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Research Paper

Host plant accessions determine bottom-up effect of snapmelon (*Cucumis melo* var. *momordica*) against melon fly (*Bactrocera cucurbitae* (Coquillett))

Shravan Manbhar Haldhar*, Dilip Kumar Samadia, Rakesh Bhargava, Balu Ram Choudhary and Dhurendra Singh

ICAR-Central Institute for Arid Horticulture, Sri Ganganagar Highway, Beechwal Industrial Area, Bikaner–334006, India

The melon fly, *Bactrocera cucurbitae* (Tephritidae: Diptera) is an important pest of snapmelon (*Cucumis melo* var. *momordica*), leading to significant losses in yield in the hot arid agro-climate of India. The accessions IC-430190 (11.21%), DKS-AHS 2011/4 (14.97%) and DKS-AHS 2011/3 (18.57%) were found to be novel resistant accessions against melon fly, *B. cucurbitae* infestation. Free amino acid and total soluble solid (TSS) were in positive correlation with percent fruit infestation whereas phenols, tannin, total alkaloids and flavonoid contents had significant negative correlation with percent fruit infestation. The percent fruit infestation had significant positive correlation with fruit length, fruit diameter and flesh thickness and negative correlation with length of ovary pubescence, rind hardness at immature stage, rind hardness at mature stage and pericarp thickness. Based on Kaiser Normalization method, two principal components (PCs) were extracted explaining cumulative variation of 82.80% in melon fly infestation. PC1 explained 53.41% of the variation while PC2 explained 29.39% of variation. The flavonoid, total alkaloid, tannins, phenols content, length of ovary pubescence and rind hardness were the novel antibiosis and antixenotic characters found in snapmelon resistant melon fly, *B. cucurbitae* and therefore, could be used as marker traits in plant breeding programs to select resistant accessions.

Key Words: *Bactrocera cucurbitae*, *Cucumis melo* var. *momordica*, intra-specific diversity, bottom-up effect, plant defense, plant-insect interactions, host arid environment.

Introduction

A goal of many integrated pest management (IPM) researchers and practitioners has been to develop sustainable management programs that are more resilient and less reliant on synthetic pesticides (Bustos-Segura *et al.* 2017, Sharma and Ortiz 2002, Tooker and Frank 2012). It has been widely recognized that biological diversity playing and a vital role in structuring communities ecosystem processes (Haddad *et al.* 2011, Tooker and Frank 2012). The genotypic variation may influence the distribution and damage levels of herbivores on focal plants through processes referred to as associational resistance or susceptibility (Barbosa *et al.* 2009, Muthusamy *et al.* 2017). The feature of bottom-up effects is that it is farmer-friendly and environment-friendly method of insect management. The attractive and beneficial feature of bottom up effect is farmers friendly and does not need much financial investment toward pest control. The identification and development of crop specific genotypes with resistance to pests is determined by the nutrients and concentrations of secondary metabolites. Host plant plays an important role in determining insect populations in respect to concentrations and proportion of nutrients and differs among species (Schoonhoven *et al.* 2005). Considerable progress has been made in identification and development of crop cultivars with resistance to the major pests in different crops. There is a need to transfer resistance genes into high-yielding cultivars with adaptation to different agro-ecosystems (Samadia and Haldhar 2017, Sharma and Ortiz 2002). Plants are generally exposed to a variety of biotic and abiotic factors that may alter their genotypic and phenotypic properties resulting in expression of different mechanisms of resistance to pest attack (Gogi *et al.* 2010, Haldhar *et al.* 2015a). Such mechanisms of plant resistance have been effectively used against insect pests in many field and horticultural crops (Dhillon *et al.* 2005, Gogi *et al.* 2010, Haldhar *et al.* 2015b). Plants having antibiosis characters like flavonoid, alkaloid, phenols, tannins etc. may cause reduced insect survival, prolonged developmental time, decreased size and reduced fitness of new generation adults (Choudhary *et al.* 2015, Gogi *et al.* 2010, Haldhar

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*Corresponding author (e-mail: haldhar80@gmail.com)

et al. 2013a, 2014, Sarfraz *et al.* 2007). Antixenosis refers to the potential plant morphological traits (length of ovary pubescence, fruit hardness, roughness, rind thickness etc.) that imparts or alters insect behavior towards host preference (Haldhar *et al.* 2015a, Moslem *et al.* 2011, War *et al.* 2012).

Snapmelon (*Cucumis melo* L. var. *momordica* (Roxb.) belongs to family *Cucurbitaceae*, that is a native of India and is used as vegetable in a variety of ways. Snapmelon is rich in nutritional attributes; 100 g edible fruit of snapmelon contains 15.6 g carbohydrates, 18.6 mg vitamin C, and provides 74.0 kcal energy (Goyal and Sharma, 2009). Immature fruits are cooked or pickled; the low sugar mature fruits are eaten raw. India being a center of snapmelon diversity is endowed with great variability in terms of morphological characters, especially fruit size and shape, fruit cracking and peeling patterns, flesh color, skin texture, and primary and secondary color of fruit skin (Haldhar *et al.* 2017, Pandey *et al.* 2011). Indian snapmelon accessions have been reported to be a good source for disease and insect pest resistance, and many of them are used as reference accessions worldwide.

