Characterization of Stable Somatic Hybrids of *Sinapis alba* and *Brassica juncea* for *Alternaria blight*, *Sclerotinia sclerotiurum* Resistance and Heat Tolerance

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ABSTRACT

The wild members of family brassicaceae are treasurers for invaluable genes of resistance. Sinapis alba *a wild member of this family was reported to carry resistance to various biotic and abiotic stresses. Therefore, we evaluated here two symmetric somatic hybrids (allohexaploids = AABBSS) of* B. juncea *(AABB) and* S. alba *(SS) developed by protoplast fusion for biotic and abiotic stresses. These two allohexaploids of brassicas referred hereafter as H1 and H2. The hybrids were evaluated against two major diseases of rapeseed-mustard i.e. Alternaria blight and Sclerotinia stem rot including tolerance to high temperature. The results of the study showed that H2 somatic hybrid showed very high degree of resistance against* Alternaria *blight. While, H1 somatic hybrid possessed complete resistance to Sclerotinia stem rot along with Alternaria blight. However, both the hybrids expressed very good tolerance to high temperature at the time of seed setting. These hybrids were screened for disease resistance against the Alternaria blight and Sclerotinia stem rot in four different environments during seven successive seasons. These two allohexaploids also remained stable and completely fertile after the eight subsequent generations. Therefore, these somatic hybrids are novel genetic stocks to develop climate resilient varieties.*

Key words: *Sinapis alba; B. juncea ; Alternaria blight; Climate resilient varieties;Sclerotinia stem rot;*

B*rassica juncea* is a major oil seed crop of India and grown into 85 per cent of total production area of cultivated Brassicas (*Darekar and Reddy, 2018*). The production of the *B. juncea* was limited by various biotic factors such as *Alternaria* blight, *Sclerotinia* stem rot, white rust and abiotic stresses such as heat and drought. It was estimated that alone Alternaria blight can reduce seed yield up to 47 per cent and in some cases much higher too (*Kolte et al. 1987, Shukla 2005*). However, *B. juncea* have many high yielding varieties but all of these lack tolerance to abruptly changing environmental conditions. Some diseases became devastating in such conditions viz. *Sclerotinia* stem rot and *Alternaria* blight. The genetic resistance source for these diseases is not available within cultivated brassicas. However, some wild relatives carried genes for resistance to these diseases and tolerance to abiotic stresses (*Conn et al.1988; Sharma et al.2002*). *S. alba* is a wild member of brassicaceae (Warwick and Black 1991) carried various desirable traits such as yellow seed colour, tolerance to high temperature and drought (*Primard et al.1988; Brown et al. 1997*) resistance for *Alternaria* blight (*Hansen and Earle, 1997*) and Sclerotinia stem rot (*Morrall and Dueck 1982, Li et al. 2009*). Therefore, with the aim of introgression of these invaluable genes into cultivated Brassicas, we have introgressed complete nuclear genome of *S. alba* into *B. juncea* maternal back ground through protoplast fusion (*Kumari et. al. 2017*). Two symmetric somatic hybrids (H1&H2) conferred the resistance to *Alternaria* blight and high temperature tolerance at the time of seed setting and germination apart from these H1 also possess resistance to *Sclerotinia stem rot*. In present investigation, we have conducted the disease screening experiments to check resistance

status in present genetic stocks (H1 & H2) in four different environments up to seven subsequent generations.

METHODOLOGY

The somatic hybrids (H1&H2) along with their fusion partners (*B. juncea* and *S. alba*) were sown in net house at National Research Centre on Plant Biotechnology, IARI, New Delhi for generation advancement and disease screening against the *Alternaria brassicae* and *Sclerotinia sclerotiorum* pathogens. The hybrids were also evaluated for high temperature tolerance and data was recorded. The following methods were adopted to screen somatic hybrids against the Alternaria blight and Sclerotinia stem rot in all environmental conditions:

Resistance screening for Alternaria brassicae: The virulent *Alternaria brassicae* culture was procured from ITCC, IARI, New Delhi (ITCC No. 2542) and maintained on brassica dextrose agar medium at 4°C temperature for artificial inoculation of back cross progeny. The conidia were harvested after 96 hours from culture in sterilized double distilled water. The conidial concentration was maintained at 10^6 ml^{-1} in dd H_2O. The somatic hybrids (H1 & H2) were inoculated on lower leaves at artificially scratched portions by pathogen spore suspension and sticking of conidial discs on the leaves. Humidity around these plants was maintained by spraying sterilized distilled water for seven successive days. The blight lesion size (Lesion size = length x width) was recorded from 7 to 10 days after inoculation of pathogen. The resistance or susceptibility of both hybrids was estimated according to per cent blighted leaf area (BLA). The grading for resistance responses was done as no lesion (immune), 0-10 per cent BLA (highly resistant), 11-20 per cent (resistant), 21-30 per cent (moderately resistant), 31-40 per cent (tolerant), 41-50 per cent (moderately tolerant), 51-60 per cent (susceptible) and Ã60 per cent (highly susceptible).

