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# Phenotyping for resistance to the sugarcane aphid *Melanaphis sacchari* (Hemiptera: Aphididae) in *Sorghum bicolor* (Poaceae)

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**Abstract.** The sugarcane aphid *Melanaphis sacchari* (Zehnt.) has become a serious pest of sorghum, particularly during the post-rainy season in India and East and Southern Africa. Therefore, we tested a number of techniques to screen sorghum genotypes for their resistance to *M. sacchari*. Infesting the plants with aphid-infested leaf cuttings and covering with a nylon net was effective in screening sorghum genotypes for their resistance to *M. sacchari*. Sprinkling the plants with aphids (filled in an 0.5 ml eppendorf tube) in the greenhouse was also used to confirm whether the resistance of genotypes selected is less susceptible to the aphids under natural infestation. Nine genotypes (Line 61510, ICSV 12001, ICSV 12002, ICSV 12003, ICSV 12004, ICSV 12005, SLR 41, PU 10-1 and DJ 6514) exhibited moderate levels of resistance to *M. sacchari*. These genotypes also exhibited a lower rate of aphid multiplication in the clip cage and leaf disc assays. The rates of aphid multiplication were lower on the genotypes IS 21807, IS 40615, IS 40616 and IS 40618 than on the susceptible check, Swarna in the clip cage assay under the field conditions. Also, lower rates of aphid increase were also recorded on IS 21807 and IS 40615 in the leaf disc assay under laboratory conditions. Some of the genotypes that exhibited resistance to aphid damage under field conditions showed comparatively higher rates of aphid increase than the susceptible check, Swarna in the clip cage assay, indicating that antixenosis could be one of the components of resistance to *M. sacchari* in these genotypes. Therefore, the clip cage assay could be used to gain further understanding of the mechanisms of resistance to *M. sacchari*. There is a need to assess the role of antixenosis and colonization in genotypic reaction against *M. sacchari* to identify the lines with different mechanisms of resistance to this pest. The results suggested that the nylon net technique could be used to screen sorghum genotypes for resistance to *M. sacchari*. The genotypes exhibiting resistance to *M. sacchari* can be used to develop aphid-resistant sorghums for sustainable crop production.

**Key words:** sugarcane aphid, *Melanaphis sacchari*, sorghum, resistance screening, clip cage, nylon net, technique

## Introduction

Sorghum, *Sorghum bicolor* (L.) Moench (Poaceae), is an important cereal crop in Asia, Africa, the Americas and Australia. Grain yields have been

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reported to be generally low (500–800 kg/ha) in farmers' fields in Asia and Africa mainly due to insect pest damage. Nearly 150 insect species have been reported as pests on sorghum (Sharma, 1993), of which the major pests worldwide include sorghum shoot fly *Atherigona soccata* (Rond.), spotted stemborer *Chilo partellus* (Swin.), oriental armyworm *Mythimna separata* (Walk.), shoot bug *Peregrinus maidis* (Ashmead), sugarcane aphid *Melanaphis sacchari* (Zehnt.), sorghum midge *Stenodiplosis sorghicola* (Coq.), mirid head bugs *Calocoris angustatus* (Leth.) and *Eurystylus oldi* (Pop.), and head caterpillars *Helicoverpa, Eublemma*, *Cryptoblabes* and *Pyroderces*. Nearly 32% of sorghum crop is lost due to insect pest damage during the rainy season (Borad and Mittal, 1983) and 26% during the post-rainy season (Daware *et al.*, 2012). Insect pests cause an estimated loss of US$ 1089 million in the semi-arid tropical regions of Asia and Africa (ICRISAT, 1992).

The sugarcane aphid *M. sacchari* (Zehnt.) (Hemiptera: Aphididae) is an important pest in Asia, Africa, Australia and the USA (Sharma and Nwanze, 1997). It is one of the vectors of the sugarcane yellow leaf virus, which occurs in most of the sugarcane-growing countries (Smith *et al.*, 2000). The nymphs and adults of *M. sacchari* suck the sap from the undersurface of the leaves, and the infested leaves dry up and turn yellow or brown. Under heavy infestation of *M. sacchari*, the plants may be severely stunted. The aphids secrete honeydew, which falls on the leaves and on the ground, on which sooty moulds grow. The aphids multiply by parthenogenesis, i.e. they give birth to apterous nymphs, which moult four times before they become adults. Under crowded conditions or when host plants are stressed, they produce winged forms (alates), which moult five times before they become adults (Meksongsee and Chawanapong, 1985). Each female produces 60 to 100 nymphs in 12 to 20 days. The adults live for about 10 to 16 days. Aphid infestation in sorghum is very high during the flowering and grain-filling stages (Fang, 1990). Long dry spells result in heavy aphid damage (Raetano and Nakano, 1994). In addition to leaf feeding, *M. sacchari* also affects grain quality in terms of diastatic power, malt loss and abrasive hardness index. This results in the poor quality of sorghum beer and milling. Reduced grain hardness may also result in increased flour losses during milling (van den Berg *et al.*, 2003).

Best agronomic practices, natural enemies, host plant resistance and synthetic insecticides have been employed for controlling insect pests. Insecticides are costly and, at times, beyond the reach of resource-poor farmers in the semi-arid tropics. The application of chemical insecticides for aphid control under subsistence farming conditions may not be economically viable. Therefore, it is important to identify sorghum cultivars that are resistant or less susceptible to this pest. Extensive efforts have been made to screen sorghum germplasm for their resistance to the sorghum shoot fly, spotted stemborer, sorghum midge and head bugs (Sharma *et al.*, 1992, 2003). However, there has been little effort to identify sorghum genotypes for their resistance to the sugarcane aphid *M. sacchari.* Therefore, there is a need to develop techniques to screen and breed sorghum genotypes with resistance to *M. sacchari*.

## Materials and methods

The experiments were conducted during the post-rainy season at the International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh, India. A total of three techniques were used to evaluate the resistance of sorghum genotypes to *M. sacchari*. These involved the following steps: (1) infesting the plants with aphid-infested leaf cuttings and covering with a nylon net, (2) confining the aphids to the leaves inside a clip cage and (3) placing leaf discs in 3% solidified agar–agar medium at the one end in a 500 ml plastic jar in the laboratory.

### *Infesting the plants with aphid-infested leaf cuttings and covering with a nylon net in the field*

A total of 31 genotypes, including resistant (IS 40618 (TAM 428)) and susceptible (CK 60 B and Swarna) checks, were planted in three replications in the field in a randomized complete block design. Each genotype was planted in two row plots of 2 m length and the rows were 75 cm apart. The seeds were planted at a depth of 5 cm with a four-cone

**Plate 1.** Sorghum leaf cutting with a colony of the sugarcane aphid *Melanaphis sacchari* used for infesting the plants grown inside the nylon net.

