INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
09–142/WIB/2009/11–6–746–750
http://www.fspublishers.org

Full Length Article

Screening of Bitter Gourd (*Momordica charantia*) Germplasm for Sources of Resistance against Melon Fruit Fly (*Bactrocera cucurbitae*) in Pakistan

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ABSTRACT

Thirteen bitter gourd germplasm per cultivars (Col-II, FSD-long, Col-Nankana sahib, Col-I, GS-51, Col-III, Col-Multan, Col-Vehari, Chaman, Sunder-F1, Janpuri, F1-484 & F1-485) were screened for sources of resistance against melon fruit fly in Pakistan. Results revealed that the percent fruit-infestation and larval density per fruit varied significantly in all tested bitter gourd genotypes. Based on the considered criteria i.e., percent fruit infestation (< 20%) and larval density per fruit (3 larvae fruit^{-1}), Col-II and FSD-long were categorized as resistant genotypes. Col-Nankana sahib, Col-I and GS-51 showed > 20% but < 50% fruit infestation and >3 but <6 larvae per fruit were categorized as moderately resistant genotypes. Col-III, Col-Multan, Col-Vehari, Chaman, Sunder-F1, Janpuri, F1-484 and F1-485, with > 50% but < 80% fruit-infestation and >6 but <10 larvae per fruit were categorized as susceptible genotypes. It was concluded that Col-II and FSD-long can be used as a source of resistance for developing bitter gourd genotypes resistant to melon fruit flies.

Key Words: Source of resistance; Bitter gourd; Germplasm; Melon fruit fly

INTRODUCTION

Plant genotypes are exposed to various types of stressors, including, nutrients imbalance, soil composition (Eckey-Kaltenbach *et al.*, 1994; Goncalves-Alvim *et al.*, 2004), microclimate, plant-genetics, plant-tissue ontogeny (Ponti, 1977; Mutikainen *et al.*, 2000; Sadrnia, 2007), herbivore (or abiotic) induction responses (Tallamy & Raupp, 1991), somatic mutations (Karban & Baldwin, 1997), plant chemistry (Feeny, 1995; Mutikainen *et al.*, 2000) and/or of the interplay between all of them (Stadler, 1992). These stressors alter not only the genotypic, but also the phynotypic and/or biochemical properties of the plants and resultantly, induce in them different mechanisms of resistance, which enable them to avoid, tolerate or recover from the effects of insect pest attacks (Eckey-Kaltenbach *et al.*, 1994; Pedigo, 1996) and toxic metals (Ghani & Wahid, 2007).

Bitter gourd (*Momordica charantia* L.; Cucurbitacae), commonly known as balsam pear, or karela, is cultivated throughout the world, especially in the tropical areas (El-Batran *et al.*, 2006). The immature fruits and the tender leafy shoots or the ripe fruits (Yamaguchi, 1983) have both nutritional as well as medicinal importance (Khan & Anderson, 2003). It is also cultivated as an important vegetable crop in many areas of Pakistan (Tahir & Haider, 2005).

Melon fruit flies (Diptera: Tephritidae: Dacinae) are economically important pests of the cucurbits and are geographically distributed throughout the tropics and subtropics of the world (Drew, 1992; Chinajariyawong *et al.*, 2003), especially in most of the countries of South East Asia (Allwood *et al.*, 1999). It has more than 81 plant species as its host (Dhillon *et al.*, 2005), but plants of family Cucurbitacae are considered to be its preferred hosts (Allwood *et al.*, 1999). Amongst cucurbits, the fruits of bitter gourd (*Momordica charantia*), muskmelon (*Cucumis melo*), snap melon (*Cucumis melo* var. *momordica*) and snake gourd (*Trichosanthes anguina* & *T. cucumeria*) have been reported as being the most preferred hosts (Doharey, 1983). It causes heavy quantitative and qualitative losses in bitter gourd, *Momordica charantia* Linn. (Cucurbitacae) (Mote, 1975; Rabindranath & Pillai, 1986). Female fruit fly deposits its eggs, preferably on young, green and tender fruits or, sometimes in the corolla of the flowers and maggots feed inside the fruit as well as on the fruit pulp (Dhillon *et al.*, 2005) or, occasionally on the flowers, taproots, stems and leaf stalks (Weems & Heppner, 2001). The infested fruits and flowers do not develop properly and fall down or rot on the plant and result in a dramatic reduction of yield (Dhillon *et al.*, 2005). Depending on the cucurbit species, season and prevailing climatic conditions, a loss of 30 to 100% can be caused by the melon fruit fly