The fruit flies (Diptera: Tephritidae) are devastating insect-pests having a foremost influence on global agricultural products affecting yield losses, and dropping the value and marketability of horticultural crops. In addition, tephritid fruit flies are amongst the mainly persistent pest species of fruits and vegetables in the world causing direct and indirect economic fatalities due to their injury (Sarwar 2006). The melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is a serious pest of snapmelon in India and its outbreak causes substantial crop losses to the growers. The melon fly has been observed to naturally infest fruits of a number of different plant species (136 plant taxa), but snapmelon is one of the most preferred hosts and has been a major limiting factor in obtaining good quality fruits and high yield (Mcquate *et al.* 2017). The extent of losses from fruit fly varies between 30 and 100%, depending on the cucurbit species, area and the season. As the maggots damage the fruits internally, it is difficult to control this pest with insecticides (Nath and Bhushan 2006). Limited studies on snapmelon accessions resistant to melon fly have been conducted in the world owing to inadequate information on the sources of plant traits associated with resistance to pest infestations. The present study was designed to identify various morphological (phenotypic mechanism) and biochemical (allelochemical compounds) fruit traits of snapmelon accessions associated with resistance against melon fly.

Materials and Methods

Preliminary screening of snapmelon accessions (summer season, 2014)

Fourty three accessions of snapmelon *viz.*, IC-430154, IC-430170, IC-430171, IC-430172, IC-430173, IC-430174, IC-430175, IC-430176, IC-430177, IC-430155, IC-430156, IC-430157, IC-430158, IC-430159, IC-430160, IC-430161, IC-430162, IC-430163, IC-430164, IC-430165, IC-430166, IC-430167, IC-430168, IC-430169, IC-430186, IC-430187, IC-430188, IC-430189, IC-430190, IC-369788, DKS-AHS 2011/1, DKS-AHS 2011/2, IC-430178, IC-430179, IC-430180, IC-430181, IC-430182, IC-430183, IC-430184, IC-430185, DKS-AHS 2011/3, DKS-AHS 2011/4 and DKS-AHS 2011/5 were sown at experimental farm of ICAR-Central Institute for Arid Horticulture, Bikaner (28°06′N, 73°21′E). The crop was sown in summer season, 2014 with three replicates (blocks) for each accession with a randomized block design.

Screening of the selected snapmelon accessions (rainy, 2014 and summer, 2015)

Seventeen selected accessions from forty three preliminary screening accessions on the basis of melon fly infestation of snapmelon, *viz.*, IC-430169, DKS-AHS 2011/3, IC-430172, IC-430175, IC-369788, IC-430160, IC-430162, IC-430171, IC-430179, IC-430180, IC-430190, DKS-AHS 2011/2, IC-430185, IC-430184, IC-430155, DKS-AHS 2011/4 and IC-430164 were sown at experimental farm of ICAR-Central Institute for Arid Horticulture (28°06′03.8″N 73°21′12.5″E), Bikaner in July, 2014 and February, 2015 following a randomized block design, with three blocks for each accession with each block representing a replication. The area of each bed was 5 m × 2 m and the plant to plant distance was maintained at 50 cm. A drip irrigation system was used to provide plant water requirements. All the recommended vegetable practices (e.g. weeding, fertilization, hoeing, etc.) were performed equally in each experimental bed. Two pickings were done during the entire growing season of snapmelon. Ten fruits were randomly selected from each picking from each experimental bed (replication) of each accession and were brought to the laboratory for microscopic examination for melon fly infestation. The infested fruits were sorted and percent fruit infestation was calculated. All the accessions were found similar in occurrence of flowering and fruiting (ranged within 10 days) which means that they reached the stage of susceptibility to melon fly at around the same time. The accessions were categorized by following the rating system given by Haldhar *et al.* (2013a) and Haldhar *et al.* (2018) for fruit infestation as: immune (no damage), highly resistant (1–10%), resistant (11–20%), moderately resistant (21–50%), susceptible (51–75%) and highly susceptible (76–100%).

Biochemical fruit traits of the snapmelon accessions

Two fresh fruits of each accession from each replication were selected, cut in to small pieces and dried. The Total Soluble Solid (TSS%) of different accessions of fresh fruit was determined by hand refractometer. For the estimation of biochemical's, the procedures used for each biochemical were flavonoid (Nabavi *et al.* 2008), phenols content (Malik and Singh 1980) and tannins content (Schandert 1970), free amino acid (Lee and Takahashi 1966) and the analysis were also determined on the basis of these procedures.

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Morphological fruit traits of the snapmelon accessions

Ten marketable fresh fruits of each of the seventeen snapmelon accessions were used to record data on the morphological traits (length of pubescence, rind hardness, pericarp thickness, flesh thickness, fruit length and fruit diameter). Length of ovary pubescence, pericarp thickness, flesh thickness, fruit diameter and fruit length were measured at five different positions of each fruit using Digital Vernier Caliper (MITU-TOYO, 300 mm, 0.01 mm reading capacity). The rind hardness of fruit at immature and mature stages was assessed using fruit pressure tester (Model FT 327, 0–14kg/cm^2). Immature stages are slightly bitter, about 20–24 days fruit after fruit setting and cannot use as vegetable purpose. Whereas mature stages are sweet, ripe fruit, about 28–30 days fruit after fruit setting and can be used for cooking vegetable and dessert salad.