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**Plate 2.** Nylon net technique used to screen sorghum genotypes for resistance to sugarcane aphid *Melanaphis sacchari*.

planter. The field was irrigated immediately after sowing. At 1 week after seedling emergence, thinning was carried out to maintain a spacing of 10 cm between the plants. A basal dose of 150 kg/ha of diammonium phosphate (DAP) was applied to the experimental plots. Inter-culture and earthing-up operations were carried out at 15 and 30 days after seedling emergence. Hand weeding was carried out as and when required. The crop was irrigated at intervals of 30 days. The test material was planted in three sets, of which one set was left uninfested under natural conditions, the second set was infested with aphids at the flag leaf stage (each plant infested with 3 × 3 cm aphid-infested leaf cuttings, stapled to the fifth leaf from the bottom; Plate 1) and the third set was infested with aphid-infested leaf cuttings at the flag leaf stage and covered with a nylon net to exclude natural enemies (Plate 2). Observations were recorded on aphid damage at physiological maturity on a 1–9 scale (Table 1). Data were also recorded on agronomic desirability at maturity on a 1–5 rating scale (1 = good and 5 = poor).

### *Nylon net screening technique to confirm the resistance of the genotypes selected under natural infestation in the field*

Seven genotypes (ICSV 12001, ICSV 12005, IS 21807, IS 21808, IS 40615, IS 40616 and IS 40618), which exhibited moderate levels of resistance to the sugarcane aphid under field conditions, were tested for their resistance to the aphids under a nylon net in the greenhouse. ICSV 745, with moderate levels of resistance to the aphids, and CK 60 B and Swarna, with high levels of susceptibility to the aphids (Sharma and Dhillon, 2005), were included as controls. The test material was planted in plastic pots (30 cm diameter and 30 cm deep) filled with a mixture of black soil, farmyard manure and sand (3:1:1). DAP (20 g per pot) was applied as a basal fertilizer just before planting. The seeds were placed 5 cm below the soil surface and watered immediately. Five seeds were planted in each pot and three seedlings were retained in each pot at 15 days after seedling emergence. The pots were watered on alternate days. The potted plants were grown inside a nylon net cage (2.5 × 3 × 2 m). There were three replications for each genotype in a randomized complete block design. The test plants were infested with 3 × 3 cm aphid-infested leaf cuttings (stapled to the fifth leaf from the bottom) or sprinkled with aphids (filled in a 0.5 ml eppendorf tube) at the flag leaf stage. The severity of aphid damage on plants was evaluated at physiological maturity on a 1–9 scale, as described in Table 1.

### *Clip cage technique*

Resistance to aphid damage in terms of severity of damage could be recorded as a visual damage rating, but it is not possible to record the data on the number of aphids on whole plants under field conditions as there are too many aphids on each plant, and they are unevenly distributed all over the plant. However, it is important to record the data on the number of aphids as a measure of host suitability to the insects, which also provides information on the antibiosis component of resistance to the insects. Therefore, we designed a clip cage to confine gravid females to the leaf and record the number of progenies produced as a measure of antibiosis or host suitability/resistance to the aphids. Initially, 11 genotypes were evaluated for their resistance to the aphids using the clip cage assay. The test material was grown under field conditions as described above. There were three replications in a randomized complete block design. The clip cage consisted of two plastic rings of 3.5 cm diameter, of which one side was covered with a 60-mesh screen and the open portion had a 0.25 mm-thick layer of foam that could be held against the leaf lamina. The two rings were held together tightly with a 2.5 mm-thick galvanized triangular iron wire. The clip cage was designed such that it could be placed in the mid-portion of the leaf (fifth leaf from the bottom, which is most suitable for aphid infestation) and covered 10 square centimetres of leaf area. The clip cage was supported on the leaf by a thin wire tied around the stem (Plate 3). Ten gravid females collected from the aphid-infested plants in the field were released inside the clip cage on the mid-portion of the fifth leaf of each plant at the milk stage. The number of nymphs/adults produced by the gravid females was counted after 5 days, which provided sufficient time for the aphids to complete one generation.

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**Table 1.** Visual damage rating scale to evaluate sorghum genotypes for resistance to sugarcane aphid *Melanaphis sacchari*

| Damage rating | Remarks |
|---|---|
| 1 | Few aphids present on the lower one to two leaves, with no apparent damage to the leaves |
| 2 | Lower one to two leaves showing aphid infestation, and 1–20% of the infested leaves/area showing damage symptoms |
| 3 | Lower two to three leaves showing aphid infestation, and 20–30% of the infested leaves/area showing damage symptoms, with moderate levels of honeydew/black moulds on the leaves/soil |
| 4 | Lower three to four leaves showing aphid infestation, and 30–40% of the infested leaves/area showing damage symptoms, with moderate levels of honeydew/black moulds on the leaves/soil |
| 5 | Lower four to five leaves showing aphid infestation, and 40–50% of the infested leaves/area showing damage symptoms, with moderate levels of honeydew/black moulds on the leaves/soil |
| 6 | Aphid infestation up to five to six leaves, and 50–60% of the infested leaves/area showing damage symptoms, and heavy honeydew/black moulds on the leaves and on the soil below |
| 7 | Aphid infestation up to six to seven leaves, and 60–70% of the infested leaves/area showing damage symptoms, and heavy honeydew/black moulds on the leaves and on the soil below |
| 8 | Aphid infestation up to seven to eight leaves, and 70–80% of the infested leaves/area showing damage symptoms, and heavy honeydew/black moulds on the leaves and on the soil below |
| 9 | Heavy aphid infestation up to the flag leaf, and >80% of the leaves showing aphid damage (drying-up symptoms), and heavy honeydew/black moulds on the leaves and on the soil below |

### *Leaf disc assay*

Since the reproduction of aphids inside the clip cage under field conditions may be influenced by the environmental conditions and the leaf turgor status of the plants, we also recorded the reproduction of the aphids on the leaf discs (7 cm leaf cuttings from the mid-portion of the leaf) of different genotypes to assess the usefulness of this technique to measure the antibiosis component of resistance to *M. sacchari*. The experiment was repeated for three seasons. As described above, 11 genotypes were evaluated for their resistance to the aphids using the leaf disc assay. The leaf discs were taken from the mid-portion of the fifth leaf from the bottom at the flag leaf stage, and kept inside a plastic jar (10.8 cm diameter and 4 cm depth). The lower portion of the leaf cuttings was inserted in 3% agar–agar medium in a slanting manner (Plate 4). There were five replications for each genotype in a completely randomized design. Ten gravid females collected from the aphid-infested plants in the field were released on each leaf disc. The number of aphids were counted after 5 days.

