BIOCHEMICAL MECHANISMS OF RESISTANCE TO SHOOT FLY, *Atherigona soccata* (RONDANI) IN SORGHUM, *Sorghum bicolor* (L.) MOENCH

Thesis submitted in partial fulfillment of the requirements

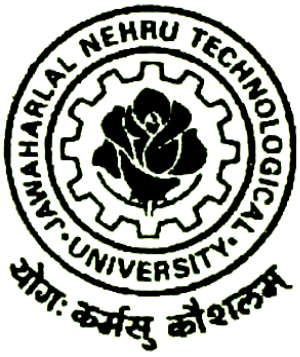
for the award of the degree of

DOCTOR OF PHILOSOPHY

IN BIOTECHNOLOGY

SIVA KUMAR CHAMARTHI

M.Sc. (Biotechnology)



CENTER FOR BIOTECHNOLOGY,

INSTITUTE OF SCIENCE AND TECHNOLOGY

JAWAHARLAL NEHRU TECHNOLOGICAL UNIVERSITY

HYDERABAD 500 072, ANDHRA PRADESH, INDIA

2008

DECLARATION

This is to declare that the contents presented in the thesis entitled, **"Biochemical Mechanisms of Resistance to Shoot Fly, *A. soccata* (Rondani) in Sorghum (*Sorghum bicolor* (L.) Moench)"**, submitted in partial fulfillment for the award of the degree of **Ph.D.** in **Bio-technology** to **Jawaharlal Nehru Technological University, Hyderabad** is a record of bonafide work carried out independently by me. Further, this is to state that the results embodied in this thesis have not been submitted to any other University or Institution for the award of any degree or diploma.

Place: **(SIVA KUMAR CHAMARHTI)**

ACKNOWLEDGEMENTS

I wish to take this opportunity to express my deep sense of gratitude and sincere thanks to my supervisor ***Dr. H. C. Sharma,*** *Principal Scientist, Entomology, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India. It is a great privilege to have been his student and be benefited by his immensely valuable and exemplary guidance. His continuous involvement, well-timed suggestions, continuous care and constant encouragement during the course of study enabled me in bringing out the thesis into the presentable form to the best of my ability.*

Words seem inadequate in expressing the thankfulness to my co-supervisor ***Prof. M. Lakshmi Narasu,*** *Head, Center for Bio-technology, Institute of Science and Technology (IST), JNTU, for her stimulating guidance, patient appraisals and the keen interest she has taken to give me the right perspective of research. I shall gratefully memorize the invaluable suggestions and scholarly advises offered by her. And also I expressed my sincere thanks to all the other staff members of the Department of Biotechnology, JNTU, Kukatpally.*

I wish to record my sincere gratitude and respectful regards to Dr. C. T. Hash, Principal scientist, Molecular breeding, ICRISAT, Patancheru, India and for their generous support and helpful suggestions to carry out molecular work and make comfort during my stay in AGL.

I sincerely thank to Dr. R. P. Thakur, Principal Scientist, Pathology, ICRISAT, Patancheru, India, and plant quarantine unit staff specially, Mr. Girish and Mr. Murali for providing facilities and their generous help to carry out my thesis work in plant quarantine unit.

I also sincerely thank to Dr. K. L. Sahrawat and his staff members, Analytical Chemistry Laboratory, ICRISAT for their constructive suggestions, and their kind co-operation in biochemical studies.

I am grateful to the AGL scientific and technical staff specially, Dr. Rajeev K Vershaney, Senior Scientist, ICRISAT, Mrs. Seetha Kannan, Scientific Officer, ICRISAT, Dr. Santhosh Deshpande, Visiting Scientist, ICRISAT, Mr. Rajaram and Mr. Ramu, and all students for their co-operation, encouragement and help in completing my lab work efficiently, expediently and smoothly.

I wish to record my sincere gratitude and respectful regards to Dr. B. U. Singh, Prncipal Scientist, NRCS, and Rajendhra Nagar, and Dr. M. K. Dhillon, Special Project Scientist, for giving excellent support, whole hearted co-operation and encouragement during the course of my doctoral study.

I feel privileged to render my heartfelt words of appreciation to Entomology Staff, Messers. Mr. G. Pampapathy, Mr. M. Rahul Wadaskar, Mr. V. S. Reddy, Mr. J. Raja Rao, Mr K. Hareendranath, Mr. M. Madhusudhan Reddy, Mr. S. V. N. Chandhra, Mr. V. Venkateshswara Rao, Mr. S. R. Venkateshswarlu, Mr. V. Vittal Reddy, Mrs. P. Ponnama, and Mrs. B. Vimala for their whole hearted help and kind co-operation in the greenhouse, field and lab work for successful completion of my doctoral research at ICRISAT.

I will take this opportunity to thank Dr. Rex L. Navaro, and Dr. Balaji, former Program Leader, Learning Systems Unit, and present Program Leader, Dr. Rupela, for their kind cooperation and help. My heartfelt

thanks are to the staff of Library, Learning Systems, Housing & Food Services, Security, Field & Medical Unit, Farm & Engineering Services, and Finance Division for their excellent cooperation during my research work at ICRISAT.

I shall be failing in my duty if I do not express my cordial thanks to all my colleagues and friends Gopala Swamy, Peter, Mohan, Vinod, Ramakrishna, Devvart, Mehtre, Parikshit, Vijay Dalvi, Velu, Bhaskaran, Gopi, Girija Shankar, Murali, Chander Rao, Narasimha reddy, Akbar Ali, A. Srinivasa Reddy, P. Srinivasa Reddy, Mahendra, Raju, Khedekhar, Anand, Rachit, Richa, Srujana, Sireesha, Lakshmi, Harini, Samatha, Padmaja, Surekha, Parama Siva, Ramani, Spurthy, Nicy, Lekha, Prathima (Cheruku & Juveda) and others who could not find mention for the help, morale support, and nice company during my stay at ICRISAT.

