Assessing and utilizing variation available in wild crucifers for alternaria blight resistance to enhance Brassica germplasm

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ABSTRACT

The black spot caused by *Alternaria brassicae* (Berk.) Sacc. is a serious disease afflicting crop *Brassicas*. Economic losses may vary up to 40 percent. Despite considerable economic advantage, attempts to breed resistant cultivars have not been successful. These studies were thus undertaken to evaluate wild crucifers for resistance to alternaria blight and hybridize the resistant genotypes with cultivated Brassica species for introgression of desirable variability in cultivated germplasm. In all 94 accessions of wild and weedy crucifers belonging to six genera (*Brassica*, *Erucastrum*, *Camelina*, *Diplotaxis*, *Eruca* and *Sinapis*) were assessed for reaction to *Alternaria brassiceae.* The wild crucifers were first challenged to the prevalent racial mixture of pathogen under aided epiphytotic conditions. The ones found promising were subsequently challenged to the most virulent pure isolates(11C and 7T) of the pathogen using detached leaf method. Majority of the wild species showed susceptible reaction and only three : *Camelina sativa*, *Diplotaxis erucoides* and *Sinapis alba* showed resistant reaction on both leaves as well as pods with disease rating of 1 as compared to 5 in the control, *B. juncea*. As the objective of the present investigation was to introgress desirable nuclear variation, the crosses were attempted using crop *Brassica* species (*B.rapa, B.nigra, B.juncea*) as female parent. Modified ovary- ovule sequential culture was used to overcome post fertilization barriers. Out of 23 combinations attempted, four hybrids could be successfully produced.These were: *Brassica nigra/ Diplotaxis erucoides, Brassica rapa/ Diplotaxis catholica, Brassica juncea/Erucastrum abyssinicum* and *Brassica juncea/Diplotaxis tennuisiliqua.* Hybridity of these hybrids was confirmed by molecular markers (ISSR/SSR) as well as cytological analysis. The hybrids involving *Brassica juncea* showed varied level of fertility. Amphiploidy could be induced in both *Brassica nigra/ Diplotaxis erucoides and Brassica rapa/ Diplotaxis catholica* through application of colchicine. No hybrid with *Camelina sativa* could be produced. A possible hybrid plant, *B. rapa /Sinapis alba* was also obtained under cultural conditions. Its hybridity has been established at molecular level. The reaction of the amphiploids/crosses to alternaria infestation was studied. The amphiploid/ hybrids of *Brassica nigra/ Diplotaxis erucoides* and *B.rapa/Sinapis alba* showed resistant reaction.

INTRODUCTION

The black spot caused by *Alternaria brassicae* (Berk.) Sacc. is a serious disease afflicting crop *Brassicas* (Sharma and Tewari 1998). The pathogen infests both foliage and silique and the disease symptoms manifest as linear to oblong necrotic lesions that are brown in center with concentric rings and chlorotic halo. Economic losses may vary up to 40 percent. Variations in intra- and inter-specific yield losses may possibly reflect differences in racial pathogencity and resistance level of the prevalent cultivars. Despite considerable economic advantage, attempts to breed resistant cultivars using intra-specific or wide hybridization, mutagenesis etc. has not been successful. Well characterized nuclear genes conferring resistance to this serious disease are simply not available in the primary gene pool. This communication summarizes work undertaken to evaluate wild crucifers for resistance to alternaria blight and hybridize the resistant genotypes with cultivated Brassica species for introgression of desirable variability in cultivated germplasm.

MATERIAL AND METHODS

As a matter of strategy, wild crucifers were challenged to the prevalent racial mixture of *Alternaria brassiceae* under aided epiphytotic conditions. The ones found promising were subsequently challenged to the most virulent pure isolate of the pathogen using detached leaf method (Vishwanath and Kolte 1999). A total of 109 isolations were made from alternaria blackspot infested *Brassica* leaves collected from wide geographic spread. These were purified using single spore culture, 38% of these were pathogenic. The isolates were subsequently grouped on the basis of morphological variation and fungicide sensitivity assays. The grouping was subsequently confirmed on the basis of assembled differential set comprising 11 Brassica genotypes. Two of the isolates namely 11C and 7T were most

virulent. Both these isolates were used to confirm the resistant reaction of the wild crucifers identified putative for resistance to alternaria under aided epiphytotic conditions.

RESULTS AND DISCUSSION

The data of aided epiphytotic conditions is presented in Table 1. As is apparent from this table, majority of the wild species showed susceptible reaction. Only three: *Camelina sativa*, *Diplotaxis erucoides* and *Sinapis alba* showed resistant reaction on both leaves as well as pods with disease rating of 1 as compared to 5 in the control, *B.juncea*. Other promising species showing promising reaction ,at least at pod stage were *Brassica nigra*, *Crambe abyssinicum, Diplotaxis tennuisiliquae* and *Erucastrum abyssinium.* All these species maintained their reaction when challenged by most virulent isolates 11C and 7T using detached leaf method. Resistance reaction of *Diplotaxis erucoides* and *Sinapis alba* has been reported previously(Warwick,2011).