To cite this paper: Gogi, M.D., M. Ashfaq, M.J. Arif and M.A. Khan, 2009. Screening of Bitter Gourd (*Momordica charantia*) Germplasm for sources of resistance against melon fruit fly (*Bactrocera cucurbitae*) in Pakistan. *Int. J. Agric. Biol*., 11: 746–750

(Dhillon *et al.,* 2005). In the bitter gourd crop, 41-95% fruit infestation, by melon fruit fly, has been recorded (Gupta & Verma, 1978; Rabindranath & Pillai, 1986; Hollingsworth *et al.,* 1997). The melon fruit fly has also been reported to infest 95% of the bitter gourd fruits, in Papua (New Guinea) (Hollingsworth *et al.*, 1997). Singh *et al.* (2000) reported 31.27% damage on bitter gourd in India.

Melon fruit fly can be managed or suppressed in the farmer fields, by local area and wide area management programmes (Dhillon *et al.,* 2005). Local area management programme, which aims at suppressing, rather than eradicating the melon fruit flies, involves the integration of various management tactics, including, bagging fruits, application of field sanitation measures, installation of protein baits and cue lure traps, growing fruit fly resistant genotypes, augmentation of bio-control agents and cover spray of soft insecticides (Chinajariyawong *et al.,* 2003; Omar & Hashim, 2004; Dhillon *et al.,* 2005).

As a result of the recent efforts, made by the Environmental Protection Agency, to reduce the use of harmful insecticides, especially, organophosphates, organochlorines, some carbamates and pyrethrides, in the agricultural crops, the trend has now been shifted towards an integrated pest management (IPM) for the control of tephritid fruit flies (Klungness *et al.,* 2005). Integrated pest management (IPM), includes, a combination of chemical, biological and cultural control tactics (Sarfraz *et al.*, 2005), with insecticides still to continue as an important components of such strategies. But, the larvae of melon fruit flies, like, other fruit flies often pupate either in the soil, inside the fruits or under the fruits, thereby avoiding the exposure to or contact with insecticides, when surface application is practised. Similarly, the maggots damage the fruits internally. Therefore, it is imperative to explore alternative methods of control of this pest.

Hence, the development of varieties resistant to melon fruit fly is an important component for an integrated pest management of this pest (Panda & Khush, 1995). The development and then the cultivation of fruit fly resistant bitter gourd cultivars has been limited, because of the lack of adequate information on the genetic variability and sources of resistance in the available bitter gourd genotypes and influence of these sources on the pest multiplication (Dhillon *et al.*, 2005). Therefore, it becomes imperative to identify sources of resistance in bitter gourd and get knowledge of their influence on oviposition preference, larval performance and pest multiplication for devising sustainable pest management strategies for the control of pest fruit. These studies were carried out to screen out the available bitter gourd germplasm per cultivars in Pakistan for the source of resistance against melon fruit fly.