I am in dearth of words to express my unbound gratitude, love and affection to my beloved parents Ramalinga Raju and Lakshmi Narasamma who have been a constant source of inspiration and encouragement at each and every step of my career. I would like to express my affection for my brothers Prasad and family, Ravi and family, and Sister, Sujatha and family, and all my family members who shared both the joys and sorrows with me.

Financial assistance received from Suri Sehgal Foundation Research Fellowship, ICRISAT, is thankfully acknowledged.

For the unbound grace and abundant blessings I am eternally indebted to The Almighty.

(Siva Kumar Chamarthi)

ABSTRACT

Sorghum is the fifth major cereal crop after wheat, rice, corn, and barley, and third important cereal crop after rice and wheat in India. Nearly 150 insect species have been reported as pests on sorghum, of which sorghum shoot fly, *Atherigona soccata,* is an important pest. Host plant resistance is one of the important components for managing this pest, and therefore, the present studies were undertaken on biochemical mechanisms of resistance to shoot fly to strengthen host plant resistance to this insect for sustainable crop production.

Genotypes IS 2312, SFCR 125, SFCR 151, ICSV 700, and IS 18551 exhibited antixenosis, antibiosis, and tolerance components of resistance to shoot fly, *A. soccata*. There was a significant variation in the leaf surface wetness, leaf glossiness, trichome density, seedling vigor, plumule and leaf sheath pigmentation, days to 50% flowering, and plant height among the test genotypes.

Transplanting and clipping of sorghum seedlings reduced shoot fly damage. There was no effect of *p*-hydroxy benzoic acid (PHBA), *p*-hydroxy benzaldehyde (PHB), Cu_2So_4, KI, and 2, 4- D on shoot fly damage. However, application of PHBA showed increase in egg laying by the shoot fly females.

Sorghum genotypes with high amounts of soluble sugars, more leaf surface wetness and fats, and better seedling vigor were susceptible to shoot fly; while those with glossy leaf trait, pigmented plumule and leaf sheath, tall with high trichome density; and high tannin, Mg, and Zn contents showed resistance to shoot fly. Leaf surface wetness, Mg, Zn, soluble sugars, tannins, fats, leaf glossiness, leaf sheath and plumule pigmentation, and trichome density explained 99.8% of the variation for deadhearts, of which leaf glossiness, plumule pigmentation, trichomes, and fat content had direct effects and correlation coefficients for

deadhearts in the same direction, and can be used to select for resistance to shoot fly.

Leaf glossiness, leaf sheath and plumule pigmentation, high trichome density, tannins, moisture, total soluble polyphenols, lignins, and Mg were associated with antibiosis to shoot fly.

Phenolic compounds: *p*-hydroxybenzaldehyde, *p*-hydroxy benzoic acid, luteolin and unknown peaks at RTs 24.38 and 3.70 were associated with susceptibility to shoot fly, whereas, protocatechuic acid, *p*-coumaric acid, cinnamic acid, and apigenin were associated with resistance to shoot fly, *A. soccata*.

Protein peaks 1, 2, 3, 4, 7, 12, 14, 16, and 17 were positively associated with susceptibility to shoot fly. Peaks 5, 8, 9, 11, and 15 were associated with resistance to shoot fly, *A. soccata*. Peaks 1, 2, 3, 4, 6, 7, 10, 12, 14, 16, and 17 were negatively correlated with developmental period, pupal period, and female pupal weight, but positively correlated with larval survival, adult emergence, and male pupal weight, indicating that those were associated with susceptibility to shoot fly. On the other hand, peaks 8, 9, 11, and 15 were associated with antibiosis to shoot fly.

Compounds undecane 5- methyl, decane 4- methyl, hexane 2, 4- methyl, pentadecane 8- hexyl and dodecane 2, 6, 11- trimethyl, present on the leaf surface of sorghum seedlings, were associated with susceptibility to shoot fly, while 4, 4- dimethyl cyclooctene was associated with resistance to shoot fly.

There was considerable diversity among the sorghum genotypes used in the present studies, based on the morphological, biochemical, and molecular characterization, and can be used in shoot fly resistance breeding program to broaden the genetic base and increase the levels of resistance to this pest.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

1. INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Moench] is an important crop in Asia, Africa, Australia, and the Americas. Sorghum belongs to the grass family, Gramineae, and is the fifth major cereal crop after wheat, rice, corn, and barley. It is grown in about 86 countries covering an area of about 42 million ha with an annual production of 58.7 million tones (FAO, 2004). In India, sorghum is the third important cereal after rice and wheat, and is currently grown in 10.4 m ha with an annual production of 8 million tons (FAS, 2005). Sorghum grain is used as a staple food in Asia and Africa (Awika and Rooney, 2004; Ratnavathi and Sashidar, 1998).

Genetic manipulation of sorghum since 1960`s has lead to development of several high-yielding varieties and hybrids. However, sorghum yields on farmer's field are quite low (500-800 kg ha^{-1}), although the potential yields are as high as 10 tons ha^{-1} (Sharma, 1985). Several biotic and abiotic factors constrain sorghum yields, of which insect pests are major factors in the semi-arid tropics.

Nearly 150 insect species have been reported as pests on sorghum (Jotwani *et al.*, 1980; Sharma, 1993). Sorghum shoot fly [*Atherigona soccata* (Rond.)], stem borers [*Chilo partellus* (Swin.), *Busseola fusca* (Ful.), *Eldana saccharina* (Wlk.), and *Diatraea* sp.], armyworms [*Mythimma separata* (Wlk.)], *Spodoptera exempta* (Wlk.), and *Spodoptera frugiperda* (J. E. Smith)], aphids [*Melanaphis sacchari* (Zehnt.), *Rhopalosiphum maidis*

(Fit.), and *Schizaphis graminum* (Rond.)], shoot bug [*Peregrinus maidis* (Ashm.)], sorghum midge [*Stenodiplosis sorghicola* (Coq.)], head bugs [*Calocoris augustatus* (Leth.), *Eurystylus immaculatus* (Odh.), *Creontiades pallidus* (Ramb.), and *Campylomma* spp.] , and head caterpillars [*Helicoverpa armigera* (Hub.), *Helicoverpa zea* (Bodd.), *Cryptoblabes* spp., *Eublemma* spp., *Euproctis* spp., etc] are the major pests worldwide.

