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Evaluation of Brassicaceous Wild Relatives for Resistance to the Large White Butterfly, *Pieris brassicae* L.

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ABSTRACT

***Pieris brassicae* (L.), a brassica specialist, is one of the most destructive and widespread pests of cruciferous crops in many countries of the world. It causes about 40 per cent damage on cruciferous crops including cabbage and cauliflower which are the two major vegetables produced and consumed in India. Development of an insect resistant cultivar is a sought after goal in insect-pest management as it provides farmers with an effective, economically sound, and environment friendly option for pest management. However, the first step in the development of an insect resistant cultivar is precise knowledge of source(s) of resistance. In this study, we screened a diverse array of 63 brassicaceous accessions (mostly wild crucifers) under field as well as laboratory conditions to determine *P. brassicae* performance under free choice (in the field) and no choice (in laboratory) conditions. Resistant accessions were identified among *Brassica barrelieri, B. fruticulosa, B. maurorum, Crambe abyssinica, Diplotaxis muralis, D. tenuisiliqua, Erucastrum abyssinicum, Raphanus rugosum, Sinapis alba* and *S. arvensis.* Biochemical analysis of the putative accessions revealed that high concentration of total glucosinolates had a significant negative impact on insect development, while reverse was true for total phenols and total flavonols. Our findings may be useful for genetic improvement of both vegetable and crop brassicas aimed at development of cultivars resistant to *P. brassicae.* This research again shows the importance of crop wild relatives for finding pest resistance.**

Keywords: Cabbage caterpillar, Genetic resistance, Host plant resistance, Wild crucifers.

INTRODUCTION

Large white butterfly, *Pieris brassicae* (L.), is one of the most important pests of cruciferous crops particularly *Brassica* vegetables. It is cosmopolitan in distribution and is found wherever cruciferous plants are grown (Hill, 1987). The pest has a Palearctic distribution from North Africa across Europe and Asia to the Himalayan Mountains (Raqib, 2004; Jainulabdeen and Prasad, 2004). It has been recorded as a serious pest of cabbage, cauliflower, broccoli, and Brussels sprouts in different parts of the world. In India, it is reported to cause about 40 per cent damage per annum to different cruciferous crops (Hasan and Ansari, 2010a, b), particularly cabbage and cauliflower which are the two major vegetables produced and consumed in India. Both crops are of considerable economic importance and are often produced under smallholder conditions in India (Weinberger and Srinivasan, 2009). Intensive cultivation of these crops over the years has resulted in high pest infestation (Chaudhuri *et al.*, 2001; Weinberger and Srinivasan, 2009). In addition to being a serious pest on vegetable brassica, *P. brassicae* is also emerging as an important pest of oilseed Brassica in India, particularly *B. juncea*, *B. carinata*, *B. rapa* and *B. napus* (Kumar, 2011; 2017) in addition to

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the already prevalent mustard aphid, *Lipaphis erysimi* (Kumar, 2015). It is a voracious feeder and inflicts heavy damage to aboveground plant parts such as leaves, inflorescence, and pods. Failure to manage the pest in a timely manner results in plants practically devoid of leaves, flowers and developing pods.

The present methods of management of this pest largely depend upon the use of toxic insecticides. Indiscriminate use of synthetic insecticides has many adverse effects such as development of insecticide resistance in insect-pests, resurgence, pesticide residues in vegetables, oil and cake, besides environmental pollution. This necessitates the development of alternate control strategies that can be made a part of Integrated Pest Management (IPM) module. Host plant resistance holds a promise in this context. An insect resistant cultivar fits well in IPM modules as it provides the growers with an ecologically sound, effective, and economical option for pest management. Even the varieties with moderate levels of resistance can be integrated with other management options to reduce the pesticide applications on a crop. The first step in the development of an insect resistant cultivar is the identification of source of resistance (Stoner and Shelton, 1988). Some attempts have been made in the past to identify sources of resistance against *P. brassicae* in primary gene pools of brassica species (Jindal, 2000). Despite these investigations and resultant publications claiming resistance, no germplasm that carries genetically characterized resistance factor(s) seems to be available.

The inability to identify suitable sources of resistance has precipitated attempts at developing Brassica transgenics (Shelton *et al.*, 2009; Liu *et al.*, 2011). Transgenic crops were once considered to be the only way to feed the burgeoning world population. However, unrelenting opposition to Genetically Modified Organisms (GMOs) due to several environmental, ethical and social issues related to their cultivation and consumption continue to hamper their greater acceptability in large areas of the globe. An alternative approach to transgenic technology is the exploitation of beneficial genes from wild relatives of crop plants using conventional or genomics aided breeding methods (Harberd, 1969; Snell, 1978; Ayotte *et al.*, 1987). This is reflected in renewed interest in moving back to wilds to hunt for genes of interest equipped with present knowledge of genomics and tools of biotechnology (Samizadeh *et al.*, 2007) that have erased the sexual boundaries of gene transfer. Extensive genetic variability for many resistance related traits is known to exist in wild and weedy crucifers as reported by Kumar *et al.* (2011) against mustard aphid and Dosdall and Kott (2006) against cabbage seed pod weevil; and attempts have been made to introgress this variation to cultivated background. Wild relatives of crop plants may serve as important source of insect resistance genes. Keeping in view the enormous genetic diversity present in wild species, the current investigation was carried out with the objective to find new source of host plant resistance against *P. brassicae* which is an important pest on both vegetable as well as oilseed brassica.