MATERIALS AND METHODS

Screening of varieties. Thirteen varieties of bitter gourd viz., Col-II, FSD-long, Col-Nankana sahib, Col-I, GS-51, Col-III, Col-Multan, Col-Vehari, Chaman, Sunder-F1, Janpuri, F1-484 and F1-485, were sown at two localities i.e., Ayub Agricultural Research Institute, Faisalabad and Chak No. 103-04/7R, Harappa, Sahiwal. The seeds of each variety were soaked in water within petridishes for two hours, before sowing. The sowing was done on 10 April, 2005, at Faisalabad and on 15 April, at Harappa. The experiment was laid out in a Randomized Complete Block Design, with three replications of each variety. The area of each experimental unit (bed) was 6 m X 2 m. In each experimental unit, the plant to plant distance was maintained at 30 cm. All the recommended agronomic practices were carried out. But none of the fruit fly management practices were carried out to check the varietals' resistance of tested bitter gourd varieties against the melon fruit fly. Picking of the fruits was started on 10 June, 2005 at Faisalabad and on 15 June, 2005 at Harappa. Totally, five pickings were done at each locality. After each picking, the fruits were weighed with a weighing balance in the field. After weighing, ten fruit were randomly taken from each replicate of each genotype and were brought into the laboratory, where they were observed for fruit infestation under a microscope. The infested fruits were counted and the percent fruit infestation was calculated. Each infested fruit was then observed under a microscope, the number of larvae in each fruit, were counted and the number of larvae per fruit were calculated. The genotypes were grouped by following the rating system, given by Nath (1966) for the fruit damage as– immune (no damage), highly resistant (1–10%), resistant (11–20%), moderately resistant (21–50%), susceptible (51–75%) and highly susceptible (76–100%).

Statistical analysis. The data collected were analyzed through a Multivariate General Linear Model (MGLM) Technique (Tabachnick & Fidell, 2001) by using factorial ANOVA test, using STATISTICA software: (i) to determine either the differences in above mentioned parameters are significant or non-significant among tested genotypes and (ii) to calculate means along with their standard deviations and upper and lower bounds at 95% confidence intervals. The means of significant parameters, among tested genotypes, were compared by using Tukey's Honestly Significant Difference (HSD) tests for paired comparisons, at a probability level of 5%.

RESULTS

The tested genotypes of bitter gourd, revealed that the percentage fruit infestation ($P < 0.001$) and larval density per fruit ($P < 0.001$), varied significantly. However, percentage fruit infestation ($P > 0.05$) and larval density per fruit ($P > 0.05$), varied non-significantly in all tested bitter gourd genotypes at both localities.

Percent fruit infestation and larval density per fruit in Col-II (18.7% & 2.4 larvae fruit^{-1}) and FSD-long (19.3% & 3.2 larvae fruit^{-1}) remained below 20% and 3 larvae per fruit, respectively at Harappa and both genotypes were

categorized as resistant genotypes, according to the resistant scales, described by Nath (1966). Percent fruit infestation and larval density per fruit in Col-Nankana sahib (36.7% & 4.7 larvae fruit^{-1}), Col-I (44.7% & 5.8 larvae fruit^{-1}) and GS-51 (46.7% & 5.8 larvae fruit^{-1}) were > 20% but < 50% and > 3 but < 6 larvae per fruit, respectively at Harappa and all these genotypes were categorized as moderately resistant genotypes (Table I). Percent fruit infestation and larval density per fruit in Col-III (55.3% & 6.6 larvae fruit^{-1}), Col-Multan (67.3% & 7.3 larvae fruit^{-1}), Col-Vehari (71.3% & 8.2 larvae fruit^{-1}), Chaman (72% & 8.3 larvae fruit^{-1}), Sunder-F^{1} (72.7% & 8.2 larvae fruit^{-1}), Janpuri (73.3% & 7.5 larvae fruit^{-1}), F^{1}-484 (73.3% & 8 larvae fruit^{-1}) and F^{1}-485 (75.3% & 9.3 larvae fruit^{-1}) were > 50% but < 80% and > 6 but < 10 larvae per fruit, respectively at Harappa and were categorized as susceptibility genotypes (Table I).