Of this, sorghum shoot fly, *A. soccata* is one of the most important constraints in sorghum production in Asia, Africa, and the Mediterranean Europe. Board and Mittal (1983) reported that nearly 32% of the actual produce is lost due to insect-pests in India. Losses due to shoot fly have been estimated to be nearly $274 million in the semi-arid tropics (ICRISAT, 1992). Losses in grain yield are directly correlated with infestation (Rai and Jotwani, 1977). Shoot fly attacks sorghum at the seedlings stage (7-30 days after seedling emergence). Infestation rates are higher in late-sown *kharif* (rainy season) and early- sown *rabi* (post-rainy season) sorghum crops.

To reduce insect damage, farmers often use chemical pesticides that are hazardous to the beneficial organisms and the environment. Shoot fly is not easily accessible to insecticides sprayed on sorghum crop because the larvae feed inside the leaf whorls. Over 30,000 germplasm accessions have been screened for resistance to shoot fly (Sharma *et al.,* 2003), and considerable progress has been made in transferring

resistance into elite breeding lines (Agarwal and Abraham, 1985; Sharma *et al.*, 1992, 2005).

A number of genotypes with resistance to shoot fly have been identified, but the levels of resistance are low to moderate (Jotwani, 1978; Taneja and Leuschner, 1985; Sharma *et al.*, 2005). With the availability of modern tools in biotechnology such as genetic engineering, molecular markers, functional genomics, and metabolomics, we can identify genotypes with diverse mechanisms of resistance to shoot fly, and transfer such genes into elite cultivars.

To develop crop cultivars with durable resistance to insect pests, it is important to study the resistance mechanisms, and identify lines with diverse combinations of factors associated with resistance to the target pest, and combine different components/mechanisms of resistance in the same genetic background. Some of the factors associated with resistance to insects can be quantified or monitored easily in plant populations, and such characters can be used as "marker traits" to screen and select for resistance to insect pests.

Several physico-chemical components of the plant affect the orientation, oviposition, development, and fecundity of insects. Sorghum contains phenolic compounds, which may be responsible for astringency of many plant materials and affect colour, appearance, and nutritional quality of the host plant (Hahn *et al.*, 1984; Murthy and Kumar, 1995). Phenolic compounds in sorghum comprise of phenolic acids, flavonoids,

and tannins, and are located primarily in leaves or outer layers of the sorghum kernel (pericarp and testa), and aleurone (Hahn *et al.*, 1984).

The females of *A. soccata* are attracted both to the volatiles emitted by the susceptible seedlings, and to phototactic (optical) stimuli that influence the orientation of adult flies to its host plant for oviposition (Nwanze *et al.*, 1998). Host plant recognition by insects cannot be easily explained by the presence or absence of a single stimulus, but it is a cumulative effect of several stimuli. The chemical subset of these stimuli or chemical search image plays an important role in host plant recognition for ovipositing. The leaf surface constitutes an interface between the external environment and the plant tissues. Most behavioral events that lead insects to lay eggs and feed on a host plant are associated with leaf surface contact sensory cues, and therefore, it is important to gain an in-depth understanding of the factors that regulate these processes.

Integration of molecular technology with conventional crop improvement approaches is important to gain an understanding of the genetics of important traits that are associated with resistance to insect pests. In the past, studies have been conducted on genetic variation in sorghum based on morphological traits or inheritance of such traits. However, this approach has its limitations as complex quantitatively inherited traits are difficult to understand solely on the basis of phenotype. For this reason, DNA-based methods have been employed in

studies on sorghum genetic diversity, and in genetic improvement of this crop. Molecular markers are identifiable DNA sequences found at specific locations of the genome and the inheritance of traits is governed by standard laws of inheritance from one generation to the next. In contrast to morphological (based on visible traits) and biochemical markers (based on proteins produced by genes), molecular markers rely on a DNA assay.

In view of the facts discussed above, the present studies were undertaken on constitutive and inducible resistance to shoot fly in sorghum, and physico-chemical traits that influence host plant resistance to shoot fly, and identify sorghum genotypes with different combinations of physico-chemical and molecular characteristics conferring resistance to this pest for use in sorghum improvement.

1.2 OBJECTIVES

1. Identification of physico-chemical traits of sorghum seedlings associated with different mechanisms of resistance to sorghum shoot fly, *A. soccata*.
2. Analyze constitutive and inducible biochemical constituents associated with resistance/susceptibility to *A. soccata*.
3. Identify physico-chemical and molecular markers associated with different components of resistance to *A. soccata*.

2. REVIEW OF LITERATURE

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth major cereal crop after wheat, rice, maize, and barley, and is the most important crop in semi-arid tropics of Asia, Africa, Australia, and the Americas. Nearly 150 insect species damage the sorghum crop, of which sorghum shoot fly, *Atherigona soccata* (Rond.) is one of the most important biotic constraints in Asia, Africa, and the Mediterranean Europe. Therefore, the present studies were undertaken on various aspects of physico-chemical mechanisms of resistance to shoot fly, to formulate appropriate strategies to develop sorghum cultivars with resistance to *A. soccata*.

2.1 Biology and population dynamics of sorghum shoot fly, *Atherigona soccata*

The shoot fly females lay white, elongated, cigar-shaped eggs singly on the abaxial leaf surface of sorghum seedlings at 5-30 days after seedling emergence. Most of the eggs are laid between 08.00 to 12.00 h, and they hatch between 04.00 to 06.00 h. On emergence, the neonate larvae crawl to the plant whorl and move downward between the folds of the young leaves. After reaching the growing point, it cuts the growing tip resulting in drying of the central leaf known as 'deadheart'. Deadheart formation leads to the seedling mortality. The damage occurs 1 to 4 weeks after seedling emergence.