MATERIALS AND METHODS

Plant Materials

In total, 63 accessions from 16 crucifer species (Table 1) were evaluated in field and laboratory bioassays. The seeds of these accessions were provided by Professor S. S. Banga, Indian Council of Agricultural Research National Professor, Oilseeds Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India.

Field Experiments

Location

Field screening of the accessions was carried out at Oilseeds Research Farm,

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Table 1. Species and accessions of 63 crucifer accessions tested for susceptibility/resistance to *Pieris brassicae.*

Crucifer species	No of accessions	Accessions
Brassica barrelieri (L.) Janka	1	WS-09-29
Brassica carinata A. Braun	2 Including one susceptible control	WS-09-27, PC-5 (Susceptible control, cultivated)
Brassica fruticulosa Cyr.	19	WS-09-34, WS-09-35, WS-09-36, WS-09-37, WS-09-38, WS-09-39, WS-09-40, WS-09-41, WS-09-42, WS-09-43, WS-09-44, WS-09-45, WS-09-46, WS-09-49, WS-09-50, WS-09-51, ATC-94716, ATC-94718, ATC-94720
Brassica fruticulosa Cirillo sub sp. *cossoniana* (Boiss. & Reut.) Maire	1	WS-09-47
Brassica fruticulosa Cirillio sub sp. *fruticulosa* [Synonym *Erucastrum fruticulosum* (Cyr.) C. Presl]	1	WS-09-48
Brassica juncea (L.) Czern.	2	ATC-93868, PBR 210 (Cultivated)
Brassica maurorum Durieu	2	WS-09-52, WS-09-61
Brassica napus L.	1	ATC 95953
Crambe abyssinica R. E. Fr.	2	EC-400059, EC-400060
Diplotaxis erucoides (L.) DC	1	*D. erucoides*
Diplotaxis muralis (L.) DC.	1	WS-09-72
Diplotaxis tennuisiliqua Del.	1	*D. tennuisiliqua*
Diplotaxis tenuifolia (L.) DC.	3	WS-09-75, WS-09-79, ATC 94832
Erucastrum abyssinicum (A. Rich.) O. E. Schulz	1	*E. abyssinicum*
Raphanus rugosum (L.)	1	ATC 94954
Raphanus sativus L.	2	WS-09-88, ATC-90769
Sinapis arvensis L.	3	WS-09-99, WS-09-100, ATC 94963
Sinapis alba L.	19	WS-09-90, WS-09-91, WS-09-92, WS-09-93, WS-09-94, WS-09-95, WS-09-97, WS-09-98, CN-30473, CN-33056, CN-33057, CN-39042, CN-40230, CN-43450, CN-43560, CN-43807, CN-45727, CN-45814, CN-91065

Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana (30.9° N and 75.85° E, 244 m above msl), India during 2013-2014 and 2014-2015 crop seasons (November to April). This part of the country is characterized by sub-tropical and semiarid climate with hot and dry spring-summer from April to June, and hot and humid summer from July to September. The crop season generally spans from October-November to April and is characterized by cold winters where the minimum temperature falls to 1^{o}C and sometimes even lower than that during December-January, while it rises to maximum of 31^{o}C and sometimes even more at the end of the season in March-April, with RH ranging from 30% to more than 90 per cent. The average annual rainfall is about 705 mm,

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most of which falls during monsoon period from July to September, while few showers fall during winter season from November to March.

Establishment of Trials

All the accessions were sown on canal or tube well irrigated sandy loam soil in 3 m paired rows with plant to plant and row to row spacing of 15 and 30 cm, respectively, as per the recommendations of Punjab Agricultural University, Ludhiana, India (Anonymous, 2014). The experiment was laid out in randomized complete block design and replicated thrice. Sowing was deliberately delayed relative to normal sowing in October and was done during November 15 and 17 in 2013 and 2014, respectively, to ensure heavy population build-up under natural conditions since late sown plants are attacked more by the pest (Kumar, 2011; 2017). At the time of sowing, a uniform dose of nitrogen (first split dose of urea at 45 kg acre^{-1}) and phosphorous (full basal dose of diammonium phosphate at 75 kg acre^{-1}) was applied to all the accessions, while the second split dose of urea (at 45 kg acre^{-1}) was applied four weeks after sowing. About 20 days after sowing, weeds were removed manually. All the recommended practices were followed for raising a good crop, except spray of insecticides as per the university recommendations (Anonymous, 2014). For screening of accessions, weekly data on *P. brassicae* larval populations were recorded starting at the pest appearance. For this purpose, 10 plants from each replication of the accession were selected at random. The data were recorded till the maturity of the crop with four and five observations during 2013-2014 and 2014-2015, respectively. To record the larval count, whole of the plant was visually observed.

Insects

Eggs and larvae of *P. brassicae* were collected from infested *B. rapa* plants in field during November - December and brought to the laboratory. These larvae were reared in glass jars (10 inch length, 5 inch diameter) on cut pieces of cabbage heads till pupation with top of jars covered with muslin cloth fastened with elastic bands. Adults emerging out of the pupae were paired in oviposition cages (30×30×30 cm) made up of wooden frames with stainless steel mesh (30 mesh size) on all the sides, with one pair per cage. The floor of the cage was lined with filter paper and a few cabbage leaves were placed at the bottom to act as oviposition substrate. Eggs thus obtained were kept in Biological Oxygen Demand (BOD) incubator at 22±1^{o}C and the larvae were used for further experiments.