### *Comparison of the nylon net, clip cage and leaf disc assays to evaluate sorghum genotypes for resistance to* Melanaphis sacchari

A total of 30 genotypes, including resistant (IS 40618) and susceptible (CK 60 B and Swarna) checks, were evaluated for their resistance to *M. sacchari* at the flag leaf stage using the nylon net, clip cage and leaf disc assays. The plants were grown under field conditions, as described above. Each genotype was planted in two rows of 2 m length. The ridges were 75 cm apart and the seedlings were spaced at 10 cm. The plants were infested with aphid-infested leaf cuttings at the flag leaf stage and immediately covered with a nylon net cage. The plants outside the nylon net were evaluated at the flag leaf stage using the clip cage and leaf disc assays. There were three replications for each genotype in a randomized complete block design. Ten gravid females were released inside each clip cage or on the leaf discs in the laboratory. The number of aphids were counted at 5 days after infestation in the clip cage assay and after 7 days after infestation in the leaf disc assays. The infested genotypes inside the nylon net were evaluated for their resistance to the aphids at physiological maturity on a 1–9 scale, as described in Table 1.


**Plate 3.** Clip cage used to confine the sugarcane aphid *Melanaphis sacchari* to the leaves of sorghum (a = aphids released inside the clip cage and b = clip cage placed on the sorghum leaf and tied with a galvanized iron wire around the stem).

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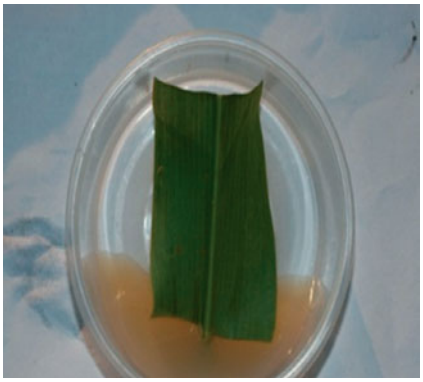

**Plate 4.** Leaf disc assay used to assess the antibiosis component of resistance to the sugarcane aphid *Melanaphis sacchari* under laboratory conditions.

### *Statistical analysis*

Data were subjected to the analysis of variance. Data on the number of aphids were subjected to square root transformation before the analysis of variance. Significance of differences between the genotypes was tested using the *F*-test, while treatment means were compared using Duncan's multiple range test at $P \leq 0.05$.

We also prepared a biplot of the test genotypes based on aphid damage rating under artificial infestation and the increase in the number of aphids inside the clip cage under the field conditions to identify the lines with antibiosis mechanisms of resistance and/or tolerance to aphid damage. The genotypes were placed in four quadrants based on aphid damage under nylon net and increase in number of aphids in the clip cage assay. The genotypes placed in quadrant I had a lower rate of increase in the number of aphids and also suffered low aphid damage rating, i.e. exhibiting antibiosis as a component of resistance. The genotypes placed in quadrant II suffered low leaf damage despite a high increase in the number of aphids, and had tolerance to aphid damage. The genotypes placed in quadrant III showed lower rates of increase in the number of aphids (antibiosis), but exhibited susceptibility to aphid damage, while the genotypes placed in quadrant IV showed high levels of increase in the number of aphids and a high susceptibility to aphid damage, and thus were categorized as highly susceptible.

## Results

### *Infesting the plants with aphid-infested leaf cuttings and covering with a nylon net in the field*

Under the field conditions, the average aphid damage severity rating was 2.46 in plots under natural infestation, 3.44 for plants infested with leaf cuttings and 5.18 for plants infested with leaf cuttings and covered with a nylon net (Fig. 1). Covering the plants with a nylon net to exclude natural enemies was effective in building-up heavy aphid infestation on the test material. Twenty-four genotypes suffered significantly lower damage than the susceptible check, CK 60 B, under natural infestation, of which nine showed a susceptible reaction when the plants were infested with aphid-infested leaf cuttings at the flag leaf stage (Table 2). Among the genotypes that exhibited resistance to the aphids when infested with aphid-infested leaf cuttings, 10 genotypes exhibited a susceptible reaction when the plants were covered with a nylon net, indicating that infestation with aphid-infested leaf cuttings and covering the plants with a nylon net was quite effective in screening sorghum genotypes for resistance to *M. sacchari*. Nine genotypes (Line 61510, ICSV 12001, ICSV 12002, ICSV 12003, ICSV 12004, ICSV 12005, SLR 41, PU 10-1 and DJ 6514) showed moderate levels of resistance (DR 3.0–4.5) when infested with aphid-infested leaf cuttings and covered with a nylon net. Of these genotypes, Line 61510, ICSV 12001, ICSV 12002, ICSV 12003 and ICSV 12004 also exhibited good agronomic desirability (agronomic score 2.0–2.33).

### *Reaction of sorghum genotypes under nylon net in the screenhouse*

There were significant differences among the genotypes infested with aphid-infested leaf cuttings or sprinkled with aphids (placed in a 0.5 ml eppendorf tube) inside the nylon net in the greenhouse. The genotypes ICSV 12001 and ICSV 12005 exhibited moderate levels of resistance (DR 4.2–4.8) to *M. sacchari* (Fig. 2), while the genotype

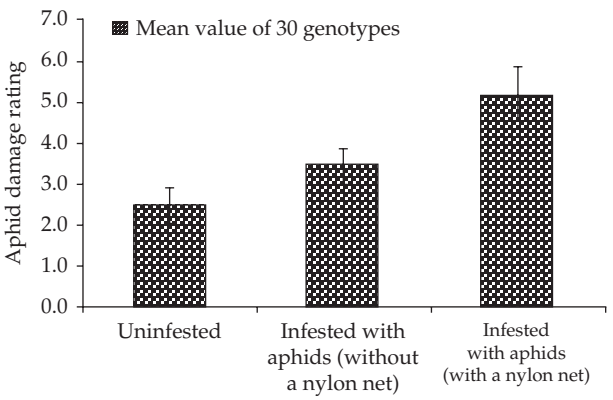


**Fig. 1.** Mean leaf damage rating of sorghum genotypes for resistance to sugarcane aphid *Melanaphis sacchari* under different infestation techniques in the field. Aphid damage rating: 1 = <10% of the leaf area infested/damaged by the aphids; 9 = > 80% of the leaf area damaged by the aphids.