The larval and pupal periods are completed in 8 to 10 days each, and the total life cycle from egg to adult is completed in 17 to 21 days (Kundu and Kishore, 1970; Zein el Abdin, 1981; Dhillon *et al.*, 2005b). The pupal period lasts for 8 days in South India and up to 14 days in North India. Pupation takes place mostly at the base of the stem, and sometimes in the soil. The adult emergence is mostly in the morning between 07.30 to 10.30 h. Males and femals live for 7 and 17 days, respectively (Raina, 1982a), while Meksongsee *et al.* (1981) recorded adult longivity of 20 and 30 days. The adults are active throughout the day. Meksongsee *et al.* (1981) reported that a female lays 238 eggs. More than 63 eggs per female were recorded by Ogwaro (1978a), while Raina (1982b) recorded 17 to 239 eggs, and Dhillon *et al.* (2005b) reported that a female lays 68 to 186.2 eggs.

The shoot fly females prefer second leaf, followed by third, first, and fourth leaves for egg laying under laboratory conditions, while third leaf, followed by second, fourth, fifth, sixth, first, and seventh leaf were preferred for oviposition under field conditions (Ogwaro, 1978b; Davies and Reddy, 1981a). In general, shoot fly females lay only one egg per plant, but under high shoot fly-pressure, there may be several eggs on the same leaf. When more than one egg is present on a plant, these are laid by different females, but under no-choice conditions, more than one egg per plant and more than one larvae per plant have also been observed by Dhillon *et al.* (2005 a,b).

Sorghum shoot fly is active throughout the year, and there may be 10 to 15 generations in a year (Jotwani, 1978). Bene (1986) recorded 5 to 6 generations in temperate areas in Italy. There is no diapause during the off-season (Priyavratha Rao and Narasimha Rao, 1956). During the off-season, the insect survives on alternate hosts (*Sorghum* spp., *Echinochloa colonum*, *E. procera*, *Cymbopogon* sp., *Paspalum scrobiculatum*, and *Pennisetum glaucum*), tillers of ratoon crop, and volunteer/fodder sorghum (Reddy and Davies, 1979; Sharma and Nwanze, 1997). The shoot fly population begins to increase in July, peaks in August-September, and declines thereafter in South central India. Infestations are also high when sorghum plantings are staggered due to erratic rainfall during the post-rainy season, and heavy shoot fly infestation occurs in the crop planted during September-October.

In India, the peak in shoot fly population has been observed during March-April and August-October at Delhi; January, May, and July-October at Pusa, Bihar; August-September at Udaipur, Rajasthan and Hyderabad, Andhra Pradesh; June-September at Dharwad, Karnataka; and November-December at Coimbatore, Tamil Nadu (Pradhan, 1971; Jotwani, 1978). Unnithan and Saxena (1990) demonstrated the potential of an oviposition stimulant for diverting shoot fly eggs onto non-host plants (maize) as a strategy for the management of the shoot fly pest.

Jotwani *et al.* (1970b) suggested that the shoot fly activity and incidence are adversely affected by extremes of temperatures (maximum

30 to 40 ^{0}C and minimum 2 to 14 ^{0}C) and continuous heavy rains. Temperatures above 35 ^{0}C and below 18 ^{0}C, and continuous rainfall reduce shoot fly abundance (Taneja *et al.*, 1986). Delobel and Unnithan (1981) observed that during dry season, shoot fly populations are usually higher on wild sorghum, *Sorghum arundinaceum* than on local cultivated varieties of *S. bicolor*.

2.2 Host plant resistance to shoot fly, *Atherigona soccata*

Host plant resistance to insects is one of the easiest and cheapest components of an integrated pest management program. It is an environmentally friendly method of pest management, and is compatible with other control strategies such as biological, cultural and chemical control.

2.2.1 Techniques to screen for resistance to shoot fly, *Atherigona soccata*

Several techniques to screen for resistance to shoot fly have earlier been described by several workers (Pradhan, 1971; Soto, 1974; Jotwani, 1978; Taneja and Leuschner, 1985; Sharma *et al.*, 1992). The interlard-fish-meal technique (Soto, 1974) is quite useful for increasing shoot fly abundance for screening the test material under field conditions. The moistened fishmeal kept in polyethylene bags are kept in interlards to attract shoot flies from the surrounding areas. Shoot fly abundance in the field can also be monitored through fishmeal-baited traps to determine its peak period of activity, which helps in decision making for

planting of the test material to expose the test material to optimum shoot fly density.

Planting density also affects oviposition and subsequent deadheart formation. High shoot fly incidence has been observed in close plant spacing (1 cm between plant to plant and 75 cm between the rows) (Davies *et al.*, 1976; Davies and Reddy, 1981a). Delobel (1982) reported 3.35 times more number of eggs under low planting density compared to dense planting density, but the larval mortality resulting from competition increased from high to low planting density. Reddy *et al.* (1981) reported that the amines resulting from the biodegradation of fishmeal serve as the chemical cues for shoot fly attraction to the fishmeal, but the amine based chemical compounds have not been identified, which could be useful to understand the mode of attraction of sorghum shoot fly females to fishmeal and/or its host plants.

Soto (1972) developed a cage-screening technique to confirm the resistance to shoot fly observed under field conditions, and to study the resistance mechanisms involved in host plant resistance to sorghum shoot fly. Soto and Laxminarayan (1971) studied shoot fly bio-ecology, mass-rearing technology under greenhouse conditions. Shoot flies can also be collected from fish-meal-baited traps in the field, stored under greenhouse conditions, and used for screening sorghums for resistance to shoot fly under multi-, dual- or no-choice tests (Sharma *et al.*, 1992;

Dhillon *et al.*, 2005b). Rapid screening can also be carried out using a top-cage technique.

This system consists of two plastic trays (40 x 30 x 14 cm), one for sowing the test material and the other used as a top-cage (fitted with fine wire-mesh) is clamped over the first tray, thus forming a cage (Dhillon, 2004). Shoot flies are released @ 2 flies per 5 plants for 24 h through an opening. The adult flies are fed with 20% sucrose solution and dry mixture of Brewer's yeast and glucose (1: 1). After 24 h, the flies are removed and the plants are observed for oviposition. Four-five days after the release of flies, the data are recorded on number of plants with deadhearts.