Laboratory Evaluation

In laboratory screening, effect of the test genotypes on various demographic parameters of *P. brassicae* was studied. To this end, 10 second instar larvae (to avoid high natural mortality in the first instar larvae (Ahmad *et al.*, 2007) per replication were released in a plastic Petri plate (2.5 cm height, 10 cm diameter) with the help of camel's hair brush and were provided with fresh food of each genotype. The experiment was set up following completely randomized design with three biological replicates. The larvae were provided fresh food i.e. fresh leaves of each genotype every other day till pre-pupation when they stopped feeding. Data on different demographic parameters viz. larval development period, larval length, larval weight, pupal period, pupal length, and pupal weight were recorded. The time taken from release of the second instar larvae till pupation was recorded as larval development period. The larval length and weight were recorded from the fully grown fifth instar larvae with the help of thread and scale and electronic weighing balance, respectively. Similar data were recorded for pupal stage. For this purpose, one day old pupae were gently removed from the sides/top of the Petri plate and, after

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recording the pupal length and weight, they were transferred to glass jars placed on a layer of filter paper. Pupal period was recorded from the date of formation of each pupa until adult emergence.

In addition, leaf area consumed during 24 hours after the release of larvae was measured for each genotype, following completely randomized design with three biological replicates. For this purpose, a single third instar larva was released on a fully expanded leaf of each genotype in a Petri plate (30 cm diameter). Leaf area before and after 24 hours of release was measured by leaf area meter and the leaf area consumed per larva was determined.

Biochemical Analysis

Since, *P. brassicae* is a foliage feeder, biochemical analysis was done for leaf samples collected from 10 plants/genotype/replicate. Leaf samples were collected from *P. brassicae* infested field plants seven days after infestation and were analyzed for total glucosinolates as per McGhee *et al.* (1965), total phenols as per Swain and Hillis (1959), ortho-dihydroxy phenols as per Nair and Vaidyanathan (1964), and total flavonols as per Balbaa *et al.* (1974).

Statistical Analysis

The field data on the larval population of different genotypes over the seasons was analyzed using independent linear mixed models for each year; genotype was considered a fixed effect in the model and blocks were a random effect using the statistical software SAS 9.1 (SAS Institute, 2005). A second analysis involved liner mixed model with genotypes as fixed effect and year as random effect. Likewise, in the laboratory evaluation, we compared the different demographic parameters among the genotypes using linear mixed model with genotype as fixed effect and Petri plate as random effect. The level of statistical significance was set at $P \leq 0.05$ for all analyses. When analysis of variance indicated significant differences among the treatments, mean comparisons were done using *LSD*. Data on the mean larval population under field condition and on different demographic parameters studied under laboratory condition were subjected to simple correlation analysis with different biochemical constituents.

RESULTS

Selection of Potentially Resistant Accessions under Field Conditions

From the 63 accessions that were evaluated in two crop seasons under field conditions, 18 were clearly susceptible. The remaining 45 accessions were those in which larval population was significantly lower than that on susceptible control *B. carinata* PC 5 (Table 2). These included putatively resistant accessions of wild species *B. barrelieri, B. fruticulosa, B. fruticulosa* sub sp. *cossoniana, B. fruticulosa* sub sp. *fruticulosa, B. maurorum, Crambe abyssinica, Diplotaxis erucoides, D. muralis, D. tenuisiliqua, D. tenuifolia,*, and *Erucastrum abyssinicum, Raphanus rugosum*, in addition to *B. napus, R. sativus, Sinapis arvensis* and *S. alba.*

Validation of Field Results under Laboratory Conditions

In the laboratory experiment, the performance of *P. brassicae* was monitored on different brassicaceous wild relatives. Significant differences in larval development period were observed among the accessions indicating their suitability as host for *P. brassicae.* Accessions with longer larval development period were less suitable as host and vice versa. The larval development period in 20 accessions was significantly longer than that in the

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Table 2. Field evaluation of Brassicaceous accessions for resistance against *Pieris brassicae* based on comparative larval population.

Species	Total number of accessions tested	Putative resistant accessions [a]
Brassica barrelieri	1	1
B. carinata	2	- [b]
B. fruticulosa	19	18
B. fruticulosa sub sp. *cossoniana*	1	1
B. fruticulosa sub sp. *fruticulosa*	1	1
B. juncea	2	-
B. maurorum	2	1
B. napus	1	1
Crambe abyssinica	2	2
Diplotaxis erucoides	1	1
D. muralis	1	1
D. tennuisiliqua	1	1
D. tenuifolia	3	3
Erucastrum abyssinicum	1	1
Raphanus rugosum	1	1
R. sativus	2	2
Sinapis arvensis	3	2
S. alba	19	8
	63	45

[a] Based on the two-years field data. Putatively resistant accessions revealed significantly lower larval population than the susceptible control *Brassica carinata* cv. PC-5. [b] No putative resistant accessions were selected.