Resistance screening for Sclerotinia sclerotiorum: The virulent culture of *S. sclerotiorum* was procured from ITCC, Indian Agriculture Research Institute, Pusa, New Delhi (Accession No: ITCC-3292). The fungal culture was multiplied on potato dextrose agar medium containing petriplates. The *S. sclerotiorum* culture was maintained for further experiments on culture media at 24±1 ºC under alternate 12 hours light and dark conditions. The sclerotia developed in the petriplates were harvested after 24-29 days and maintained at 4ºC for further use. The hybrids (H1 & H2) and parents (for resistant and susceptible checks) were screened with *S. sclerotiorum*. A total of five plants from each hybrid were selected for pathogen inoculation after initiation of flowering. The selected plants were inoculated with 6 mm discs of actively growing virulent pathogen on agar plates at just above the first node and tied with parafilm (*Li et al. 2007*). The disease progress was monitored regularly and final stem lesion lengths were recorded after 15-18 days of inoculation. The plants were categorized according to lesion size in immune (no disease sign), highly resistant (0-2.0 cm), resistant (2.1-4.0 cm), moderately resistant (4.1-6.0 cm), tolerant (6.1-.8.0 cm), susceptible (8.1-10.0 cm) and highly susceptible (more than 10.0 cm) categories.

RESULTS AND DISCUSSION

The two somatic hybrids (H1&H2) of *B. juncea* and *S. alba* carried complete nuclear genome of the parents. These symmetric somatic hybrids have very good male and female fertility. However, all the previous hybrids of *B. juncea* and *S. alba* were reported male sterile (*Gaikwad et al., 1996*). We have reported first time both the somatic hybrids (H1&H2) not only governed high degree male and female fertility but they were also stable over the generations (*Kumari et al., 2017*). All the somatic hybrids of the *B. juncea* + *S. alba* reported earlier were not survived due to poor fertility (*Gaikwad et al., 1996*). Therefore, the hybrids reported here are very important genetic resources for identification of genes responsible for durable resistance against *Alternaria* blight and *Sclerotinia* stem rot pathogens. The screening through *in –vitro* detached leaf assay revealed that *B. juncea* leaves showed the development of necrotic spots with the yellowness at the site of inoculation on the third day after inoculation whereas somatic hybrid H1, H2 and *S. alba* were not developed any disease symptom. We obtained similar results under *in-vivo* conditions in net house during 2015-2016 when same set of experiment was conducted. Both the somatic hybrids proved their resistance nature against the highly virulent strain of *A. brassiceae* in season II after challenging *in-vivo* conditions at the IWBR, Regional station, Shimla while the *B. juncea* showed the development of the concentric rings after the fifth day

of inoculation. *S. alba* plants were developed only two-three pin head like spots in some inoculated leaves. In season III, both the somatic hybrids showed hypersensitive reactions upon challenging with highly virulent strain of *A. brassiceae* on the fifth day of inoculation and no necrotic region was developed while the *B. juncea* RLM-198 again showed severe disease symptoms after fifth day of inoculation. Both the somatic hybrids revealed resistance against the *A. brassiceae* pathogen when inoculated in high humid and suitable temperature for disease development at the IARI, Regional station, Katrain while the *B. juncea* RLM-198 again scored as susceptible in season IV.

The genetic resistance of both somatic hybrids (H1&H2) again tested *in-vitro* and *in-vivo* when challenged with virulent strain of the *A. brassiceae* and as in previous experiments, both hybrids again not developed any disease symptom under *in-vitro* and *in-vivo* conditions in season V. However, the *B. juncea* revealed their susceptibility and acquired concentric rings on leaves and stems scored under susceptible category after 7th day of inoculation. The subsequent generations of the both hybrids along with parents again screened at IARI, Regional Station, Katrain during Jun to September 2018 when high humidity maintained due to heavy rain in season VI. The initial disease symptoms appeared from 3rd day of inoculation on the susceptible *B. juncea* RLM-198 and lesions spread severally from 4th to 8th day while the resistant donor *S. alba* developed pin head like lesions and rated at highly resistant scale. Likewise the previous generation, all replicate of the H1 and H2 were not attained any disease symptom and score as highly resistant on the 8th day of inoculation.

In season VII, both somatic hybrids along with their parents were screened at hot spot of Alternaria blight. The susceptible parent *B. juncea* RLM-198 was infected at the age of 75 days and complete leaves of the all replicates mated with concentric rings and all the leaves felled at the age of 85th days and silique were also infected and seed setting hampered due to disease. The stems of susceptible plants were also showed severe infection of the pathogen. However, both somatic hybrids were not acquired any disease symptom at the age of 90th days. However, 1-3 pin head like lesion observed after the 95th days of sowing on the leaves but these lesions were not converted into concentric rings and leaves persist till harvesting. The stems and silique were completely free from any disease symptom.