Statistical analysis

Transformations (angular and square root transformed value) were used to achieve normality in the data before analysis but untransformed means were also presented in all the tables. The data on percentage fruit infestation and larval density per fruit and biochemical fruit traits were analyzed through one-way ANOVA using SPSS 16 software (O'Connor 2000). The means of significant parameters among tested accessions were compared using Tukey's honestly significant difference (HSD) tests for paired comparisons at probability level of 5%. Correlations between melon fly parameters (percent fruit infestation and larval density per fruit) with biophysical and biochemical fruit traits were determined using correlation analysis at the 95 and 99% significance level.

Results

Screening of snapmelon accessions

The 43 snapmelon accessions were taken for preliminary screening against *B. cucurbitae* and significant differences were found in percentage fruit infestation and larval density per fruit. The accessions IC-430190, DKS-AHS 2011/4, DKS-AHS 2011/3 and IC-430176 were found resistant; IC-430160, IC-430162, IC-430163, IC-430165, IC-430185, IC-430188, IC-430189, IC-369788, IC-430166, IC-430167, IC-430173, IC-430174, IC-430175, IC-430179, IC-430180, IC-430181, DKS-AHS 2011/2 and DKS-AHS 2011/5 were moderately resistant whereas IC-430154, IC-430155, IC-430156, IC-430157, IC-430161, IC-430164, IC-430168, IC-430169, IC-430170, IC-430171, IC-430172, IC-430177, IC-430158, IC-430159, IC-430178, IC-430182, IC-430183, IC-430184, IC-430186, IC-430187 and DKS-AHS 2011/1 were the susceptible accessions against melon fruit fly. The larval densities ranged from 8.3 to 18 larvae per fruit and were found significantly lower in resistant accessions than in the susceptible accessions. The fruit infestation ranged from 10.8 to 70.6% which was significantly lower in resistant accessions and higher in susceptible accessions. The 17 accessions selected for final evaluation trials against *B. cucurbitae* resistance during rainy, 2014 and summer, 2015, the accessions; IC-430190, DKS-AHS 2011/4, and DKS-AHS 2011/3 were resistant; IC-430160, IC-430162, IC-430175, IC-430179, IC-430180, IC-430185, IC-369788, and DKS-AHS 2011/2 were found moderately resistant whereas IC-430155, IC-430164, IC-430169, IC-430171, IC-430172 and IC-430184 were susceptible accessions in both seasons (**Table 1**). The percentage fruit infestation increased with an increase in larval density per fruit and there was a significant positive correlation ($r = 0.988$; $p < 0.01$) between per cent fruit infestation and larval density per fruit. The fruit infestation in rainy season of 2014 ranged from 11.6 to 70.4% whereas in the 2015 summer season, it ranged from 10. 8 to 68.9%. Pooled data of fruit infestation in both seasons (11.2–69.7%) was significantly low in resistant and high in susceptible accessions. In pooled data, the per cent fruit infestation was the highest in IC-430184 (69.7%) and the lowest in IC-430190 (11.2%) followed by DKS-AHS-2011/4 (15.0%). The larval density ranged from 8.6 to 17.6 and 8.3 to 17.2 larvae per fruit in the rainy season, 2014 and summer season, 2015, respectively. Pooled data of larval density per fruit in both seasons (8.5–17.4 larvae per fruit) were significantly lower in resistant and higher in susceptible accessions. In pooled data, the larval density were maximum in IC-430169 (17.4 larvae per fruit) and minimum in IC-430190 (8.5 larvae per fruit) followed by DKS-AHS 2011/3 (8.8 larvae per fruit) (**Table 1**, **Supplemental Fig. 1**).

Biochemical fruit traits of the snapmelon accessions

Flavonoid, tannins, total alkaloid and phenols contents ranged from 0.6 to 2.3 mg/g, 6.1 to 11.8 mg/g, 0.5 to 2.2% and 9 to 14.8 mg/g (on dry weight basis), respectively with values significantly higher in resistant and lower in susceptible accessions (**Table 2**). The flavonoid content (2.3 mg/g) and total alkaloid content (2.2%) were found maximum in IC-430190 and minimum in IC-430169 (flavonoid content 0.7 mg/g and total alkaloid content 0.5%). The tannin (12.9 mg/g) and phenols content (16.0 mg/g) were found the highest in DKS-AHS 2011/4 and the lowest in IC-430184 (6.1 & 9 mg/g). The free amino acid and total soluble solid (TSS) of different accessions fruits ranged from 4.6 to 9.5 (mg/g on dry weight basis) and 4 to 8.1%, respectively with values lower in resistant and higher in susceptible accessions. The percentage of fruit infestation and the larval density per fruit with free amino acid (0.97 & 0.96) and TSS (0.3 & 0.3) of fruit had a significant positive correlation with free amino acid and nonsignificant positive correlation with TSS whereas flavonoid (–0.98 & –0.96), tannins (–0.97 & –0.95), phenols (–0.96 & –0.95) and total alkaloid (–0.97 & –0.94) had significant negative correlation (**Table 3**).