2.3 Identification and utilization of host plant resistance to shoot fly, *Atherigona soccata*

2.3.1 Mechanisms of resistance

All the three major mechanisms of resistance viz, oviposition non-preference (antixenosis), antibiosis, and recovery contribute to host plant resistance to sorghum shoot fly (Doggett *et al.*, 1970; Soto, 1974; Raina *et al.*, 1981; Taneja and Leuschner, 1985; Sharma and Nwanze, 1997; Dhillon *et al.,* 2005a, b, 2006).

2.3.1.1 Antixenosis for oviposition

Jain and Bhatnagar (1962) first reported ovipositional non-preference by shoot fly in resistant cultivars. Later, several workers considered it as the primary mechanism of resistance to shoot fly in sorghum (Blum, 1967;

Sharma *et al.*, 1977; Singh and Jotwani, 1980a; Taneja and Leuschner, 1985; Kumar *et al.*, 2000; Singh *et al.*, 2004; Dhillon *et al.*, 2005b).

Behavioral responses of shoot fly have shown that initial choice of the host was random, but the females spent less time on the resistant cultivars (Raina *et al.*, 1984). Deadheart formation was low and the expression of resistance was stable across different seedling growth stages in the germplasm lines IS 1054 and IS 2146 (Singh *et al.*, 2004). Females laid eggs on non-preferred cultivars only after laying several eggs on the seedlings of susceptible cultivar (Dhillon, 2004). However, this mechanism of resistance was not stable, and tends to breakdown under no-choice conditions (Dhillon *et al.*, 2005b). Under no-choice conditions in the cage, oviposition was similar on resistant and susceptible varieties (Taneja and Leuschner, 1985; Dhillon *et al.*, 2005b, 2006). Varieties preferred for oviposition show a high degree of deadheart formation (Rana *et al.*, 1975; Dhillon *et al.*, 2005b).

2.3.1.2 Antibiosis

The resistance to sorghum shoot fly is a cumulative effect of non-preference and antibiosis (Raina *et al.*, 1981). Antibiosis to shoot fly was reported by Jotwani and Srivastava (1970a), Blum (1972), Soto (1974), Sharma *et al.* (1977), Singh and Jotwani (1980b), and Dhillon *et al.* (2005b). Retardation of growth and development, prolonged larval and pupal periods, and poor emergence of adults on resistant varieties provides direct evidence of antibiosis (Narayana, 1975; Singh and

Jotwani, 1980b; Raina *et al.*, 1981; Dhillon *et al.*, 2005b). Raina (1985) suggested that biochemical deficiencies or the presence of chemical factors in resistant cultivars might adversely affect the development and survival of *A. soccata* larvae. Patil *et al.* (2006) observed high enzyme activity (peroxidase and polyphenol oxidase) in resistant lines as well as resistant x resistant and resistant x susceptible crosses. The higher enzyme activity might be inducing and activating the antibiosis mechanism, leading to reduction in damage caused by the shoot fly.

The fecundity of shoot fly females was greater when raised on susceptible variety Swarna, than on moderately resistant varieties IS 2123 and IS 5604 (Singh and Narayana, 1978). However, reverse trend was observed by Dhillon *et al.* (2005b). There is prolongation in larval and pupal periods, and lower larval survival on resistant cultivars (Singh and Jotwani, 1980b; Jadhav and Mote, 1986; Dhillon *et al.*, 2005b). Larval survival and rate of larval development are also dependent on the age of the host plant. Larval and total growth indices were significantly low on resistant varieties compared to the susceptible ones. The percentage pupation on resistant lines was significantly lower compared to that on susceptible lines (Dhawan *et al.*, 1993; Dhillon *et al.*, 2005b). The larvae on the resistant varieties are sick and smaller as compared to susceptible varieties.

2.3.1.3 Tolerance/recovery resistance

Tiller development consequent to deadheart formation in the main shoot and its survival depend on the level of primary resistance as well as shoot fly pressure (Doggett *et al.*, 1970). Tiller survival is related to its faster growth rate with a better chance to escape deadheart formation. Seedling vigor and high rate of recovery are important characteristics of resistant cultivars (Sharma *et al.*, 1977), which may not be related with seedling height, because some of the tolerant germplasm lines are dwarf, medium tall or very tall (Shivankar *et al.*, 1989; Dhillon *et al.*, 2005b). Blum (1972) suggested that faster tiller growth leads to the minimizing shoot fly infestation in tillers.

The shoot fly-resistant genotypes have significantly less tiller deadhearts than the susceptible ones. Varieties with high recovery resistance compensate for yield loss under shoot fly infestation (Rana *et al.*, 1985). The Serena and Namatrare varieties recovered well even when more than 90% of the main plants were killed by shoot fly attack (Doggett and Majisu, 1965; Doggett *et al.*, 1970). Recovery resistance does not appear to be useful mechanisms of resistance particularly when shoot fly population increases progressively as the rainy season continues (Singh and Rana, 1986). The damaged plants produce axial tillers, which serve as a mechanism of recovery resistance. However, the axial tillers often mature later than the main plants, and often suffer greater damage by sorghum midge, *S. sorghicola* (Coq.), head bugs, *C. angustatus* (Leth.),

and birds, or may not be able to produce grain under drought stress (Dhillon, 2004).

2.3.2 Morpho-physiological traits associated with resistance to shoot fly, *Atherigona soccata*

2.3.2.1 Seedling vigor

Faster seedling growth and toughness of the leaf sheath are associated with resistance to shoot fly (Singh and Jotwani, 1980d; Kamatar and Salimath, 2003). Blum (1972) observed that shoot fly-resistant sorghum lines grew faster than susceptible ones. Seedling vigor was significantly and negatively associated with deadhearts and oviposition (Taneja and Leuschner, 1985).