Li et al. (2009) produced somatic hybrids of *B. napus* and *S. alba* and screened BCF_4 lines of somatic hybrids against *Sclerotinia* stem rot disease. They observed variable reaction against the pathogen and found some lines strongly resistant than known resistant rapeseed variety 'Zhongshuang 9'. We observed that *B. juncea* lines were naturally infected with S. sclerotiorum and powdery mildew pathogens during the Alternaria blight screening experiment in second week of February while the somatic hybrid H1 planted near these infected lines devoid of any infection of both pathogens. However, replicates of H2 got infected up to some extent with S. sclerotiorum only. Thus along with *Alternaria* blight resistance, the somatic hybrid H1 showed high level resistance to S. sclerotiorum while the H2 displayed moderately resistance to the stem rot pathogen during 2015-16 in net house conditions. However, *B. juncea* found susceptible in the same environment and produced fungal sclerotia within stem. *You et al. (2016)* screened *Brassica oleracea* var. *capitata*, *Brassica juncea* and *B. napus* for field resistances against *Sclerotinia* rot. The result indicated that only one genotype of Indian *B. juncea* with weedy introgression showed significant resistance. In 2016-17, we again inoculated both hybrids with highly virulent strain of *S. sclerotiorum* at the age of 100 days when plants started seed setting and environmental conditions found favorable in the first week of the February in north India. The pathogen was not grown on stems of all three replicates of somatic hybrid H1 up to harvesting in field conditions. Similarly H2 was not showed typical disease infection up to some extent but few replicates of this hybrid developed a white colony on infected areas of the stems. However, all the replicates of *B. juncea* started to develop the lesions on the stems after 6th day of infection and these lesions developed up to 13 to 16 cm on 10th day of infection. Both the somatic hybrids along with their parents were screened during off season at the IARI, Regional Station, Katrain (H.P.). Due to rainy season the temperature and humidity favored the disease development. The inoculation and scaling remained same as adopted earlier. The somatic hybrid H1 consistently failed to grow the pathogen on stem while H2 again acquired small colonies of pathogen after the 9th day of inoculation. Similarly, *S. alba* also acquired small colonies along with the infected areas. *B. juncea* again developed typical disease

symptoms (12-16.5 cm) and stems were completely rotten up and sclerotia accumulate within the stems. We repeatedly inoculated both the somatic hybrids (H1&H1) along with their parents with highly virulent strain of the S. sclerotiorum. Again pathogen was failed to grow on all replicates of H1 while H2 acquired very small colonies but disease symptoms were not developed. Similarly, *S. alba* showed resistance like H2. However, *B. juncea* stems completely rotted and showed white cottony growth on the surface. The somatic hybrids and their parents again screened for S. Sclerotiorum during off season crop at IARI, Regional Station, Katrain (H.P.) and scored as a resistant to the Sclerotinia stem rot pathogen. While the *B. juncea* scored as a susceptible to the Sclerotinia stem rot disease and sclerotia developed within the stems. Similar results were obtained during seasonal screening under field conditions at the age 112 days. At maturity, *B. juncea* developed more sclerotia after the 12th day of the infection. H1 again scored highly resistant and H2 showed moderately resistance to the Sclerotinia stem rot disease. The somatic hybrid grown in different seasons at agricultural field of IARI showed flowering at 37 degree Celsius while seed setting was recorded >40^{0}**C**.

CONCLUSION

Although the brassica genus contains both diploid (2n=2x one set of the chromosomes/genome) and allotetraploids (2n=4x two set of the chromosomes/ genome) species but natural occurrence of allohexaploids is not known. We have synthesized two novel allohexaploid brassicas from *B. juncea* and *S. alba* somatic hybridization. In present investigation, we have evaluated both symmetric somatic hybrids (Allohexaploids: H1&H2) which are the novel genetic resource besides they carried very good fertility generation after generation. This is first report when somatic hybrids/ allohexaploids carried gametic fertility more than 90 per cent. The success rate of somatic hybridization was forfeit due to pollen immovability into somatic hybrids. These genetic stocks also displayed high degree of resistance against the most devastating pathogens such as *A. brassicae* and *S. sclerotiorum*. However, the cultivated *Brassicas* has no resistant source for such diseases. While, the durable source for high temperature tolerance was also not available within the cultivated germplasm. Therefore, these are desirable genetic stocks under cultivation for resistant breeders and farmers in the era of fungicide and changing climatic conditions. Fungicidal or cultural control measures offer inconsistent and expensive disease management options that were also not effective always, especially for high input and low return cropping systems. Effective host resistance is the most cost-effective and reliable means of disease control. Therefore, both the genetic stocks and their derived large population will used in identification of genes responsible for resistance/ tolerance for major biotic and abiotic stresses. In addition to above, the somatic hybrids are genetically stable therefore resistance of these somatic hybrids (H1&H2) will be maintained naturally generation after generation. These genetic stocks are valuable genetic resources for resistant breeding programs and novel genes searching. These new allohexaploid brassicas will hopefully uncover the resistant nature of the genetic stocks.

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