Morphological fruit traits of the snapmelon accessions

The length of ovary pubescence, rind hardness at immature stage, rind hardness at mature stage and pericarp

Table 1. Larval density and percent fruit infestation of melon fruit fly on different accessions of snapmelon, *C. melo* var. *momordica* during final screening trials

Accession	Institute	Larval density/fruit	Fruit infestation (%)	Resistance category
IC-430169[#]	ICAR-CIAH	17.4^{f}	68.5^{j}	S
DKS-AHS 2011/3	ICAR-CIAH	8.8^{ab}	18.6^{bc}	R
IC-430172	ICAR-CIAH	17.8^{f}	66.3^{ij}	S
IC-430175	ICAR-CIAH	11.9^{cd}	41.1^{f}	MR
IC-369788	ICAR-CIAH	9.3^{ab}	25.3^{de}	MR
IC-430160	ICAR-CIAH	8.9^{ab}	22.5^{cd}	MR
IC-430162	ICAR-CIAH	9.6^{ab}	26.4^{de}	MR
IC-430171	ICAR-CIAH	14.9^{e}	53.6^{gh}	S
IC-430179	ICAR-CIAH	13.7^{de}	47.6^{fg}	MR
IC-430180	ICAR-CIAH	14.6^{e}	48.3^{g}	MR
IC-430190	ICAR-CIAH	8.5^{a}	11.2^{a}	R
DKS-AHS 2011/2	ICAR-CIAH	10.6^{bc}	29.1^{e}	MR
IC-430185	ICAR-CIAH	14.5^{e}	48.5^{g}	MR
IC-430184	ICAR-CIAH	17.4^{f}	69.7^{j}	S
IC-430155	ICAR-CIAH	14.5^{e}	54.1^{gh}	S
DKS-AHS 2011/4	ICAR-CIAH	8.9^{ab}	14.9^{ab}	R
IC-430164	ICAR-CIAH	15.2^{e}	59.9^{hi}	S
Mean ± SD		12.7 ± 3.3	41.5 ± 19.5	
SEm±		0.6	1.4	
LSD (P = 0.05)		1.8	4.0	
F calculated		27.2	70.7	
Error degree of freedom		32	32	

[#] The 'IC' number was given by ICAR-National Bureau of Plant Genetics and Resources.
Values in columns with different letters are significantly different using Tukey's HSD test, R: resistant, MR: moderately resistant and S: susceptible.

Table 2. Biochemical (allelochemical) fruit traits of different accessions of snapmelon, *C. melo* var. *momordica*

Accession	Flavonoid content (mg/g)	Tannins content (mg/g)	Total alkaloid content (%)	Phenols content (mg/g)	Free amino acid (mg/g)	TSS (%)	Resistance category
IC-430169	0.7^{ab}	6.9^{abc}	0.5^{a}	10.2^{ab}	9.5^{k}	7.6^{gh}	S
DKS-AHS 2011/3	2.0^{ijk}	11.3^{gh}	1.8^{gh}	14.3^{fg}	5.0^{ab}	8.1^{hi}	R
IC-430172	0.9^{abc}	7.2^{abc}	0.6^{ab}	10.3^{ab}	8.9^{ijk}	6.6^{de}	S
IC-430175	1.6^{fgh}	9.9^{efg}	1.3^{e}	13.1^{def}	7.6^{ef}	6.2^{bcd}	MR
IC-369788	1.9^{ij}	10.8^{fgh}	1.5^{f}	13.8^{efg}	5.7^{c}	4.7^{ab}	MR
IC-430160	1.7^{hi}	11.3^{ghi}	1.7^{g}	14.2^{fg}	5.7^{c}	6.0^{bc}	MR
IC-430162	1.7^{hij}	10.6^{efg}	1.5^{f}	14.0^{fg}	6.4^{d}	5.5^{ab}	MR
IC-430171	1.1^{cde}	8.1^{cd}	0.9^{c}	11.1^{bc}	8.4^{hi}	6.8^{ef}	S
IC-430179	1.4^{efg}	9.4^{def}	1.2^{de}	12.6^{de}	7.7^{efg}	6.6^{cde}	MR
IC-430180	1.3^{def}	8.3^{cd}	1.1^{d}	11.4^{bc}	8.0^{fgh}	4.0^{a}	MR
IC-430190	2.3^{l}	11.8^{hi}	2.2^{i}	14.8^{gh}	5.4^{bc}	6.9^{ef}	R
DKS-AHS 2011/2	1.7^{ghi}	10.6^{efg}	1.4^{ef}	$13.5d^{efg}$	6.9^{d}	8.0^{hi}	MR
IC-430185	1.1^{cd}	9.1^{de}	1.1^{d}	12.0^{cd}	8.4^{ghi}	7.2^{fg}	MR
IC-430184	0.6^{a}	6.1^{a}	0.7^{ab}	9.0^{a}	9.2^{jk}	8.5^{i}	S
IC-430155	1.1^{cde}	8.0^{bc}	0.8^{bc}	11.2^{bc}	8.9^{ijk}	8.0^{hi}	S
DKS-AHS 2011/4	2.2^{kl}	12.9^{i}	1.9^{h}	16.0^{h}	4.6^{a}	5.5^{ab}	R
IC-430164	0.9^{bc}	6.47^{ab}	0.8^{bc}	9.5^{a}	8.6^{ij}	6.5^{cde}	S
Mean ± SD	1.4 ± 0.5	9.3 ± 2.0	1.2 ± 0.5	12.4 ± 2.0	7.9 ± 1.6	6.6 ± 1.2	
SEm±	0.1	0.5	0.1	0.5	0.2	0.2	
LSD (P = 0.05)	0.3	1.5	0.2	1.5	0.7	0.6	
F calculated	28.8	14.5	78.4	16.0	49.8	41.6	
Error degree of freedom	32	32	32	32	32	32	