However, the trails conducted at Faisalabad, revealed that the percent fruit infestation and larval density per fruit in Col-II (16.7% & 2.4 larvae fruit^{-1}) and FSD-long (20% & 3.7 larvae fruit^{-1}) remained ≤ 20% and < 4 larvae per fruit, respectively at Faisalabad and both genotypes were categorized as resistant (Table II). Percent fruit infestation and larval density per fruit in Col-Nankana sahib (33.3% & 4.6 larvae fruit^{-1}), Col-I (46.7% & 5.9 larvae fruit^{-1}) and in GS-51 (50% & 6.1 larvae fruit^{-1}), were > 20% but ≤ 50% and > 3 but ≤ 6 larvae per fruit, respectively at Faisalabad and were categorized as moderately resistant. Percent fruit infestation and larval density in Col-III (55.3% & 6.5 larvae fruit^{-1}), Col-Multan (63.3% & 7.3 larvae fruit^{-1}), Col-Vehari (66.7% & 8.2 larvae fruit^{-1}), Chaman (70% & 8.4 larvae fruit^{-1}), Sunder-F^{1} (70% & 8.2 larvae fruit^{-1}), Janpuri (73.3% & 8.6 larvae fruit^{-1}), F^{1}-484 (73.3% & 8.9 larvae fruit^{-1}) as well as in F^{1}-485 (73.3% & 9.4 larvae fruit^{-1}), were > 50% but < 75% and > 6 but < 10 larvae per fruit, respectively at Faisalabad and were susceptible (Table II). Screening trails on both locality indicated that Col-II and FSD-long were resistant genotypes; whereas Col-Nankana sahib, Col-I and GS-51 were moderately resistant and Col-III, Col-Multan, Col-Vehari, Chaman, Sunder-F^{1}, Janpuri, F^{1}-484 and F^{1}-485 were susceptible.

DISCUSSION

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 (Thronsteinson, 1953; Rafiq *et al.*, 2008). This is primarily regulated by

Table I. The percentage fruit-infestation and larval-density, per fruit of the melon fruit fly, on different genotypes of bitter-gourd, during screening trails, at Harappa, Punjab, Pakistan

Factor	% Fruit infestation		Larval density per fruit		Resistance category
	Means±SD	95% C.I.	Means±SD	95% C.I.	
Col-II	18.7±3.1 e	11.1-26.3	2.4±0.6 f	0.95-3.85	R
FSD-long	19.3±2.3 e	13.6-25.1	3.2±0.3ef	2.47-3.97	R
Col-Nankana Sahib	36.7±2.3 d	30.91-42.4	4.7±0.4de	3.65-5.75	MR
Col-I	44.7±9.1 cd	22.3-67.1	5.8±0.7cd	4.20-7.45	MR
GS-51	46.7±8.3 cd	25.98-67.4	5.8±0.8cd	3.76-7.82	MR
Col-III	55.3±8.1 bc	35.3-75.4	6.6±1.2bc	3.52-9.67	S
Col-Multan	67.3±16.2 ab	27.2-107.5	7.3±0.9bc	5.22-9.45	S
Col-Vehari	71.3±23.4 ab	13.3-129.3	8.2±2.7ab	1.5-15.02	S
Chaman	72±15.9 ab	32.6-111.4	8.3±1.9ab	3.7-13.02	S
Sunder-F1	72.7±6.1 a	57.5-87.8	8.2±0.37ab	7.27-9.12	S
Janpuri	73.3±8.1 a	53.3-93.4	7.5±0.5 bc	6.33-8.60	S
F1-484	73.3±9.0 a	50.9-95.7	8.0±0.27ab	7.34-8.69	S
F1-485	75.3±5.1 a	62.8-87.8	9.3±0.97 a	6.9-11.72	S

Means sharing similar letters, column-wise, do not differ significantly at 5% significant level

Table II. The percentage fruit-infestation and larval-density, per fruit, of the melon fruit fly, on different genotypes of bitter-gourd, during screening trails, at Faisalabad, Punjab, Pakistan

Factor	% Fruit infestation		Larval density per fruit		Resistance category
	Mean±SD	95% C.I.	Mean±SD	95% C.I.	
Col-II	16.7±5.77g	2.3-31.0	2.4±0.8g	0.36-4.5	R
FSD-long	20±0.0 fg	20-20	3.7±0.5fg	2.4-5.0	R
Col-Nankana Sahib	33.3±5.8ef	18.99-47.7	4.6±0.7ef	2.9-6.4	MR
Col-I	46.7±5.8de	32.3-61.0	5.9±0.4de	4.9-6.97	MR
GS-51	50±0.0 cd	50-50	6.1±0.8de	4.1-8.1	MR
Col-III	53.3±5.8bcd	38.9-67.7	6.5±1.5cde	2.8-10.2	S
Col-Multan	63.3±15.3abc	25.4-101.3	7.3±1.8bcd	2.8-11.8	S
Col-Vehari	66.7±11.5ab	37.98-95.4	8.2±0.2abc	7.9-8.6	S
Chaman	70±10a	45.2-94.8	8.4±1.6abc	4.4-12.4	S
Sunder-F1	70±10a	45.2-94.8	8.2±0.6abc	6.7-9.6	S
Janpuri	73.3±20.8a	21.6-125	8.6±2.8ab	1.7-15.5	S
F1-484	73.3±5.8a	58.99-87.7	8.9±0.7ab	7.1-10.7	S
F1-485	73.3±11.5a	44.6-102	9.4±1.4a	5.9-12.9	S