Faster seedling growth and longer shoot length causes the larvae to take more time to reach the base of the shoot. Singh (1998) concluded that rapid seedling growth and long and thin leaves during the seedling stages makes plants less susceptible to shoot fly. Karanjkar *et al.* (1992) suggested that seedling vigor can be used to select for resistance to *A. soccata.*

Jayanthi *et al.* (2002) showed that shoot fly resistant parental lines and their hybrids showed significantly high seedling vigor compared to susceptible parental lines and their hybrid groups. The negative association of seedling vigor and plant height with shoot fly resistance seems to be influenced by shoot fly damage in resistance screening trails, rather than the direct effect of seedling vigor on shoot fly damage. The

seedling vigor scores in shoot fly screening trails are affected by shoot fly damage. Under shoot fly damage the shoot fly susceptible lines apparently apper to be less vigorous as a result of deadheart formation. However, if the seedling vigor is scored under un-infested conditions, there is no direct effect of seedling vigor on expression of resistance to shoot fly. In fact, vigorously growing plants are more attractive to the shoot fly oviposition, which ultimately may result in greater shoot fly damage (Sharma, H.C. unpublished; Dhillon, 2004).

2.3.2.2 Trichomes

Trichomes or plant hairs are common anatomical features on leaves, stem and/or reproductive structures in higher plants. Levin (1973) had described the role of trichomes in plant defense and pointed out that in numerous species, there is negative association between trichome density and insect feeding, oviposition responses, and nutrition of larvae. Trichome density has a positive correlation with resistance to shoot fly in sorghum (Moholkar, 1981; Omori *et al.*, 1983; Jadhav *et al.*, 1986; Patel and Sukhani, 1990b; Dhillon *et al.*, 2005b, 2006). Per cent plants with eggs and number of eggs per plant were negatively correlated with trichome density at 14 days after emergence (Dhillon *et al.*, 2005b, 2006; Patil *et al.*, 2006).

Karanjkar *et al.* (1992) suggested that although there are highly significant and negative correlation between the trichome density and shoot fly infestation, it seems that trichomes do not have a direct role in

reducing the deadhearts, but are associated with reduced oviposition. Maiti (1994a) suggested that trichomes on both leaf surfaces can be used as a reliable selection criteria to select for resistance to *A. soccata.* Biradar *et al.* (1986) reported that the intensity of trichomes on the adaxial surface was 2 to 6 times more than that on the abaxial leaf surface. Trichomes on the abaxial and adaxial leaf surfaces may inhibit the movement of young larvae in the whorl, which may prolong the time to reach the growing point or result in mortality of the neonate larvae (Maiti *et al.*, 1980; Gibson and Maiti, 1983; Raina, 1985). Level of resistance to shoot fly have been reported to be higher when both glossy and trichome traits occurred together (Agrawal and House, 1982, Dhillon *et al.*, 2005b, 2006a, b; Sharma *et al.*, 2006).

2.3.2.3 Glossiness

The leaf glossiness (pale green and shiny leaves) at seeding stage probably has a strong influence on the orientation of shoot fly females due to reflection of light in sorghum (Blum, 1972; Maiti and Bidinger, 1979; Agarwal and Abraham, 1985; Kamatar and Salimath, 2003). However, the expression of glossiness is better in rainy season than in post-rainy season (Jayanthi *et al.*, 1999). Genotypes with glossy leaf trait are resistant to shoot fly, and observed high leaf glossiness on *A. soccata* resistant genotypes (Maiti, 1994a; Dhillon *et al.*, 2005 a,b; 2006 a,b; Sharma *et al.*, 2006).

The lower amount of chlorophyll in the leaves renders them less attractive to the shoot fly females for oviposition (Patil *et al.*, 2006). Tarun Verma and Singh (2000) suggested that genotypes having glossy leaves during the seedling stage are comparatively resistant to shoot fly. Patil *et al.* (2006) reported that deadheart incidence was positively correlated with non-glossiness. Differences between glossy and non-glossy genotypes can be detected by the adherence of water sprayed on leaf blades (Nwanze *et al.*, 1990b). There is a negative correlation between leaf glossiness, oviposition, and deadhearts (Jadhav *et al.*, 1986; Vijayalakshmi, 1993; Dhillon, 2004; Dhillon *et al.*, 2005b, 2006a; Patil *et al.*, 2006). Maiti (1980) suggested that presence of trichomes and glossy traits are independent, and apparently have an additive effect in reducing the incidence of shoot fly.

2.3.2.4 Leaf surface wetness (LSW)

The role of leaf surface wetness in plant resistance to insects was first studied by Rivnay (1960), who observed the role of morning dew in the movement of freshly hatched shoot fly larvae through the leaf sheath to the growing point. Sree *et al.* (1992) suggested that LSW originates from the plant, and it is not due to condensation of atmospheric moisture. This was further confirmed by radioactive labeling using tritinium and Carbon-14 (Sivaramakrishnan *et al.*, 1994). Tritiated water applied to the soil of potted seedlings was translocated to the surface of the whorl leaf. There were significant differences in the amount of

tritiated water collected from susceptible (CSH 5) and resistant (IS 18551) genotypes. Nwanze *et al.* (1990a) reported that leaf surface wetness is associated with shoot fly resistance. Cultivars with a high transpiration rate are preferred for oviposition (Mate *et al.*, 1988).

The dew or moisture accumulation in the central whorl leaf, through which the larvae move downward from the site of oviposition to the growing point has an important role in shoot fly resistance (Blum, 1963; Raina, 1981b). The shoot fly larvae spend less than 30 min from egg hatch to arrival at the funnel, and >3 h from the funnel to the growing point. Larval survival is affected by the wetness of the central shoot than the central expanded leaves on which eggs are laid. Admittedly, initial contact with moisture enhances larval movement and survival. A waxy surface will permit an even spread of water on leaf surface, but may not retain water in large droplets as a non-waxy surface does. A smooth amorphous wax layer and sparse wax crystals characterize shoot fly resistant and moderately resistant genotypes, while susceptible genotypes possess a dense mesh of crystalline epicuticular wax (Nwanze *et al.*, 1992). Hence, a highly waxy leaf retains more water as droplets than a non-waxy leaf and vice-versa (Nwanze *et al.*, 1990b). Sree *et al.* (1994) suggested that LSW could be the result of some form of cuticular movement of water to the leaf surface.