Hybridization of wild crucifers with *B.rapa/B.juncea*

In the past most of the wide hybrids involving wild species were developed using wild species as female (Prakash et.al.,2010). Recurrent backcrossing with the recurrent crop Brassica species invariably lead to cytoplasmic male sterility. As the objective of the present investigation was to introgress desirable nuclear variation the crosses were attempted using crop *Brassica* species (*B.rapa, B.nigra, B.juncea*) as female parent. Basic crossing strategy was to develop a bridging species for ultimate development of introgression lines. The basic sceme involved hybridization of *B. rapa* (AA)/ *B. nigra* (BB) as female parent.

Table1: Alternaria blight Reaction of wild crucifer species

Wild crucifer	Alternaria blight reaction *in vitro*		Alternaria blight reaction *in vivo* (Scale Rating)	
	Spot Size(mm)	Halo Size (mm)	Leaves	Pods
Brassica.chinensis	70	20	3	3
B.fruticulosa	70	20	3	3
B.maurorum	80	20	3	2
B .nigra	65	20	4	1
B.pekinensis	70	20	4	4
B.tournifortii	70	20	3	3
Crambe abyssinicum -57	60	20	5	1
C. abyssinicum -58	70	20	5	1
C. abyssinicum -59	70	20	5	1
C. abyssinicum -60	70	20	5	1
C. abyssinicum -61	70	20	5	1
C. abyssinicum -87	70	20	5	1
Camelina sativa EC-481684	70	20	1	1
Diplotaxis assurgens	70	20	3	3
D.barthauti	70	20	3	3
D.catholica	62	20	2	2
D. cossoniana	62	20	3	3
D.erucoides	60	20	1	1
D.tennuisiliquae	65	20	4	2
D.muralis	60	20	3	1
Erucastrum abyssinium	70	20	2	2
E.cardaminoides	55	20	2	2
Eruca sativa	65	20	3	3
Sinapis alba	80	20	1	1
Control	90	20	5	5

To facilitate it, a large number of parental genotypes of the target crop brassica species were used as female. Only those genotypes whose stigma showed germination of wild species pollen were used further to produce wide hybrids. Modified ovary- ovule sequential culture was used to overcome post fertilization barriers. Out of 23 combinations attempted, hybrids could be successfully produced involving : *Brassica nigra/ Diplotaxis erucoides, Brassica rapa/ Diplotaxis erucoides, Brassica rapa/ D.catholica, Sinapis alba/Brassica nigra, Brassica juncea/ Erucastrum abyssinicum* and *Brassica juncea/ Diplotaxis tennuisiliqua.*

Their hybridity was confirmed by molecular markers (ISSR/SSR) as well as cytological analysis. Hybrids *Brassica nigra/ Diplotaxis erucoside, Brassica rapa/ Diplotaxis erucoides* and *Brassica rapa/ Diplotaxis catholica* were highly sterile and showed aberrant meiosis. Varied number of bivalents (2-4) were observed. This level of bivalency was indicative of homoeology between the crop brassica and wild genomes. The hybrids involving *Brassica juncea* showed higher level of fertility as was indicated by greater pollen grain stainability of the hybrids. These also had higher seed fertility than that observed in hybrids between monogenomics .Amphiploidy could be induced in both *Brassica rapa/ Diplotaxis erucoides and Brassica rapa/ Diplotaxis catholica* through application of

colchicine.The hybrid, *Sinapis alba/ Brassica nigra* was highly sterile and despite repeated efforts amphiploidy could not be induced. The reaction of the amphiploids/crosses to alternaria infestation using detached leaf method is given below(Table 2).

Table 2: Reaction of newly developed amphiploids/hybrids to Alternaria isolates 11C and 7T

Hybrid/Amphiploid	Alternaria	
	Leaves	Pods
Brassica nigra/ Diplotaxis erucoides	2	1
Brassica rapa/ Diplotaxis erucoides	2	2
Brassica rapa/ D.catholica	3	2
Brassica juncea/Erucastrum abyssinicum	3	2
Brassica juncea/D.tennuisiliqua	3	2
Sinapis alba / *Brassica rapa*	1	1

No hybrid with *Camelina sativa* could be produced. A possible hybrid plant, *B.rapa /Sinapis alba* has been obtained under cultural conditions. Its hybridity has been established at molecular level .Cytological confirmation is still awaited. BC1F3 generation of a cross *D.erucoides*/*B.rapa* was also screened for resistance to alternaria under aided epiphytotic conditions. Although most of the plants in the segregating generation were CMS as a consequence of sterilizing cytoplasm of the wild species. The disease reaction using detatched leaf meathod from this cross was indicative of substantial variability for resistance in the available alloplasmic segregants involving wild crucifers.

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