susceptible *B. carinata* variety PC 5, i.e. the control. These included one accession of *B. barrelieri* (WS-09-29), 12 of *B. fruticulosa* (WS-09-34, WS-09-36, WS-09-37, WS-09-38, WS-09-39, WS-09-40, WS-09-41, WS-09-42, WS-09-43, WS-09-45, WS-09-46, WS-09-50), one each of *B. maurorum* (WS-09-61), *D. muralis* (WS-09-72), *D. tenuifolia* (WS-09-75), three of *S. alba* (WS-09-92, WS-09-98, CN 39042) and one of *C. abyssinica* (EC 400060). Larval length of full grown larvae in six accessions (*B. fruticulosa* ATC 94716, *D. erucoides, D. muralis* WS-09-72, *Sinapis alba* WS-09-98, CN-45814 and *S. arvensis* WS-09-99) was significantly lower than that in susceptible control PC 5, while larval weight of full grown larvae in 26 accessions was significantly lower than that in PC-5. These accessions were: *B. fruticulosa* (10) WS-09-34, WS-09-37, WS-09-39, WS-09-40, WS-09-41, WS-09-42, WS-09-43, WS-09-46, ATC-94716, ATC-94720, *B. fruticulosa* sub sp. *cossoniana* (1) WS-09-47, *B. maurorum* (2) WS-09-52, WS-09-61, *D. erucoides* (1), *D. tenuifolia* (2) WS-09-75, WS-09-79, *D. tenuisiliqua* (1), *E. abyssinicum* (1), *R. rugosum* (1) ATC-94954, *R. sativus* (1) ATC-90769, *S. alba* (3) WS-09-98, CN-45727, CN-45814 and *S. arvensis* (3) WS-09-99, WS-09-100 and ATC-94963. Only two accessions exhibited a negative effect on pupal period, which was significantly higher than that on PC-5. These included one accession of *Crambe abyssinica* (EC-400059) and one of *D. tennuisiliqua* (*D. tennuisiliqua*). The adverse effect on pupal length was observed in ten accessions in which it was significantly lower than that in the susceptible control PC 5. These included one accession of *B. barrelieri* (WS-09-29), two of *B. fruticulosa* (ATC-94716, ATC-94720), one each of *D. tenuisiliqua* (*D. tenuisiliqua*), *E. abyssinicum* (*E. abyssinicum*) and *S. alba* (WS-09-98), two accessions each of *S. arvensis* (WS-09-100, ATC-94963) and *C. abyssinica* (EC-400059, EC-400060). Similarly, adverse effect on pupal weight was observed in 20 accessions which included one accession of *B.*

[DOR: 20.1001.1.16807073.2018.20.4.1.9]

barrelieri (WS-09-29), three of *B. fruticulosa* (WS-09-41, ATC-94716, ATC-94720), one of *B. juncea* (ATC-93868), two of *B. maurorum* (WS-09-52, WS-09-61), one each of *D. muralis* (WS-09-72), *D. tenuifolia* (WS-09-79), *E. abyssinicum* (*E. abyssinicum*), *R. rugosum* (ATC-94954), *R. sativus* (WS-09-88), and three each of *S. alba* (WS-09-98, CN-39042, CN-45727) and *S. arvensis* (WS-09-99, WS-09-100, ATC-94963), and two accessions of *C. abyssinica* (EC-400059, EC-400060). Larvae consumed significantly less leaf area than PC-5 after 24 hours of release on ten accessions. These included one accession of *B. barrelieri* (WS-09-29), five of *B. fruticulosa* (WS-09-34, WS-09-39, WS-09-42, WS-09-43, WS-09-45), one each of *B. maurorum* (WS-09-61), *D. muralis* (WS-09-72), *D. tenuifolia* (WS-09-75), and *R. rugosum* (ATC-94954).

Biochemical Analysis

The total glucosinolates content in all the accessions was significantly lower than that in the susceptible control PC 5, except *Raphanus rugosum* accession (ATC-94954) and *Sinapis alba* accession CN-33057 (Table 3). The total phenols content in 13 accessions was significantly lower than that in PC 5 while it was significantly higher in 28 accessions. The accessions with low total phenols content were *B. fruticulosa* (ATC 94718), *C. abyssinica* (EC-400059), *B. juncea* (ATC-93868), *R. rugosum* (ATC-94954), *D. erucoides* (*D. erucoides*) and *S. alba* (WS-09-93, CN-40230, CN-43450, CN-43560, CN-43807, CN-45727, CN-45814, CN-91065), while those with high total phenols content were *B. carinata* (WS-09-27), *B. fruticulosa* (WS-09-34, WS-09-36, WS-09-38, WS-09-40, WS-09-41, WS-09-42, WS-09-49, WS-09-50, WS-09-51), *B. fruticulosa* sub sp. *cossoniana* (WS-09-47), *B. maurorum* (WS-09-52, WS-09-61), *D. tenuifolia* (WS-09-79, ATC-94832), *R. sativus* (WS-09-88, ATC-90769), *B. juncea* (PBR-210), *S. arvensis* (WS-09-99, ATC-94963), *S. alba* (WS-09-90, WS-09-91, WS-09-95, WS-09-97, WS-09-98, CN-30473, CN-33056, CN-33057). Similarly, the orho-dihydroxy phenols content was significantly lower than susceptible control PC 5 in 11 accessions, which included *B. barrelieri* (WS-09-29), *B. fruticulosa* (WS-09-35, WS-09-37, WS-09-45), *S. arvensis* (WS-09-100, ATC-94963) and *S. alba* (WS-09-92, WS-09-93, WS-09-94, WS-09-95, WS-09-98), while it was significantly higher in 22 accessions. These included: *B. fruticulosa* (WS-09-36, WS-09-41, WS-09-43, WS-09-44, WS-09-50, WS-09-51, ATC-94716), *B. fruticulosa* sub sp. *cossoniana* (WS-09-47), *B. fruticulosa* sub sp. *fruticulosa* (WS-09-48), *B. maurorum* (WS-09-61), *D. tenuifolia* (WS-09-79, ATC-94832), *R. sativus* (WS-09-88, ATC-90769), *C. abyssinica* (EC-400060), *S. arvensis* (WS-09-99), *E. abyssinicum* (*E. abyssinicum*), *D. erucoides*), *D. tennuisiliqua* (*D. tennuisiliqua*) and *S. alba* (WS-09-91, CN-33057, CN-43807). The flavonols content was significantly lower in 10 accessions including *B. barrelieri* (WS-09-29), *B. fruticulosa* (WS-09-34, WS-09-35, ATC-94718), *D. muralis* (WS-09-72), *C. abyssinica* (EC-400059), *B. juncea* (PBR-210, ATC-93868), *S. arvensis* (ATC-94963) and *R. rugosum* (ATC-94954). On the other hand, it was significantly high in 31 accessions, namely, *B. carinata* (WS-09-27), *B. fruticulosa* (WS-09-39, WS-09-40, WS-09-41, WS-09-43, WS-09-44, WS-09-51, ATC-94716, ATC-94720), *B. fruticulosa* sub sp. *cossoniana* (WS-09-47), *B. maurorum* (WS-09-61), *D. tenuifolia* (WS-09-79, ATC-94832), *R. sativus* (WS-09-88, ATC-90769), *C. abyssinica* (EC-400060), *S. arvensis* (WS-09-99), and *S. alba* (WS-09-90, WS-09-91, WS-09-92, WS-09-97, WS-09-98, CN-33056, CN-33057, CN-39042, CN-40230, CN-43450, CN-43560, CN-43807, and CN-45727, CN-91065).