Values in columns with different letters are significantly different using Turkey's HSD test.

thickness which ranged from 1.4 to 2.7 mm, 10.1 to 14.8 kg/cm^2, 4.8 to 11.0 kg/cm^2 and 0.4 to 1.2 mm, respectively, were significantly higher in resistant and lower in susceptible accessions. However, the flesh thickness (11.2 to 22.3 mm), fruit length (10.4 to 19.0 cm) and fruit diameter (6.4 to 10.1 cm) were significantly shorter in resistant and longer in susceptible accessions (Plate-1, **Table 4**). The length of ovary pubescence (–0.99 & –0.96), rind hardness at immature stage (–0.93 & –0.90), rind hardness at mature stage (–0.59 & –0.54) and pericarp thickness (–0.78 & –0.75) had significant negative correlations whereas flesh thickness (0.62 & 0.61), fruit length (0.61 & 0.62) and fruit diameter (0.76 & 0.77) had significant positive correlations with the percentage fruit infestation and the larval density per fruit (**Table 5**).

Table 3. Correlation coefficient (r) between percent fruit infestation and larval density per fruit with different allelochemical fruit traits of snapmelon, *C. melo* var. *momordica* accessions

	Percent damage	Larval density	FC	TC	TA	PC	FAA
Larval density	0.99**						
FC	–0.98**	–0.96**					
TC	–0.97**	–0.95**	0.96**				
TA	–0.97**	–0.94**	0.97**	0.95**			
PC	–0.96**	–0.94**	0.96**	0.99**	0.94**		
FAA	0.97**	0.96**	–0.96**	–0.95**	–0.95**	–0.94**	
TSS	0.30^{NS}	0.30^{NS}	$–0.36^{NS}$	$–0.31^{NS}$	$–0.27^{NS}$	$–0.32^{NS}$	0.33^{NS}

** Significant at P = 0.01 (two-tailed).
* Significant at P = 0.05 (two-tailed).
FC- flavonoid content (mg/g), TC: tannins content (mg/g), PC: phenols content (mg/g), TA: total alkaloid (%), FAA: free amino acid (mg/g), TSS: total soluble solid (%).

Based on Kaiser Normalization method

Based upon the above morphological and biochemical characters individually it was impossible to group the entries as variables were not in agreement to each other. Hence, principal component analysis was performed to achieve parsimony and reduce the dimensionality by extracting the smallest number of components that accounted for most of the variation in the original multivariate data. Taking into consideration fifteen parameters viz., flavonoid content, TSS, free amino acid, total alkaloid, tannins content, phenols content length of ovary pubescence, rind hardness at immature stage, rind hardness at mature stage, fruit length, flesh thickness and fruit diameter principal component analysis (PCA) was performed. Two principal components (PCs) were extracted with eigen value ≥1.0, after varimax rotation with Kaiser Normalization procedure which converged in

Table 4. Morphological (antixenotic) fruit traits of different accessions of snapmelon, *C. melo* var. *momordica*

Accession	Length of ovary pubescence (mm)	Rind hardness at immature stage (Kg/cm^2)	Rind hardness at mature stage (Kg/cm^2)	Pericarp thickness (mm)	Flesh thickness (mm)	Fruit diameter (cm)	Fruit length (cm)	Resistance category
IC-430169	1.4^{a}	10.4^{ab}	5.9^{b}	0.5^{ab}	18.7^{f}	9.7^{ij}	17.2^{f}	S
DKS-AHS 2011/3	2.5^{j}	13.9^{ijk}	9.8^{ef}	1.2^{h}	11.2^{a}	6.4^{a}	10.5^{ab}	R
IC-430172	1.5^{bc}	11.5^{bcd}	5.4^{ab}	0.5^{ab}	17.5^{ef}	9.7^{ij}	15.1^{bcde}	S
IC-430175	2.1^{h}	12.8^{efgh}	7.6^{cd}	0.9^{f}	18.3^{ef}	9.1^{ghi}	16.8^{ef}	MR
IC-369788	2.3^{i}	13.7^{hij}	7.2^{cd}	1.1^{gh}	17.7^{ef}	7.7^{bcd}	15.0^{bcd}	MR
IC-430160	2.3^{i}	13.6^{ghij}	6.8^{c}	0.4^{ab}	13.6^{bc}	7.5^{bc}	14.8^{bcd}	MR
IC-430162	2.2^{i}	12.7^{efg}	5.7^{ab}	0.9^{f}	15.1^{cd}	7.4^{bc}	13.8^{b}	MR
IC-430171	1.7^{ef}	12.9^{efgh}	5.0^{a}	0.4^{a}	22.3^{g}	8.9^{fgh}	17.3^{fg}	S
IC-430179	1.9^{g}	12.2^{def}	6.2^{b}	0.8^{ef}	17.4^{ef}	8.5^{efg}	15.6^{cdef}	MR
IC-430180	1.9^{g}	12.5^{def}	7.8^{d}	0.7^{de}	15.0^{cd}	7.4^{b}	16.1^{def}	MR
IC-430190	2.6^{k}	14.3^{l}	11.0^{g}	1.1^{h}	12.7^{ab}	7.4^{bc}	14.1^{bc}	R
DKS-AHS 2011/2	2.1^{h}	13.0^{fghi}	6.2^{b}	0.7^{ef}	18.2^{ef}	7.1^{b}	10.4^{a}	MR
IC-430185	1.8^{fg}	12.4^{def}	6.2^{b}	0.8^{f}	20.5^{g}	10.1^{j}	19.0^{g}	MR
IC-430184	1.4^{ab}	10.1^{a}	4.8^{a}	0.4^{ab}	20.7^{g}	9.3^{hi}	17.0^{f}	S
IC-430155	1.7^{cde}	11.9^{cde}	10.6^{fg}	0.6^{cd}	15.3^{cd}	9.5^{hij}	17.0^{f}	S
DKS-AHS 2011/4	2.7^{k}	14.8^{k}	9.5^{e}	1.0^{g}	16.7^{de}	8.0^{cde}	15.7^{cdef}	R
IC-430164	1.6^{cd}	10.9^{abc}	5.9^{b}	0.5^{bc}	17.1^{ef}	8.3^{def}	16.6^{def}	S
Mean ± SD	2.0 ± 0.4	12.6 ± 1.3	7.1 ± 1.9	0.7 ± 0.3	16.9 ± 2.9	8.4 ± 1.1	15.4 ± 2.3	
SEm±	0.03	0.4	0.3	0.04	0.6	0.2	0.6	
LSD (P = 0.05)	0.1	1.02	0.8	0.1	1.2	0.7	1.7	
F calculated	47.6	14.0	45.8	47.9	22.8	23.4	14.5	
Error degree of freedom	32	32	32	32	32	32	32	