Leaf surface wetness trends are also positively associated with shoot fly abundance, crop infestation, rainfall, temperature, and relative

humidity (Nwanze *et al.*, 1992). Soman *et al.* (1994) reported that there was no consistent variation in the relationship between plant water potential and soil metric potential of resistant and susceptible sorghum genotypes. However, soil metric potential affects the water status of the shoot fly susceptible plant, which is associated with the appearance of water droplets in the central leaf whorl of the susceptible cultivar, CSH 1. No water droplets were observed on the central whorl leaf of the resistant genotypes indicating that the production of water droplets is not solely the result of internal water status of the plant.

2.3.2.5 Other plant traits associated with resistance

No relationship was observed between moisture content of sorghum seedlings and shoot fly resistance (Singh *et al.*, 2004). However, Rao and Panwar (2002) reported that moisture content was low in maize genotypes resistant to shoot fly damage. The shoot fly incidence has also been found to be positively correlated with days to flowering and days to maturity, but negatively correlated with number of leaves per plant and plant height (Rao *et al.*, 2000).

The taller varieties with more leaves are desirable for minimizing the shoot fly incidence. Rao and Panwar (2001) reported that the leaf width and stem thickness were positively associated and number of leaves per plant, and leaf length was negatively associated with shoot fly deadhearts in maize, while there was no significant influence of these plant characters on the oviposition. Maiti *et al.* (1994b) indicated that

tall, late-maturing genotypes with high glossy intensity were the most resistant to *A. soccata*. Patil *et al*. (2006) reported that per cent plants with eggs and number of eggs per plant were negatively correlated with seedling height at 14 days after emergence.

Tarun Verma and Singh (2000) observed shoot fly oviposition was negatively correlated with seedling height, leaf length, and stem length, but positively correlated with number of leaves per plant, leaf width, stem girth, and panicle initiation, while shoot fly deadhearts were negatively correlated with seedling height, leaves per plant, leaf length, leaf width, and stem length but positively correlated with stem girth and panicle initiation. The plumule and leaf sheath pigmentation in sorghum were found to be associated with resistance to shoot fly (Dhillon, 2004; Dhillon *et al.*, 2005 a, b, 2006).

2.4 Biochemical mechanisms of resistance

Secondary metabolites play an important role in host plant resistance to insects (Bell and Charwood, 1980; Van wettstein and Chua, 1987). Plants are known to produce certain chemical compounds in different quantities and proportions, which affect the behavior and biology of phytophagous insects (Painter, 1958; Beck, 1965; Schoonhoven, 1968), and be attractants (oviposition and feeding stimulants) or repellents (oviposition and feeding deterrents) or antibiotic (reduced survival, growth, and fecundity). Cultivars that lack these defense mechanisms are often too vulnerable to damage by insect pests. An important group

of defense chemicals in sorghum is the polyphenols, particularly flavonoids and their oligomers, and the condensed tannins.

2.4.1 Polyphenols

Polyphenols are widely distributed in plants, but they are not directly involved in any metabolic process, and therefore, are considered to be secondary metabolites. Phenolic compounds in sorghum caryopsis are associated with resistance to insects and fungal pathogens (Dreyer *et al.*, 1981). Plant phenolics have attracted great interest in relation to their diversity in chemistry and functionality in biology, chemistry, medicine, ecology, and agriculture. In agriculture, they have long been recognized as allelochemicals, (Rice, 1984; Putnam *et al.*, 1986), and constitutive and induced plant defense mechanisms (Vidhyasekaran, 1988). Out of the several polyphenols produced by sorghum, individual components responsible for a particular type of resistance are being identified.

The presence of phenolic compounds in young sorghum seedlings and their decline at later stages of crop growth plays a significant role in the physiological relationships between shoot fly larvae and sorghum seedlings (Woodhead and Berneys, 1978; Woodhead and Cooper-Driver, 1979; Woodhead *et al.*, 1980). Shoot fly resistance has earlier been reported to be associated with high amounts of phenolic compounds in sorghum seedlings (Khurana and Verma, 1983; Kumar and Singh, 1998).

Hahn *et al.* (1983) identified eight main free and bound phenolic acids with different polarities in sorghum grain extracts by reverse phase

HPLC (high performance liquid chromatography). They are gallic acid (3, 4, 5-hydraxybenzoic acid), protocatechuic acid (3, 4,-dihydroxybenzoic acid), *p*-hydroxybenzoic acid (4-hydroxybenzoic acid), vannillic acid (4-hydroxy-3-methoxybenzoic acid), caffeic acid (3, 4- dihydroxycinnamic acid), *p*-coumaric acid (4-hydroxycinnamic acid), ferulic acid (4-hydoxy-3-methoxycinnamic acid), and cinnamic acid (trans-cinnamic acid).

Resistance to sorghum shoot fly is associated with low levels of polyphenol oxidase and peroxidase (Bhise *et al.,* 1996). Studies on chemical composition of sorghum cultivars and their relationship with shoot fly resistance suggested that amounts of protocatechuic acid, syringic acid, and *p*-coumaric acid were negatively correlated with shoot fly deadhearts, while *p*-hydroxybenzoic acid, vanillic acid, and ferulic acid contents were positively correlated with deadheart incidence (Pandey *et al.*, 2005).