No significant correlations were observed between the larval population under field conditions and different biochemical parameters analyzed. However, in laboratory evaluation, there was a significant positive correlation of total glucosinolates content in

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Table 3. Biochemical constituents in leaf tissue of various *Brassica* genotypes.

Sr. No.	Species	Genotype	Glucosinolates (µmoles g^{-1} dry weight of leaf tissue) (Mean±SE)	Total phenols (mg g^{-1} dry weight of leaf tissue) (Mean±SE)	O-dihydroxy phenols (mg g^{-1} dry weight of leaf tissue) (Mean±SE)	Flavonols (mg g^{-1} dry weight of leaf tissue) (Mean±SE)
1	*Brassica carinata*	WS-09-27	32.06±1.41	16.59±0.45	1.60±0.04	16.24±0.87
2		PC-5 (Susceptible control)	36.61±3.00	11.05±1.02	1.64±0.09	12.54±0.28
3	*B. barrelieri*	WS-09-29	25.60±1.74	10.89±0.26	0.63±0.05	5.58±0.48
4	*B. fruticulosa*	WS-09-34	30.41±0.81	14.19±0.78	1.82±0.29	8.96±0.5
5		WS-09-35	19.27±0.52	9.64±0.86	0.95±0.08	7.35±0.40
6		WS-09-36	25.49±0.63	17.83±0.61	2.33±0.05	13.33±0.21
7		WS-09-37	23.20±0.18	13.82±0.57	0.87±0.07	10.35±0.73
8		WS-09-38	25.15±0.14	14.83±0.68	1.91±0.01	12.85±1.41
9		WS-09-39	23.02±0.36	8.67±1.13	1.43±0.09	17.30±0.60
10		WS-09-40	21.68±1.30	14.92±1.61	1.63±0.07	16.46±1.17
11		WS-09-41	23.33±0.18	14.63±2.15	2.06±0.01	17.06±0.21
12		WS-09-42	23.64±0.03	15.63±1.72	1.92±0.15	14.87±0.28
13		WS-09-43	25.54±1.24	13.25±1.55	2.13±0.00	15.61±1.46
14		WS-09-44	25.75±0.60	13.36±2.11	2.11±0.01	18.61±0.93
15		WS-09-45	27.67±1.14	13.70±0.88	1.28±0.01	11.00±0.08
16		WS-09-46	26.76±0.29	13.75±0.16	1.50±0.06	14.72±2.12
17		WS-09-49	13.43±1.97	14.54±0.96	1.67±0.01	10.13±0.13
18		WS-09-50	22.84±0.31	17.06±0.71	2.06±0.04	13.58±0.95
19		WS-09-51	24.84±0.75	22.41±2.40	3.42±0.03	23.72±0.44
20		ATC-94716	22.71±0.38	9.90±0.01	2.12±0.03	15.38±0.26
21		ATC-94718	23.97±0.70	6.53±0.15	1.79±0.22	8.32±0.24
22		ATC-94720	25.49±0.31	13.00±0.04	1.67±0.11	16.22±0.19
23	*B. fruticulosa* sub sp. *cossoniana*	WS-09-47	18.06±0.56	15.46±0.29	2.71±0.40	15.43±0.99
24	*B. fruticulosa* sub sp. *fruticulosa*	WS-09-48	12.75±0.24	12.07±0.45	2.35±0.07	12.35±0.33
25	*B. juncea*	ATC-93868	23.08±2.46	15.94±0.42	1.86±0.13	6.67±0.48
26		PBR 210	24.20±0.92	5.52±0.13	1.36±0.11	6.11±0.79
27	*Diplotaxis muralis*	WS-09-72	30.00±0.42	8.47±0.18	1.54±0.03	4.12±0.09
28	*D. maurorum*	WS-09-52	16.69±0.16	16.53±1.29	1.68±0.15	14.11±0.71
29		WS-09-61	21.46±0.03	18.27±2.11	2.52±0.12	19.24±0.21
30	*D. tenuifolia*	WS-09-75	25.21±0.32	13.42±0.56	1.60±0.06	11.81±1.29
31		WS-09-79	29.74±0.51	18.49±0.24	2.52±0.05	24.29±0.35
32		ATC-94832	15.48±0.14	14.59±0.14	2.30±0.06	17.30±0.21

Continued…

Continued of Table3.