Values in columns with different letters are significantly different using Turkey's HSD test.

Table 5. Correlation coefficient (r) between percent fruit infestation and larval density per fruit with different antixenotic fruit traits of snapmelon, *C. melo* var. *momordica* accessions

	Percent damage	Larva density	Length of ovary pubescence	Rind hardness at immature stage	Rind hardness at mature stage	Pericarp thickness	Flesh thickness	Fruit diameter
Larval density	0.99**							
Length of ovary pubescence	–0.99**	–0.96**						
Rind hardness at immature stage	–0.93**	–0.90**	0.94**					
Rind hardness at mature stage	–0.59*	–0.54*	0.61**	0.60*				
Pericarp thickness	–0.78**	–0.75**	0.82**	0.72**	0.66**			
Flesh thickness	0.62**	0.61**	–0.59*	–0.48*	–0.70**	–0.51*		
Fruit diameter	0.76**	0.77**	–0.72**	–0.63**	$–0.36^{NS}$	–0.54*	0.68**	
Fruit length	0.61**	0.62**	–0.56*	$–0.46^{NS}$	$–0.26^{NS}$	$–0.47^{NS}$	0.59*	0.81**

** Significant at P = 0.01 (two-tailed).
* Significant at P = 0.05 (two-tailed).

Table 6. Component loadings of parameters for resistance against melon fruit fly in snapmelon, *C. melo* var. *momordica* fruits

S. No.	Parameters	Principal components	
		1	2
1	Fruit infestation (%)	–0.84	–0.53
2	Larval density per fruit	–0.82	–0.53
3	Flavonoid content	0.86	0.49
4	Tannins content	0.88	0.42
5	Total alkaloid	0.82	0.52
6	Phenols content	0.88	0.42
7	Free amino acid	–0.83	–0.51
8	Total soluble solid	–0.66	0.39
9	Length of ovary pubescence	0.88	0.47
10	Rind hardness at immature stage	0.89	0.35
11	Rind hardness at mature stage	0.43	0.52
12	Pericarp thickness	0.67	0.47
13	Flesh thickness	–0.32	–0.74
14	Fruit diameter	–0.48	–0.68
15	Fruit length	–0.18	–0.86

Rotation method: Varimax with Kaiser Normalization.
Rotation converged in 3 iterations.

three iterations. The extraction communalities for all the variables tested were ≥0.5 indicating that the variables were well represented by the extracted PCs which together explained a cumulative variation of 82.8%. PC1 explaining 53.41% of the variation while PC2 explained 29.39% of variation. PC1 had the loadings for flavonoid content (0.86), tannins content (0.88), total alkaloid (0.82), phenols content (0.88), free amino acid (–0.83), total soluble solid (–0.66), length of ovary pubescence (0.88), pericarp thickness (0.67) and rind hardness at immature stage (0.89). Rind hardness at mature stage (0.52), flesh thickness (–0.74), fruit length (–0.86) and fruit diameter (–0.68) were loaded in PC2 (**Table 6**).