Means sharing similar letters, column-wise, do not differ significantly at 5% significant level

chemoreception (Jeremy & Szentesi, 2003). Plant genotypes, either due to the environmental stress or genetic make up possess physiological and biochemical variations, which alter the nutritional values (primary metabolites) for herbivores (Eckey-Kaltenbach *et al.*, 1994; Mısırlı *et al.*, 2000; Siemens *et al.*, 2002; Goncalves-Alvim *et al.*, 2004; Rafiq *et al.,* 2008) and may also cause changes in the levels of either qualitative or quantitative secondary metabolites (Theis & Lerdau, 2003), that could affect the behaviour and physiology of insects (Karban *et al.*, 1997; Mısırlı *et al.*, 2000; Stadler, 2002; Theis & Lerdau, 2003; Goncalves-Alvim *et al.*, 2004; Aslam *et al.,* 2005). Some times, the combined nutritional and allelochemical changes either improve the quality of the host plant, as a source of food and can, therefore be considered favourable to herbivorous insects (Baur *et al.*, 1998; Goncalves-Alvim *et al.*, 2004) or make the quality of host plant, as source of food, unfavorable to herbivorous insects (De Jong *et al.*, 2000; Stadler, 2002; Goncalves-Alvim *et al.*, 2004; Aslam *et al.,* 2005; Rafiq *et al.,* 2008). Screening trails, conducted at Harappa and Faisalabad, also showed significant differences in the genotypic resistance/susceptibility for fruit infestation and larval density of melon fruit fly in bitter gourd genotypes. Similar reports have been documented by Dhillon *et al.* (2005), Srinivasan (1991), Thakur *et al.* (1992, 1994 & 1996) and Tewatia *et al.* (1997), who evaluated the genotypic susceptibility of bitter gourd genotypes, different from those evaluated in these studies. These variations can be attributed to several, environmentally or genetically, induced physiological and biochemical variations in plant traits (Eckey-Kaltenbach *et al.*, 1994; Mısırlı *et al.,* 2000; Siemens *et al.*, 2002; Theis & Lerdau, 2003; Goncalves-Alvim *et al.,* 2004; Aslam *et al.,* 2005).

Screening trails, conducted at Harappa and Faisalabad, revealed non-significant differences for fruit infestation and larval density per fruit between localities. These non-significant differences can be attributed to the similarity in the genotypic resistance/susceptibility responses of bitter gourd genotypes and/or population buildup of melon fruit fly, at both localities, which are directly or indirectly influenced by the yearwise variations in abiotic factors, like, temperature, relative humidity, rain fall etc. and plantation activity. Su (1986) and Lee *et al.* (1992) documented the similar reasons behind the fluctuation in population density of *Bactrocera cucbitae*, in Taiwan. Percentage fruit infestation and larval density per fruit were found significantly lower in resistant genotypes and higher in susceptible genotypes of bitter gourd. In agreement Dhillon *et al.* (2005) reported a lower percentage fruit infestation and larval density per fruit in wild genotypes (resistant) and higher in cultivated genotypes (susceptible) of bitter gourd. Col-II and FSD-long, were found resistant genotypes to melon fruit flies, whereas Col-Nankana sahib, Col-I and GS-51 were seen to be moderately resistant and Col-III, Col-Multan, Col-Vehari, Chaman, Sunder-F^1, Janpuri, F^1-484 and F^1-485 were susceptible to the melon fruit fly.

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(Received 29 April 2009; Accepted 22 May 2009)