Sr. No.	Species	Genotype	Glucosinolates (µmoles g^{-1} dry weight of leaf tissue) (Mean±SE)	Total phenols (mg g^{-1} dry weight of leaf tissue) (Mean±SE)	O-dihydroxy phenols (mg g^{-1} dry weight of leaf tissue) (Mean±SE)	Flavonols (mg g^{-1} dry weight of leaf tissue) (Mean±SE)
33	*Raphanus sativus*	WS-09-88	25.95±1.95	20.95±1.11	2.44±0.15	22.72±1.49
34		ATC-90769	17.91±1.04	18.43±1.62	2.02±0.02	21.43±0.52
35	*Sinapis arvensis*	WS-09-99	20.77±0.68	17.05±1.69	2.46±0.13	21.00±0.08
36		WS-09-100	29.89±0.41	12.67±1.04	1.20±0.05	12.78±0.35
37		ATC-94963	21.06±0.53	14.43±0.23	1.05±0.09	6.97±0.25
38	*Crambe abyssinica*	EC-400059	26.36±0.88	2.85±0.38	1.35±0.09	7.74±1.08
39		EC-400060	28.38±1.68	8.78±0.53	3.75±0.22	16.55±1.08
40	*R. rugosum*	ATC-94954	37.87±0.25	5.87±0.02	1.37±0.03	8.21±0.20
41	*B. napus*	ATC-95953	30.53±0.75	8.55±0.34	1.47±0.03	10.77±0.91
42	*Erucastrum abyssinicum*	*E. abyssinicum*	20.67±0.10	11.99±0.26	5.04±0.04	14.79±0.27
43	*D. erucoides*	*D. erucoides*	21.73±1.05	6.70±0.06	2.86±0.02	15.06±1.08
44	*D. tenuisiliqua*	*D. tenuisiliqua*	21.84±0.83	8.91±0.40	3.78±0.16	13.84±0.45
45	*S. alba*	WS-09-90	25.47±1.11	17.20±0.05	1.29±0.01	16.02±0.24
46		WS-09-91	29.23±1.09	23.97±2.08	2.00±0.06	20.73±0.40
47		WS-09-92	29.82±1.43	10.50±0.19	1.16±0.21	18.93±0.14
48		WS-09-93	26.69±1.77	7.11±0.03	1.25±0.09	10.87±0.95
49		WS-09-94	15.46±1.64	12.84±0.12	0.65±0.09	10.02±0.09
50		WS-09-95	16.52±1.51	22.15±2.08	1.13±0.13	14.97±0.39
51		WS-09-97	14.03±1.57	23.68±0.93	1.87±0.13	23.72±0.78
52		WS-09-98	21.50±0.17	19.36±1.21	1.14±0.14	18.13±0.80
53		CN-30473	25.73±1.46	14.81±0.35	1.61±0.06	14.09±0.57
54		CN-33056	31.40±1.57	15.10±0.58	1.80±0.22	16.32±0.84
55		CN-33057	37.66±2.96	24.31±2.09	2.02±0.23	20.19±1.14
56		CN-39042	24.94±0.32	13.45±1.23	1.77±0.01	16.61±0.60
57		CN-40230	19.90±0.20	4.29±1.16	1.34±0.08	23.17±1.90
58		CN-43450	26.46±0.21	4.62±0.50	1.76±0.01	24.78±3.18
59		CN-43560	24.79±0.93	4.15±0.20	1.77±0.08	19.50±1.49
60		CN-43807	26.53±0.62	6.25±1.10	3.14±0.07	19.41±0.54
61		CN-45727	27.01±2.29	3.02±0.75	1.54±0.19	18.65±1.20
62		CN-45814	30.63±1.81	2.81±1.07	1.55±0.30	12.29±1.68
63		CN-91065	26.95±0.64	4.36±0.40	1.86±0.17	19.75±1.24
	LSD at *P*= 0.05		3.25	2.82	0.36	2.58

leaf tissue with pupal period (Table 4), indicating the negative effect of glucosinolates on insect development, though this effect was not observed on other parameters. Likewise, significantly positive correlation of total phenols content was observed with larval weight indicating its positive effect on the insect, while the correlation between ortho-dihydroxy phenols and pupal period was negative, further a positive effect. Total flavonols exhibited a significantly negative correlation with larval development period (positive effect), while it was positive with the leaf area consumed (positive effect).

Thus, out of the 63 accessions evaluated both under field and laboratory conditions, five (*Brassica barrelieri*: WS-09-29, *B. fruticulosa*: ATC-94716, *Diplotaxis muralis*: WS-09-72, *B. maurorum*: WS-09-61, *Sinapis alba*: WS-09-98) were highly resistant, while 14 (*B. fruticulosa*: WS-09-39, WS-09-41, WS-09-42, WS-09-43, WS-09-46, ATC-94720, *B. maurorum*: WS-09-75, *S. arvensis*: WS-09-99, ATC-94963, *Crambe abyssinica*: EC-400059, EC-400060, *R. rugosum*: ATC-94954, *E. abyssinicum*: *E. abyssinicum*, and *D. tennuisiliqua*: *D. tennuisiliqua*) were resistant.