Discussion

Plant defense strategies against insect herbivores may involve the synthesis of a plethora of biologically active compounds (allelochemicals) which are phylogenetically conserved in specific plant families or genera (Mithofer and Boland 2012). Many compounds act directly on the herbivores (bottom-up control), whereas others act indirectly, via the attraction of organisms from other trophic levels (i.e. parasitoids and predators) which, in turn, protect the plants (plant mediated top-down control) (Ode 2006). Furthermore, host quality also depends on differences between the genotypes of the plant, including differences in morphological traits, nutrient contents, and the concentration of secondary compounds (Cartea *et al.* 2014, Haldhar *et al.* 2013b, 2015a). The results show the overall bottom-up effect in a study on snapmelon against the melon fly, *B. cucurbitae*. While analyzing the effect of biochemical and morphological traits on melon fly in different accession of snapmelon, significant differences in melon fly incidence were observed. Direct defenses are mediated by plant characteristics such as mechanical protection on the surface of the plants (e.g., hairs, trichomes, thorns, spines, and pericarp thickness). But the productions of toxic chemicals (such as terpenoids, alkaloids, anthocyanins, phenols, and quinones) are supposed to be "indirect defenses", as they are only active through the insect feeding being part of the secondary metabolism of plants. And the plant defense mechanism can affect the herbivores through antibiosis and antixenosis, and not just through biology, killing or retarding their development (Hanley *et al.* 2007). Host plant selection by insects is either expressed by the occurrence of a population of insects on the plant in nature or by feeding, oviposition or use of the plant for complete offspring development (Rafiq *et al.* 2008). In the present final study, the accessions IC-430190, DKS-AHS 2011/4, and DKS-AHS 2011/3 were resistant; IC-430160, IC-430162, IC-430175, IC-430179, IC-430180, IC-430185, IC-369788, and DKS-AHS 2011/2 were moderately resistant whereas IC-430155, IC-430164, IC-430169, IC-430171, IC-430172 and IC-430184 were found the susceptible accessions to melon fly infestation. The percentage fruit infestation and larval density were found to be significantly lower in resistant and higher in susceptible accessions of snapmelon (**Table 1**). Numerous studies have shown that varieties/genotypes of the same species could significantly differ in their resistance to insect pests (Cartea *et al.* 2014, Haldhar *et al.* 2013a, 2015a, 2015d, Kousik *et al.* 2007, Moslem *et al.* 2011, Simmons *et al.* 2010) and it is influenced by morphological and biochemical traits of plants. Similar to our findings, Gogi *et al.* (2010) and Haldhar *et al.* (2015b) observed lower fruit infestation and larval densities on resistant genotypes of cucurbits than on their susceptible genotypes.

Study on secondary metabolites (allelochemicals) could lead to the identification of new signaling molecules involved in plant resistance against herbivores and other stresses. Ultimately genes and enzymes involved in the biosynthesis of these metabolites could be identified. The allelochemical compounds of fruit were significantly different among the tested snapmelon accessions. The free amino acid and total soluble solid was the lowest in resistant and the highest in susceptible accessions, whereas flavonoid, tannins, phenols, and total alkaloid contents were the highest in resistant and lowest in susceptible accessions of snapmelon (**Tables 2**, **3**). No reports have been made previously on the correlation of biochemical traits in snapmelon with infestation rate by melon fly, but some data have been reported for other insect-crop interactions. Phenols act as a defensive mechanism not only against herbivores but also against microorganisms and competing plants. Qualitative and quantitative alterations in phenols and elevation in activities of oxidative enzyme in response to insect attack was a general phenomenon (War *et al.* 2011). Lignin, a phenolic heteropolymer played a central role in plant defense against insects and pathogens (Barakat *et al.* 2010). Flavonoids were cytotoxic and interacted with different enzymes through complexation. Both flavonoids and isoflavonoids protected the plant against insect pests by influencing the

behavior, and growth and development of insects (Simmonds 2003). Similar finding also showed that pH was the lowest in resistant genotypes and tannin, flavanol and phenol contents were the highest in resistant genotypes of bitter gourd against melon fly (Gogi *et al.* 2010). Tannins had a strong deleterious effect on phytophagous insects and affected the insect growth and development by binding to the proteins, reduced nutrient absorption efficiency, and cause midgut lesions (Barbehenn and Constabel 2011). Total soluble solid and pH of fruit had a significant positive correlation whereas tannins, phenols, alkaloids and flavonoid contents had significant negative correlation with the percentage fruit infestation and the larval density per fruit. The biochemical characters such as total sugar and crude protein were positively correlated with melon fly infestation, whereas total phenols had negative correlation (Haldhar *et al.* 2013a, 2015b, Sharma and Singh 2010, War *et al.* 2012). Similar to our findings, it has been demonstrated that phenols, tannins, and flavonoids enhanced plant defenses against insects (Gogi *et al.* 2010, Haldhar *et al.* 2013a, 2015a, War *et al.* 2012).

