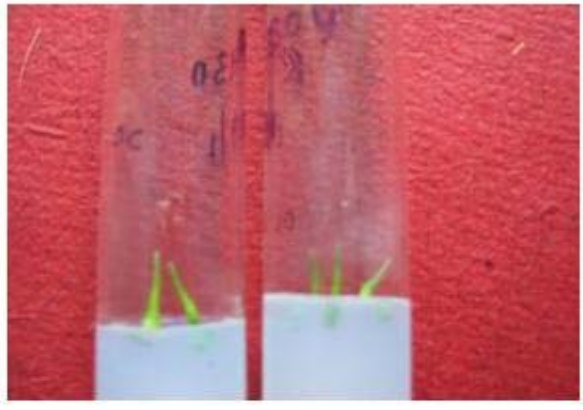

Fig.1 Cultured ovaries of Interspecific cross between *Brassica juncea* L. RH 8812 and *Brassica alba*

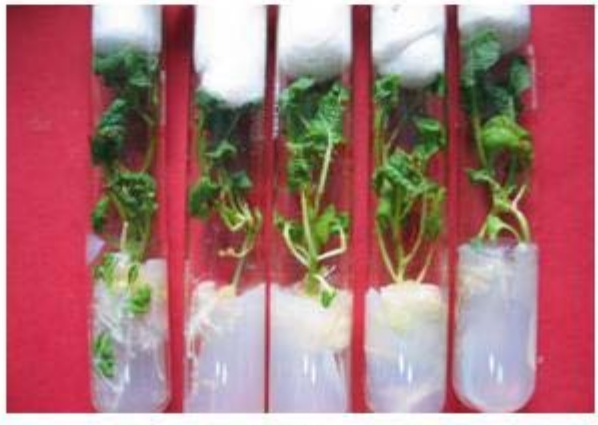

Fig 2 Plant regeneration from cultured ovules of Interspecific cross between *Brassica juncea* L. RH 8812 and *Brassica alba*

Fig 3 Regenerated Interspecific hybrid plants multiplied through node culture ready to be transferred to soil