DISCUSSION

Though field screening can be used to screen a large germplasm collection against insect-pests, the effect of environmental conditions on the outcome of evaluations cannot be ruled out (Porter *et al.*, 1991, Kumar *et al.*, 2011). To minimize this effect, we screened all the accessions for two successive years. However, in field screening, there is always a risk of susceptible plants escaping infestation (Kumar *et al.*, 2011) which ultimately get selected as putatively resistant ones. To prevent this, we evaluated putative resistant accessions from field evaluation in the laboratory under no choice conditions, which showed that many of them were indeed resistant. However, there were many accessions that were resistant under field conditions, but showed susceptibility under laboratory conditions. Whether the differences in resistance/susceptibility of accessions under field and laboratory conditions were due to intact plants or detached leaves is not known and needs further investigation.

The life history parameters of insects are affected by type of host plant (Pearl and Parker, 1921; Morgan *et al.*, 2001; Kim and Lee, 2002; Liu *et al.*, 2004). Shorter larval

Table 4. Correlation of different biochemical constituents (represented by correlation coefficient r) with different demographic parameters of *Pieris brassicae.*

Insect parameter	Biochemical constituent			
	Total Glucosinolates	Total Phenols	o-dihydroxy phenols	Total Flavonols
Larval development period	-0.13 *P*= 0.30	0.05 *P*= 0.66	-0.16 *P*= 0.19	-0.25* *P*= 0.04
Larval length	-0.16 *P*= 0.20	-0.16 *P*= 0.20	0.17 *P*= 0.16	-0.06 *P*= 0.59
Larval weight	-0.02 *P*= 0.87	0.28* *P*= 0.02	-0.04 *P*= 0.73	0.0006 *P*= 0.99
Pupal period	0.25* *P*= 0.04	-0.20 *P*= 0.11	-0.32* *P*= 0.009	-0.03 *P*= 0.81
Pupal length	0.005 *P*= 0.97	0.21 *P*= 0.09	0.02 *P*= 0.85	0.12 *P*= 0.33
Pupal weight	0.0003 *P*= 0.99	0.10 *P*= 0.38	-0.16 P= 0.20	-0.04 *P*= 0.74
Leaf area consumed	0.04 *P*= 0.71	0.09 *P*= 0.44	0.19 *P*= 0.12	0.43* *P*= 0.0004

*: Singnificant at *P*= 0.05.

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development time with bigger and heavier larvae and pupae on a host indicate greater suitability of a host and vice versa (Awmack and Leather, 2002). Out of the 45 putatively resistant accessions from field evaluation, 38 showed adverse effect on one or more demographic parameter(s) of *P. brassicae*, and 19 were resistant even under laboratory condition including 5 highly resistant. These included accessions from *Brassica barrelieri, B. fruticulosa, B. maurorum, Crambe abyssinica, Diplotaxis muralis, D. tenuisiliqua, Erucastrum abyssinicum, Raphanus rugosum, Sinapis alba*, and *S. arvensis.* Crop Wild Relatives (CWRs) may serve as important source for insect resistance genes when resistance within the cultivated germplasm is absent (Kumar *et al.*, 2011). Out of the 19 resistant accessions, maximum number (7) was from *B. fruticulosa.* This species has already been reported to be resistant to cabbage aphid, *Brevicoryne brassicae* (Cole 1994a,b; Ellis and Farrel, 1995; Ellis *et al.*; 2000), mustard aphid, *Lipaphis erysimi* (Kumar *et al.*, 2011) and cabbage root fly, *Delia radicum* (Jenson *et al.*, 2002). Exceptionally high level of resistance was observed in one accession of *Sinapis alba* (WS-09-98) which resulted in adverse effect on all the demographic parameters studied. *Sinapis alba* was densely covered with trichomes which suggests that resistance may be caused by presence of trichomes (Lamb, 1980; Traw and Dawson, 2002).

In the present study, only glucosinolates have shown a significantly negative effect on the insect development. Brassica plants synthesize a number of secondary metabolites that play role in plant defense and glucosinolates are one such group of compounds. Glucosinolates mostly act as defense chemicals against insect-pests, concentration of which increases in response to insect damage and results in varied effects on insects (Kumar, 2017). Although, *P. brassicae* is a brassica specialist and has developed ways to use these compounds to its advantage (Thorsteinson, 1953; David and Gardiner, 1966; Renwick *et al.*, 1992; Smallegange *et al.*, 2007), their higher concentrations, as well as their fission products, can still have adverse effects on this specialist (Agrawal and Kurashige, 2003; Hopkins *et al.*, 2009). Wild relatives of Brassicas contain more glucosinolates and specialists as well as generalists perform worse on them (Gols *et al.*, 2008; Griffiths *et al.*, 2001). Agrawal and Kurashige (2003) analyzed the classical interaction between *P. rapae* and isothiocyanates formed after glucosinolate hydrolysis. Using whole plants, root extracts and a microencapsulated formulation of allyl isothiocyanates, it was shown that isothiocyanates reduce insect survival and growth and increase development time in a dose dependent manner. However, in the present study, susceptible control PC 5 itself had very high concentration of glucosinolates compared to most of the genotypes, which may be responsible for increased susceptibility of this genotype to this brassica specialist. Glucosinolates are known to stimulate larval feeding and oviposition by adults in the large white butterfly, *P. brassicae* and small white butterfly, *P. rapae* (Thorsteinson, 1953; David and Gardiner, 1966; Renwick *et al.*, 1992; Smallegange *et al.*, 2007; Kumar; 2017). Similarly, a positive correlation was found between total glucosinolate concentration and plant damage by *Psylliodes chrysocephala* and *P. rapae* on *B. napus* (Giamoustaris and Mithen; 1995) suggesting the possible role of other factors in addition to glucosinolates in imparting insect resistance.