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**Table 2.** Evaluation of sorghum genotypes for resistance to the sugarcane aphid *Melanaphis sacchari* under different modes of infestation

| Genotype | Aphid damage rating[1] | | | Agronomic score[2] (uninfested conditions) |
|---|---|---|---|---|
| | Uninfested under natural conditions | Infested with aphids without a nylon net | Infested with aphids + covering plants with a nylon net) | |
| Line 61510 | 1.67ab | 2.67abc | 4.50abc | 2.00a |
| ICSV 12001 | 1.67ab | 3.00abcd | 4.50abc | 2.00a |
| Line 61579 | 2.67bcd | 3.67cdef | 5.00bc | 2.67ab |
| ICSV 12002 | 3.00cd | 3.33bcde | 4.00ab | 2.33ab |
| ICSV 12003 | 2.33bcd | 4.33ef | 4.50abc | 2.00a |
| ICSV 12004 | 2.33bcd | 2.00a | 3.00a | 2.33ab |
| ICSV 12005 | 1.00a | 2.00a | 4.00ab | 3.00b |
| Line 61602 | 2.00abc | 4.00def | 5.50bc | 3.00b |
| IS 40615 | 2.33bcd | 2.67abc | 5.50bc | 2.67ab |
| IS 40616 | 2.67bcd | 4.00def | 5.50bc | 2.67ab |
| IS 40617 | 3.33de | 3.33bcde | 5.00bc | 3.00b |
| IS 40618 (TAM 428) | 3.00cd | 3.67cdef | 5.50bc | 2.67ab |
| IS 40620 | 1.67ab | 3.00abcd | 6.50c | 3.00b |
| SLR 8 | 2.00abc | 3.33bcde | 6.00c | 5.00d |
| SLR 27 | 2.67bcd | 3.67cdef | 6.00c | 5.00d |
| SLR 28 | 2.00abc | 3.33bcde | 5.00bc | 5.00d |
| SLR 31 | 2.33bcd | 4.00def | 6.00c | 5.00d |
| SLR 35 | 2.00abc | 3.00abcd | 5.00bc | 5.00d |
| SLR 39 | 2.00abc | 4.00def | 5.50bc | 4.33cd |
| SLR 41 | 3.00cd | 3.00abcd | 4.50abc | 4.00c |
| SLV 25 | 3.00cd | 3.67cdef | 5.50bc | 4.33cd |
| IS 33722 | 2.00abc | 3.33bcde | 6.00c | 5.00d |
| IS 3420 | 2.33bcd | 3.33bcde | 6.00c | 5.00d |
| EC 8-2 | 2.33bcd | 2.67abc | 5.00bc | 5.00d |
| PU 10-1 | 2.00abc | 2.33ab | 4.50abc | 5.00d |
| IS 21807 | 3.00cd | 4.67ef | – | 2.67ab |
| IS 21808 | 2.67bcd | 3.67cdef | – | 2.33ab |
| Swarna – Local check | 4.33e | 6.33 g | 8.00d | 2.00a |
| DJ 6514 | 2.67bcd | 3.00abcd | 4.00ab | 5.00d |
| CK 60 B | 4.33e | 4.67f | 8.00d | 2.67ab |
| ICSV 745 | 2.00abc | 3.00abcd | – | 2.33ab |
| Mean | 2.46 | 3.44 | 5.18 | 3.48 |
| SE ± | 0.44 | 0.42 | 0.655 | 0.31 |
| Fp (60, 30) | <0.001 | <0.001 | 0.004 | <0.001 |

Values followed by the different letters within a column are significantly different at $P \leq 0.05$.
[1] Aphid damage rating (1 = <10% of the leaf area damaged by the aphids and 9 = >80% of the leaf area damaged by the aphids).
[2] Agronomic score: 1 = good and 5 = poor.

CK 60 B showed a susceptible reaction (DR 9.0) under both the infestation systems. The genotypes IS 21807, IS 21808, Swarna and ICSV 745 exhibited a susceptible reaction when infested with the aphid-invested leaf cuttings, but suffered complete damage when sprinkled with 0.5 ml of aphids per plant. The genotypes IS 40616 and IS 40618 exhibited moderate levels of resistance under both the infestation methods. The results suggested that leaf cuttings stapled to the fifth leaf from the bottom at the flag leaf stage inside the screenhouse could be used to confirm whether the plants/genotypes selected are resistant to aphid damage under natural infestation in the field.

### *Evaluation of sorghum genotypes using the clip cage technique*

To gain an understanding of the effect of resistant genotypes on the survival and development of aphids, 11 genotypes, including the resistant and susceptible checks, were tested using the clip cage technique. The rates of aphid multiplication were lower (15.0–16.0 aphids per

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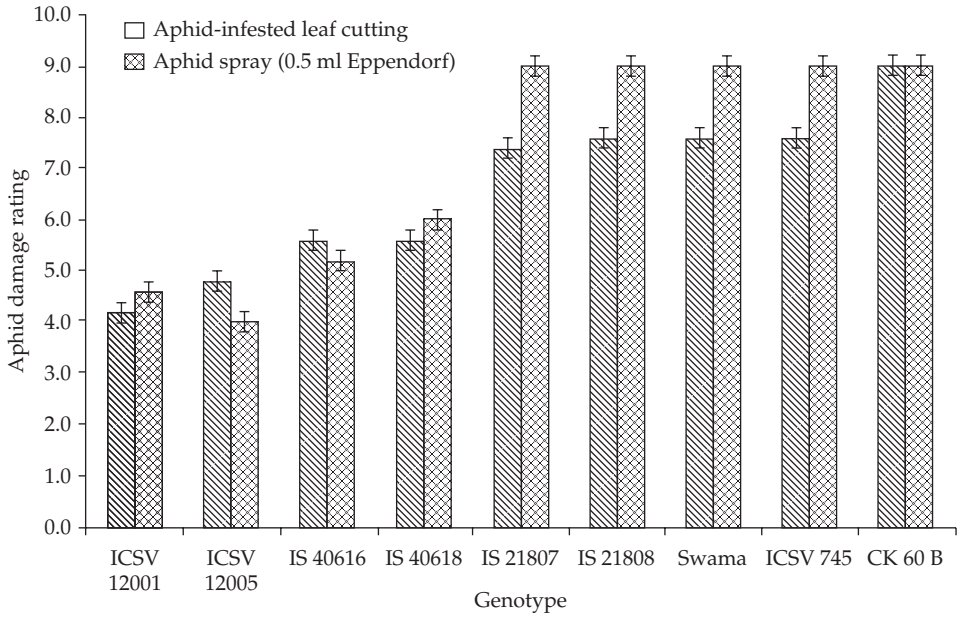


**Fig. 2.** Mean leaf damage rating of sorghum genotypes evaluated for resistance to the sugarcane aphid *Melanaphis sacchari* under screenhouse conditions by infesting the plants with aphid-infested leaf cuttings and by spreading the aphids on the plants. Aphid damage rating: 1 = <10% of the leaf area infested/damaged by the aphids; 9 = >80% of the leaf area damaged by the aphids.