The phenotypic (antixenotic) mechanisms of fruit traits were significantly different among the tested snapmelon accessions. Fruit length, flesh thickness and fruit diameter had significant positive correlations whereas rind hardness at immature stage, rind hardness at mature stage, pericarp thickness and length of ovary pubescence had significant negative correlations with the percent fruit infestation and larval density. In these findings, biophysical fruit-traits were also found significantly different among genotypes (Dhillon *et al.* 2005, Haldhar *et al.* 2015b, 2015c, Gogi *et al.* 2010, Simmons *et al.* 2010). Glandular trichomes secrete secondary metabolites including flavonoids, terpenoids, and alkaloids that could be poisonous, repellent, or trap insects and other organisms, thus forming a combination of structural and chemical defense (Sharma *et al.* 2009). Structural traits such as spines and thorns (spinescence), trichomes (pubescence), toughened or hardened leaves (sclerophylly), incorporation of granular minerals into plant tissues, and divaricated branching (shoots with wiry stems produced at wide axillary angles) played a leading role in plant protection against herbivory (Chamarthi *et al.* 2010, He *et al.* 2011). Similar results were documented by Haldhar *et al.* (2015b) that length of ovary pubescence, rind hardness, fiber content and rind thickness had significant negative correlations whereas fruit length and fruit diameter had significant positive correlations with the percentage fruit infestation and the larval density per fruit in different genotypes of ridge gourd. These variations in measurements of biophysical fruit-traits might be attributed to differences in the tested genotypes and/or stage of the fruits selected for measuring these traits, as reported in earlier studies (Dhillon *et al.* 2005, Gogi *et al.* 2010, Haldhar *et al.* 2015a, 2017, Kumara *et al.* 2006).

Based on Kaiser Normalization method, two principal components (PCs) were extracted explaining cumulative variation of 82.8% in melon fly infestation. The PC1 and PC2 were plotted and the plot showed three discrete classes of accessions which could be grouped into resistant (R), moderately resistant (MR) and susceptible (S) as depicted in **Fig. 1**. According to Gogi *et al.* (2010) maximum variation in fruit infestation was explained by tannin and flavanol contents whereas, rest of the biochemical fruit traits explained <0.2% variation in the fruit infestation. The maximum variation in larval density per fruit was explained by tannin followed by flavanol whereas, rest of the biochemical fruit traits explained <0.1% variation in larval density. In morphological characters, the maximum variation was explained by rind hardness followed by fruit diameter and number of longitudinal ribs. Haldhar *et al.* (2013) examined that the total alkaloid and pH contents explained 97.96% of the total variation in melon fly infestation and 92.83% of the total variation in larval density per fruit due to alkaloids and total sugar contents in muskmelon. Haldhar *et al.* (2015a) found that two principal components (PCs) were extracted explaining cumulative variation of 90% in melon fly infestation and length of ovary pubescence, rind thickness, flavonoid content, ascorbic acid, free amino acid, tannins content, and phenols content were the reliable variables for characterization of resistance. Ridge gourd varieties/accessions AHRG-57, Pusa Nasdar and AHRG-29 were classified as resistant to *B. cucurbitae* and these could be used in future breeding program as resistant sources. Prasad *et al.* (2015), the important sorghum, *Sorghum bicolor* (Family: Gramineae) grain characteristics viz., 100 seed weight was significantly positively correlated with grain hardness (0.55) and median development period (0.47) and significantly negatively correlated with grain weight loss (–0.43). However, the grain hardness was significantly negatively correlated with oviposition (–0.49), adult emergence (–0.75) and grain weight loss (–0.82) and was significantly positively

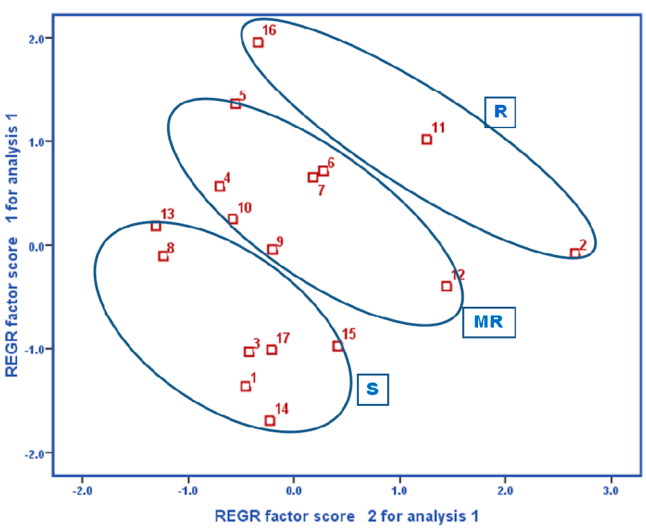

Fig. 1. Plot of PC1 and PC2 showing clusters of snapmelon, *C. melo* var. *momordica* accessions show resistance to melon fruit fly, *B. cucurbitae* (R: Resistance; MR: Moderately Resistance & S: Susceptible).

correlated with median development period (0.85). Two principal components (PCs) were extracted explaining cumulative variation of 76.2%. Seed weight, grain hardness, oviposition, adult emergence, median development period and grain weight loss were the reliable variables for characterization of resistance to *Sitophilus oryzae*. The sorghum lines EC 24, EC 22, PEC 8, PEC 7, EP 78, EP 57, AKR 354 were classified as resistant to *S. oryzae*.

Thus, from the foregoing account, it could be argued that reduction in melon fly infestations on resistant accessions could be due to phenotypics (biophysical) and antibiosis (allelochemicals). Snapmelon accessions IC-430190, DKS-AHS 2011/4, and DKS-AHS 2011/3 were classified as resistant to *B. cucurbitae* and these could be used in future breeding program as resistant sources. Certain biophysical traits (e.g. length of ovary pubescence, rind hardness at immature stage and pericarp thickness) and biochemical traits (e.g. flavonoid, tannins, phenols content and total alkaloid) were linked to resistance of snapmelon against *B. cucurbitae* and therefore, could be used as marker traits in plant breeding programs to select resistant accessions.

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