The present knowledge of modern breeding techniques has restricted plant breeders to a few advanced technologies, in which they are trained for creating and using novel genes, but the infinite wealth of desirable genes in crops wild relatives is often ignored (Edwards *et al.*, 2012). Exploration of genetic diversity provides information on desirable genes and on species/genera that can be used as potential parents in breeding programmes such as those aimed at development of insect resistant cultivars. There is a growing body of literature that suggests that almost all of the genetic variation necessary for crop improvement can be found in their CWRs that was lost over the

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course of domestication (Tanksley and McCouch, 1997; Fernie *et al.*, 2006; Vaughan *et al.*, 2007; Burger *et al.*, 2008, Pelgrom *et al.*, 2015). The use of CWRs is continuously increasing over the years for a range of beneficial traits including pest and disease resistance (Prescott-Allen and Prescot-Allen, 1986, 1988; Hajjar and Hodgkin, 2007). In a comprehensive survey by Hajjar and Hodgkin (2007) about the use of CWRs in crop improvement for the period 1986 to 2005, over 80 per cent of the beneficial traits involved pest and disease resistance. Although the use of CWRs is increasing, their contributions in providing useful genes for improvement of crop plants have been less than expected, keeping in view the improvement in technology for crossing species from different gene pools, increased number of wild accessions in gene banks and advances in molecular techniques. In the present study, we attempted to identify a few wild accessions that can serve as potential donors of genes conferring resistance to *P. brassicae*, an important pest of vegetable and oilseed Brassica.

CONCLUSIONS

By screening a collection of wild crucifers, we identified 5 accessions from 5 species highly resistant to *P. brassicae.* These included *Brassica barrelieri* (1) WS-09-29, *B. fruticulosa* (1) ATC-94716, *Diplotaxis muralis* (1) WS-09-72, *B. maurorum* (1) WS-09-61, and *Sinapis alba* (1) WS-09-98. In addition, 14 accessions from 7 species were resistant to this pest which included *B. fruticulosa* (6) WS-09-39, WS-09-41, WS-09-42, WS-09-43, WS-09-46, ATC-94720, *B. maurorum* (1) WS-09-75, *S. arvensis* (2) WS-09-99, ATC-94963, *Crambe abyssinica* (2): EC-400059, EC-400060, *R. rugosum* (1) ATC-94954, *E. abyssinicum* (1) *E. abyssinicum* and *D. tennuisiliqua* (1) *D. tennuisiliqua*. However, there is a need to study the genetics of resistance in these accessions which will provide detailed information on the complexity of the trait and generate molecular markers linked to this. It will give an idea about the ease with which resistance trait may be introgressed into cultivated backgrounds to produce *P. brassicae* resistant vegetable and oilseed *Brassica*.

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ارزیابی اقوام وحشی کلم سانان (Brassicaceous) برای مقاومت به بزرگ پروانه سفید *Pieris brassicae*

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چکیده

پروانه تخصصی *Pieris brassicae* (L.) یکی از مخرب ترین و گسترده ترین آفات گیاهان کلم سان دربسیاری از کشورهای جهان است. صدمات این آفت می تواند به گیاهان کلم سان شامل کلم و گل کلم که از سبزیجات اصلی تولیدی و مصرفی هند می باشند تا 40٪ صدمه بزند. ایجاد کولتیوار مقاوم به آفات از اهداف مورد تقاضا در مدیریت حشرات آفت می باشد زیرا برای مدیریت آفات توسط کشاورزان انتخابی موثر، اقتصادی، و دوستدار محیط فراهم می کند. اما، اولین گام در ایجاد کولتیوار مقاوم به آفت، کسب دانش دقیق از منبع(منابع) مقاومت است. در این پژوهش، 63 نمونه ثبت شده (accession) متنوع از brassicaceous(غالبا از کلم سانان وحشی) در شرایط مزرعه و آزمایشگاه غربال شد تا عملکرد *P. brassicae*در شرایط انتخاب آزاد (در مزرعه) و بدون حق انتخاب (در آزمایشگاه) تعیین شود. نمونه های مقاومی که شناسایی شدند در میان*Brassica barrelieri* ، *B. fruticulosa*، *B.maurorum*، *Crambe abyssinica*، *Diplotaxis muralis*، *D. tenuisiliqua*، *Erucastrum abyssinicum*، *Raphanus rugosum*، *Sinapis alba* و *S. arvensis* بودند. تجزیه بیوشیمیایی نمونه های ظاهرا مقاوم آشکار ساخت که غلظت های بالای گلوکوزینولات ها تاثیر منفی معناداری روی رشد حشره داشتند در حالیکه عکس این مطلب در مورد فنل کل و فلاونول ها صدق میکرد. یافته های ما

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ممکن است برای اصلاح ژنتیکی هر دو نوع سبزی و گیاه کلم سان و با هدف ایجاد کولتیوار مقاوم به *P. brassicae* مفید باشد. این پژوهش مجددا اهمیت اقوام وحشی گیاهان را برای مقاومت به آفات نشان می دهد.

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