10 females) on the genotypes ICSV 12001, IS 40615 and IS 40616 during the 2009 post-rainy season (Table 3). However, the differences between the genotypes were not significant as the temperatures during the experimental period were very high (>40°C). During the 2010 post-rainy season, the differences between the genotypes were significant, and the number of aphids were significantly lower (27–45 aphids per 10 females) on the genotypes ICSV 12005, IS 21807, IS 40615, IS 40616 and IS 40618 when compared with the susceptible check Swarna (68 aphids per 10 females). The increase in the

**Table 3.** Evaluation of sorghum genotypes for resistance to sugarcane aphid *Melanaphis sacchari* using clip cage and leaf disc assays under the field and laboratory conditions, respectively

| Genotype | No. of aphids (nymphs + adults) | | | |
|---|---|---|---|---|
| | Clip cage | | Leaf disc assay | |
| | 2009 | 2010 | 2009 | 2010 |
| ICSV 12001 | 16.00 (4.06)a | 59.00 (7.69)bc | 17.00 (4.07)bc | 81.20 (9.03)ab |
| ICSV 12005 | 27.20 (5.19)a | 34.20 (5.71)ab | 8.60 (3.00)ab | 61.00 (7.79)a |
| IS 21807 | 32.00 (5.62)a | 27.00 (5.13)a | 11.40 (3.23)ab | 59.80 (7.68)a |
| IS 21808 | 29.80 (5.06)a | 59.00 (7.51)bc | 25.40 (4.74)c | 68.40 (8.25)a |
| IS 40615 | 16.00 (4.05)a | 27.20 (5.17)a | 9.60 (3.13)ab | 75.40 (8.70)ab |
| IS 40616 | 15.60 (3.84)a | 44.40 (6.37)abc | 8.00 (2.91)ab | 132.20 (10.99)bc |
| IS 40618 (TAM 428) | 27.20 (5.24)a | 42.20 (6.36)abc | 4.80 (2.22)a | 136.20 (11.66)c |
| ICSV 745 | 20.80 (4.57)a | 73.20 (8.30)c | 11.20 (3.30)ab | 83.40 (9.07)ab |
| CK 60 B | 20.60 (4.48)a | – | 15.00 (3.81)bc | – |
| DJ 6514 | – | 45.00 (6.63)abc | – | 163.60 (12.78)c |
| Swarna | 26.60 (4.95)a | 68.00 (8.26)c | 11.40 (3.37)ab | 169.60 (12.61)c |
| Mean | 4.71 | 6.71 | 3.38 | 9.85 |
| SE ± | 0.58 | 0.76 | 0.44 | 0.87 |
| Fp (9, 36) | 0.41 | 0.03 | 0.03 | <0.01 |

Values followed by the different letters within a column are significantly different at $P \leq 0.05$.

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number of aphids on the genotypes ICSV 12001, IS 21808 and ICSV 745 was similar to that on the susceptible check, Swarna.

### *Leaf disc assay*

In the leaf disc assay, low rates of aphid multiplication were recorded on the genotypes ICSV 12005, IS 40615, IS 40616 and IS 40618 when compared with that on the susceptible checks Swarna and CK 60B during the 2009 post-rainy season (Table 3). During the 2010 post-rainy season, lower rates of increase in the number of aphids (59–83 aphids per 10 females) were recorded on the genotypes ICSV 12001, ICSV 12005, IS 21807, IS 21808, IS 40615 and ICSV 745 when compared with that on the susceptible check Swarna (169 aphids per 10 females). However, relatively higher rates of aphid increase were recorded on the genotypes IS 40616 and IS 40618 during the 2010 post-rainy season than during the 2009 post-rainy season, while the reverse was true in the case of the genotypes ICSV 12001 and IS 21808. The results suggested that the leaf disc assay is not a reliable technique to measure genotypic resistance to *M. sacchari*, probably because of possible

**Table 4.** Evaluation of sorghum genotypes for resistance to sugarcane aphid *Melanaphis sacchari* using the clip cage and leaf disc assays under the field and laboratory conditions, respectively

| | No. of aphids (nymphs + adults) | | |
|---|---|---|---|
| Genotype | Clip cage | Leaf disc assay | Aphid damage rating inside nylon net in the field[+] |
| ICSB 205 | 28.50 (5.32)ab | 50.33 (7.01)d–k | 7.30efgh |
| ICSB 215 | 26.00 (4.70)ab | 25.67 (4.74)a–g | 4.70abc |
| ICSB 321 | 40.00 (5.76)ab | 63.33 (7.96)h–k | 5.00abcd |
| ICSB 323 | 16.50 (4.12)a | 11.67 (3.45)ab | 4.30ab |
| ICSB 695 | 30.33 (5.41)ab | 55.67 (7.29)e–k | 8.00fgh |
| ICSB 724 | 45.33 (6.72)abcd | 26.67 (5.11)a–h | 4.30ab |
| ICSR 161 | 119.00 (10.59)e | 76.67 (8.72)k | 9.00 h |
| ICSR 165 | 27.00 (5.10)ab | 18.33 (4.28)a–e | 4.00a |
| Line 61510 | 84.00 (9.08)de | 36.33 (5.95)b–k | 8.00fgh |
| ICSV 12001 | 37.50 (5.89)abc | 37.00 (5.96)b–k | 4.30ab |
| ICSV 12004 | 22.00 (4.63)ab | 53.00 (7.11)d–k | 4.70abc |
| RS 29 | 36.33 (5.97)abc | 31.67 (5.45)a–i | 6.30cdef |
| RSV 1093 | 54.00 (7.28)bcd | 33.33 (5.79)a–k | 6.00bcde |
| RSV 1211 | 44.33 (6.38)abcd | 23.67 (4.69)a–f | 7.30efgh |
| RSV 1338 | 54.00 (7.20)bcd | 17.33 (3.93)abc | 6.70defg |
| IS 40615 | 28.50 (5.39)ab | 62.67 (7.74)f–k | 5.00abcd |
| IS 40617 | 30.50 (5.53)ab | 47.00 (6.58)c–k | 6.00bcde |
| C 43 | 42.33 (6.32)abcd | 80.33 (8.22)ijk | 7.30efgh |
| DSV 5 | 53.50 (7.27)bcd | 13.33 (3.65)abc | 5.30abcd |
| EC 8-2 | 39.67 (5.92)abc | 33.33 (5.52)a–h | 6.00bcde |
| Hathi Kuntha | 52.00 (7.23)bcd | 77.67 (8.66)j | 6.70defg |
| Local 453 | 35.00 (5.90)abc | 32.33 (5.64)a–j | 6.00bcde |
| Long SPS 43 | 30.50 (5.23)ab | 32.67 (5.41)a–h | 8.70 h |
| M-35-1 | 56.67 (7.47)bcd | 9.33 (2.87)a | 8.30gh |
| M-35-1 x 9808 | 25.67 (4.77)ab | 29.67 (5.13)a–h | 6.70defg |
| Parbhani Moti | 51.50 (7.20)bcd | 69.00 (8.01)h–k | 6.30cdef |
| PU 10-1 | 53.00 (7.21)bcd | 24.00 (4.89)a–g | 6.00bcde |
| IS 40618 – R | 28.33 (4.88)ab | 36.00 (5.88)a–k | 5.70abcde |
| CK 60 B – S | 45.00 (6.74)abcd | 64.00 (7.78)g–k | 8.30gh |
| Swarna – S | 83.33 (8.94)cde | 17.33 (4.20)a–d | 9.00 h |
| Mean | 6.34 | 5.92 | 6.40 |
| SE ± | 1.08 | 1.08 | 0.60 |
| Fp (29, 58) | 0.03 | 0.004 | <0.001 |

Values followed by the different letters within a column are significantly different at $P \leq 0.05$.
R, resistant check; S, susceptible check.
[+]Aphid damage rating: 1 = <10% of the leaf area damaged by the aphids and 9 = >80% of the leaf area damaged by the aphids.

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desiccation of the leaf discs during the hot and dry season.

*Comparison of nylon net, clip cage and leaf disc assays to evaluate sorghum genotypes for their resistance to* Melanaphis sacchari

To gain an understanding of the comparative usefulness of different methods, 30 genotypes were evaluated for resistance to the aphids under a nylon net in the field, and their suitability for reproduction of aphids was assessed using the clip cage and detached leaf disc assays during the 2011 post-rainy season. Nine genotypes (ICSB 215, ICSB 321, ICSB 323, ICSB 724, ICSV 12001, ICSV 12004, IS 40615, DSV 5 and IS 40618) exhibited moderate levels of resistance (DR 4.3–5.7) to *M. sacchari*, when infested with the aphid-infested leaf cuttings under a nylon net in the field (Table 4; Plate 5).

The genotypes ICSB 323, ICSB 215, ICSV 12004, ICSR 165, IS 40615, ICSV 12001, ICSB 321 and ICSB 724 placed in quadrant I suffered low aphid damage under the nylon net in the field and also exhibited a low rate of aphid increase in the clip cage assay. These genotypes exhibited antibiosis component of resistance to *M. sacchari* (Fig. 3). The genotype DSV 5 placed in quadrant II suffered low aphid damage, but exhibited a relatively higher rate of aphid increase in the clip cage assay, while the reverse was true in the case of the genotypes placed in quadrant III (SPS 43, ICSB 695, CK 60 B, ICSB 205, C 43, RSV 1211, M-35-1 x 9808, RS 29, IS 40617, IS 40618, EC 8-2 and Local 453). The genotypes placed in quadrant IV (ICSR 161, Swarna, M-35-1, Line 61510, RSV 1338, Hathi Kuntha, Parbhani Moti, RSV 1093 and PU 10-1) suffered severe damage in the field and also exhibited a high rate of aphid increase in the clip cage assay, and thus were highly susceptible to *M. sacchari*.

**Plate 5.** Reaction of sorghum genotypes against the sugarcane aphid *Melanaphis sacchari* inside the nylon net under field conditions (left: Swarna, susceptible reaction and right: ICSB 323, resistant reaction)

## Discussion

A total of 21 genotypes suffered significantly less damage than the susceptible check CK 60 B, of which 10 genotypes exhibited a susceptible reaction when infested with aphid-infested leaf cuttings and covered with a nylon net, indicating that infesting the plants with aphid-infested leaf cuttings and covering the plots with a nylon net to exclude the natural enemies is quite effective in screening and breeding genotypes for resistance to *M. sacchari*. The genotypes EC 434430, CSH 16 and 9728 have earlier been reported to be resistant to *M. sacchari* in India (Ghuguskar *et al.*, 1999; Sarath Babu *et al.*, 2000), while the genotypes PAN 8446, SNK 3939 and NS 5511 have been reported to be tolerant to aphid damage in South Africa (van den Berg, 2002). The genotypes ICSV 197, ICSV 745 and ICSV 112 have been reported to show moderate levels of resistance to *M. sacchari* and to have low density of alates (Sharma and Dhillon, 2005). Under the nylon net in the screenhouse, the genotypes ICSV 12001 and ICSV 12005 exhibited moderate levels of resistance to *M. sacchari*, while CK 60 B showed a susceptible reaction. However, the genotypes IS 21807, IS 21808, Swarna and ICSV 745 showed a susceptible reaction when infested with aphid-infested leaf cuttings, but suffered complete plant damage when sprinkled with aphids inside the nylon net in the screenhouse. This method could be used to confirm whether the plants/genotypes selected are resistant to aphid damage under natural infestation in the field.

Aphid density and damage to the plants have been reported to be highly correlated (Hagio, 1992), although some of the genotypes that suffered high aphid damage in the present study showed lower rates of aphid increase in the clip cage and leaf disc assays. It has been reported that both winged and apterous forms exhibit a strong preference for susceptible sorghums (Kawada, 1995), and hence, there is a need to assess the antixenosis component of resistance to *M. sacchari* to identify the lines with diverse mechanisms of resistance to this pest Aphids reared on resistant sorghums have been reported to exhibit an increase in nymphal period and mortality, and a reduction in longevity and fecundity (Liu *et al.*, 1990; Kawada, 1995). In the present study, the rates of aphid multiplication were lower on the genotypes IS 21807, IS 40615, IS 40616 and IS 40618 than on the susceptible check Swarna, while the rates of aphid increase on the genotypes ICSV 12001, ICSV 12005, IS 21808 and ICSV 745 were comparable to that on the susceptible check, Swarna, although these genotypes

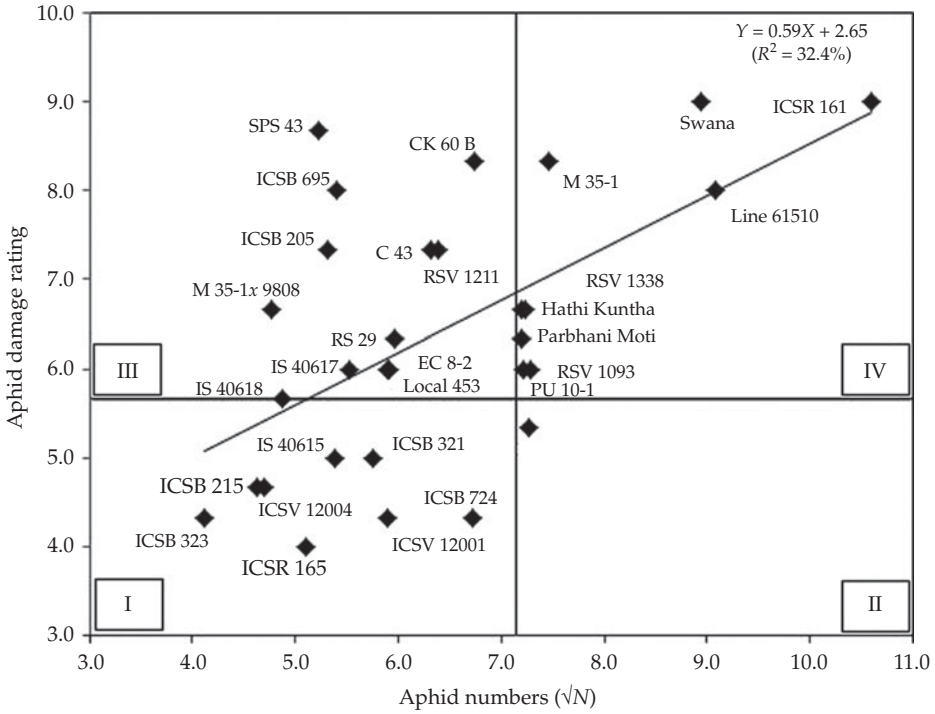


**Fig. 3.** Relationship between the genotypic reaction against sugarcane aphid under nylon net in the field and increase in aphid numbers under the clip cage. Aphid damage rating: 1 = <10% of the leaf area infested/damaged by the aphids; 9 = >80% of the leaf area damaged by the aphids.

suffered lower damage than Swarna under the field conditions. This indicates that non-preference/tolerance to aphid feeding could be one of the components of resistance to aphid damage in these genotypes, suggesting that there is a need to assess non-preference for host selection and the effect of aphid-resistant genotypes on the development and biology of *M. sacchari*.

A lower number of aphids were recorded on the genotypes ICSV 12005, IS 40615, IS 40616 and IS 40618 when compared with that on the susceptible checks, Swarna and CK 60 B. The results suggested that the leaf disc assay might not be a reliable technique to measure genotypic resistance to *M. sacchari*, probably because of rapid drying of the leaf discs during the hot and dry seasons. The leaf discs of sorghum might not be able to obtain water from the agar–agar medium as is the case with the detached leaf assay with chickpea, pigeonpea and cotton (Sharma *et al.*, 2005), as there is no distinct petiole which could be immersed in the agar–agar medium to avoid drying and chemical changes in the leaf.

The genotypes ICSB 323, ICSB 215, ICSV 12004, ICSR 165, IS 40615, ICSV 12001, ICSB 321 and ICSB 724, which suffered low aphid damage under the field conditions and exhibited a low rate of aphid increase in the clip cage assay, showed antibiosis as a component of resistance to *M. sacchari*; these genotypes will be quite useful for sorghum improvement. Some of the genotypes that exhibited a lower rate of aphid increase under the clip cage or leaf disc assay showed a susceptible reaction under field conditions and vice versa. The results suggested that infesting the plants with aphid-infested leaf cuttings and covering the plots with a nylon net is quite effective in evaluating sorghum genotypes for resistance to *M. sacchari*. The clip cage assay could be used to gain further understanding of the antibiosis component of resistance to *M. sacchari*. In addition, there is a need to assess the role of antixenosis and tolerance to aphid feeding in genotypic resistance to *M. sacchari* to identify the lines with different mechanisms of resistance to this pest.

Genotypes with a greater height, longer distance between the leaves, smaller leaf angle and presence of waxy bloom have been reported to be less susceptible to aphid damage. Studies have reported that the aphids multiply at a faster rate on genotypes with higher contents of nitrogen, sugar, free amino acids and total chlorophyll (Mote and Shahane, 1994; Tsumuki *et al.*, 1995), while genotypes with high contents of phosphorus, potassium and polyphenols are less preferred by the aphids (Mote and Shahane, 1994). Aconitic acid has also been reported to have an antifeedant effect on aphids (Rustamani *et al.*, 1992). Aphid

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infestation resulted in an 18.5 to 55.8% decrease in total phenol content over the healthy leaves, suggesting the induction of stress in the infested plants. However, this is contrary to the response to insect damage in other plants, where insect damage often leads to an increase in the phenol content of the infested plants/plant parts (War *et al*., 2012). The tannin content of grains has a relatively poor correlation with the phenol content of aphid-infested leaves when compared with healthy leaves (Sharma and Dhillon, 2005). There is a need to assess the relative contribution of various morphological and biochemical traits conferring resistance/susceptibility to *M. sacchari*, and use them as marker traits to screen and select the genotypes for their resistance to this pest. Cytoplasmic male sterility also influences the expression of resistance to *M. sacchari* (Dhillon *et al*., 2006), and restorer lines have a dominant effect on the expression of resistance to aphids in $F_1$ hybrids (Sharma *et al*., 2004, 2006). This information could be used for developing hybrid parents and varieties with resistance to aphids. The information on sources of resistance, factors associated with resistance to aphids and inheritance of resistance to *M. sacchari* can be used to develop resistant sorghum cultivars for deployment in regions prone to aphid outbreaks.

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## References

Borad P. K. and Mittal V. P. (1983) Assessment of losses caused by pest complex of sorghum hybrid, CSH-5, pp. 271–278. In *Crop Losses due to Insect Pests* (edited by B. H. Krishnamurthy Rao and K. S. R. K. Murthy). Entomological Society of India, Rajendranagar, Hyderabad.

Daware D. G., Bhagwat V. R., Ambilwade P. P. and Kamble R. J. (2012) Evaluation of integrated pest management components for the control of sorghum shoot pests in *Rabi* season. *Indian Journal of Entomology* 74, 58–61.

Dhillon M. K., Sharma H. C., Pampapathy G. and Reddy B. V. S. (2006) Cytoplasmic male-sterility affects expression of resistance to shoot bug, *Peregrinus maidis*, sugarcane aphid, *Melanaphis sacchari*, and spotted stem borer, *Chilo partellus* in sorghum. *International Sorghum and Millets Newsletter* 47, 66–68.

Fang M. N. (1990) Population fluctuation and timing for control of sorghum aphid on variety Taichung 5. *Bulletin of Taichung District Agricultural Improvement Station* 28, 59–71.

Ghuguskar H. T., Chaudhari R. V. and Sorte N. V. (1999) Evaluation of sorghum hybrids for tolerance to aphids, *Melanaphis sacchari* (Zehntner) in field conditions. *PKV Research Journal* 23, 55–56.

Hagio T. (1992) Host plant resistance and its inheritance in sorghum to sugarcane aphid (*Melanaphis sacchari* Zehntner). *Bulletin of the Chugoku National Agricultural Experiment Station* 10, 17–26.

ICRISAT (1992) *The Medium Term Plan*. Vol. II. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh.

Kawada K. (1995) Studies on host selection, development and reproduction of *Melanaphis sacchari* (Zehntner). *Bulletin of the Research Institute for Bioresources, Okayama University* 3, 5–10.

Liu J., He F. G., Qu G. M. and Zhang G. X. (1990) The effect of resistant sorghum on the fecundity and mortality of the sorghum aphid. *Acta Phytophylactica Sinica* 17, 343–347.

Meksongsee B. and Chawanapong M. (1985) Breeding sorghum for resistance to shoot fly and midge, pp. 57–64. In *Proceedings of the International Sorghum Entomology Workshop*, 15–21 July 1984, Texas A&M University, College Station, Texas, USA. International Crops Research Institute for the Semi-Arid Tropics, Patancheru.

Mote U. N. and Shahane A. K. (1994) Biophysical and biochemical characters of sorghum varieties contributing resistance to delphacid, aphid and leaf sugary exudations. *Indian Journal of Entomology* 56, 113–122.

Raetano C. G. and Nakano O. (1994) Influence of climatic conditions on the occurrence of sugarcane aphid, *Aphis sacchari* (Zehntner) (Hemiptera: Aphididae) on sugarcane. *Cientifica Jaboticabal* 22, 303–306.

Rustamani M. A., Kanehisa K., Tsumuki H. and Shiraga T. (1992) Further observations on the relationship between aconitic acid contents and aphid densities on some cereal plants. *Bulletin of the Research Institute for Bioresources, Okayama University* 1, 9–20.

Sarath Babu B., Sharma H. C., Surender A., Prasada Rao R. D. V. J., Chakravarty S. K., Singh S. D. and Girish G. A. (2000) Sorghum germplasm from Thailand showing resistance to sugarcane aphid, *Melanaphis sacchari* Zehntner. *Indian Journal of Plant Genetic Resources* 13, 186–187.

Sharma H. C. (1993) Host plant resistance to insects in sorghum and its role in integrated pest management. *Crop Protection* 12, 11–34.

Sharma H. C. and Dhillon M. K. (2005) Reaction of different sorghum genotypes to infestation by the sugarcane aphid, *Melanaphis sacchari* Zehntner. *Indian Journal of Entomology* 67, 291–296.

Sharma H. C. and Nwanze K. F. (1997) Insect pests of sorghum: biology, extent of losses, and economic thresholds, pp. 9–23. In *Plant Resistance to Insects in Sorghum* (edited by H. C. Sharma, F. Singh and K. F. Nwanze). International Crops Research Institute

for the Semi-Arid Tropics, Patancheru, Andhra Pradesh.

Sharma H. C., Dhillon M. K. and Pampapathy G. (2006) Multiple resistance to sorghum shoot fly, spotted stem borer, and sugarcane aphid in sorghum. *International Journal of Tropical Insect Science* 26, 239–245.

Sharma H. C., Pampapathy G., Dhillon M. K. and Ridsdill-Smith T. J. (2005) Detached leaf assay to screen for host plant resistance to *Helicoverpa armigera*. *Journal of Economic Entomology* 98, 568–576.

Sharma H. C., Taneja S. L., Kameswara Rao N. and Prasada Rao K. E. (2003) Evaluation of sorghum germplasm for resistance to insect pests, *Information Bulletin No. 63*. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh. 184 pp.

Sharma H. C., Taneja S. L., Leuschner K. and Nwanze K. F. (1992) Techniques to screen sorghums for resistance to insects. Information Bulletin No. 32. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh. 48 pp.

Sharma H. C., Dhillon M. K., Naresh J. S., Ram Singh, Pampapathy G. and Reddy B. V. S. (2004) Influence of cytoplasmic male-sterility on the expression of resistance to insects in sorghum, pp. 6. In *New Directions for a Diverse Planet*. *Proceedings of the 4th International Crop Science Congress*, 26 September–1 October 2004, Brisbane, Queensland, Australia (edited by T. Fisher, N. Turner, J. Angus, L. McIntyre, M. Robertson, A. Borrell and D. Lloyd). BPA Print Group Pty Ltd, Victoria. Available at: www.cropscience.org.au

Smith G. R., Borg Z., Lockhart B. E. L., Braithwaite K. S. and Gibbs M. J. (2000) Sugarcane yellow leaf virus: a novel member of the *Luteoviridae* that probably arose by inter-species recombination. *Journal of General Virology* 81, 1865–1869.

Tsumuki H., Kanehisa K. and Moharramipour S. S. (1995) Sorghum resistance to the sugarcane aphid, *Melanaphis sacchari* (Zehntner) amounts of leaf surface wax and nutritional components. *Bulletin of the Research Institute for Bioresources, Okayama University* 3, 27–34.

van den Berg J. (2002) Status of resistance of sorghum hybrids to the aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae). *South African Journal of Plant and Soil* 19, 151–155.

van den Berg J., Pretorius A. J. and van Loggerenberg M. (2003) Effect of leaf feeding by *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), on sorghum grain quality. *South African Journal of Plant and Soil* 20, 41–43.

War A. R., Paulraj M. G., Ignacimuthu S. and Sharma H. C. (2012) Defensive responses in groundnut against chewing and sap-sucking insects. *Journal of Plant Growth Regulation* 32, 259–272. DOI: 10.1007/s00344-012-9294-4.