Table 2.1 phenolic acids in sorghum grains

Phenolic acid	References
Hydroxybenzoic acid	
Gallic	Hahn *et al.* (1983), Subba Rao and Muralikrishna (2002)
Protocatechuic and Vanillic	Hahn *et al.* (1983), McDonough *et al.* (1986), Subba Rao and Muralikrishna (2002)
p-Hydroxybenzoic	Hahn *et al.* (1983), McDonough *et al.* (1986)
Gentisic	McDonough *et al.* (1986), Waniska *et al.* (1989)
Salicylic	Waniska *et al.* (1989)
Syringic	Waniska *et al.* (1989), McDonough *et al.* (1986)
Hydroxycinnamic acids	
Ferulic, Caffeic, and p-Coumaric	Hahn *et al.* (1983), McDonough *et al.* (1986), Subba Rao and Muralikrishna (2002)
Cinnamic	Hahn *et al.* (1983), McDonough *et al.* (1986)
Sinapic	Waniska *et al.* (1989), McDonough *et al.* (1986)

2.4.2 Flavonoids

Flavonoids and isoflavonoids are known to confer resistance against insect attack in several plant species (Hedin and Waage, 1986; Grayer *et al.*, 1992). Flavonoids in soybean contribute to genotypic resistance against plant pathogens (Ingham *et al.*, 1981; Ebel, 1986) and insects (Khan *et al.*, 1986; Sharma and Noris, 1991). Flavonoids are derivatives of the monomeric polyphenol flavan-4-ol, and are known as anthocyanidins. The two flavonoids identified to be abundant in sorghum grains are luteoforol (Bate Smith, 1969) and apiforol (Watterson and Butler, 1983). The latter compound was also found in sorghum leaves.

Flavonoids play a vital role in insect feeding and oviposition behavior. Insect can discriminate among flavonoids, and these modulate the feeding and oviposition behavior of insects (Simmonds, 2001). The flavonoid pathway, derived from the phenylpropanoid and acetyl CoA and malonyl CoA pathways gives rise to a diverse array of compounds such as isoflavonoids, anthocyanins, proanthocyanidins, etc. that have a multitude of biological functions.

Table 2.2 Flavonoids and proanthocyanidins in sorghum grains

Compound	References
Anthocyanins	
Apigeninidin and Luteolinidin	Nip and Burns (1971), Gous (1989)
Apigeninidin 5-Glucoside	Nip and Burns (1969, 1971), Wu and Prior (2005)
5-Methoxyluteolinidin	Seitz (2004), Wu and Prior (2005)
5-Methoxyluteolinidin 7-glucoside and 7-Methoxyapigeninidin 5-glucoside	Wu and Prior (2005)
7-Methoxyapigeninidin	Pale *et al.* (1997), Seitz (2004), Wu and Prior(2005)
Luteolinidin 5- glucoside	Nip and Burns (1971), Wu and Prior (2005)
5-Methoxyapigeninidin and 7-Methoxyluteolinidin	Seitz (2004)
Flavan-4-ols	
Luteoforol	Bate-Smith (1969)
Apiforol	Watterson and Butler (1983)
Flavones	
Apigenin	Gujer *et al.* (1986), Seitz (2004)
Luteolin	Seitz (2004)
Flavanones	
Eriodictyol	Kambal and Bate-Smith (1976)
Eriodictyol 5-glucoside and Naringenin	Gujer *et al.* (1986)
Flavonols	
Kaempferol 3-rutinoside-7-glucuronide	Nip and Burns (1969)
Dihydroflavonols	
Taxifolin and Taxifolin 7-glucoside	Gujer *et al.* (1986)
Proanthocyanidin monomers/dimers	
Catechin, Procyanidin B-1, and Epicatechin-(epicatechin) –catechin	Gupta and Haslam (1978), Gujer *et al.* (1986)
Prodelphinidin	Brandon *et al.* (1982), Krueger *et al.* (2003)
Proapigeninidin and Proluteolinidin	Krueger *et al.* (2003)

Many compounds of the flavonoid biosynthetic pathway (flavanones, flavones, flavanols, and isoflavonoids) accumulate in response to biotic and abiotic stresses (Ebel, 1986; Sharma and Norris, 1991; Heller and Forkman, 1993). C-glycosyl flavone isolated from the silk of resistant maize have been shown to inhibit the growth of the corn earworm,

Helicoverpa zea (Boisd.) (Waiss *et al.*, 1979). Genetic engineering can be used to change the metabolic pathways to increase the amounts of various flavonoids, which play an important role in host plant resistance to insect pests.

2.4.3 Tannins

Tannins are polymers resulting from condensation of flavan-3-ols. Gupta and Haslam (1980) referred to sorghum tannins as procyanidins because they thought that cyanidin was usually the sole anthocyanidin involved. Tannin content in sorghum decreases after germination (Osuntogun *et al.*, 1989). However, the tannin content of the germinated sorghum increased again upon drying. Kumar and Singh (1998) studied the inheritance of tannin content as a component of resistance to *A. soccata*. Kamatar *et al.* (2003) suggested the exploitation of heterosis to increase tannin content to confer shoot fly resistance.

Diawara *et al.* (1992) reported that the sorghum genotypes IS 1056C, IS 2177C, IS 2246C, IS 4023C, IS 7399C and IS 12680C had a significantly higher antibiotic resistance and high amounts of acid detergent, and neutral detergent fibre or tannin content in the leaves. Short floral parts, faster rate of grain development and high tannin content of grain were apparently associated with resistance to sorghum midge, *S.sorghicola* (Sharma *et al.*, 1990). Tannin content was generally 2x higher in sorghum midge resistant genotypes as compared to the susceptible ones. Shi ZhongLiang *et al.* (2002) reported that tannins are

important secondary metabolites for induced resistance to blossom midge, *Sitodiplosis mosellana (Gelin.)* in wheat.

2.4.4 Cyanogenic glycosides

The presence of cyanogenic glucosides in young sorghum seedlings reaches 6% of the dry weight (Akazawa *et al.*, 1960; Halkier and Moller, 1989). To increase food and feed safety, it is important to lower the content of cyanogenic compounds in these plants through crop improvement. The cyanide content in both seedlings and older plants also depends on growth conditions and genetic background (Gillingham *et al.*, 1969; Gorz *et al.*, 1987). Chavan *et al.* (1990) reported that sorghum cultivars with low shoot fly infestation had low HCN in leaves.

There is a significant turnover of dhurrin in seedlings (Bough and Gander, 1971; Adewusi, 1990), suggesting that dhurrin content could be regulated both by changes in synthesis and through breakdown. The occurrence of *p*-hydroxybenzaldehyde, produced by enzymatic degradation of dhurrin in sorghum seedlings of CSH 1, on the leaf surface was suspected to act as oviposition stimulant for adults and/or feeding activator for the maggots of shoot fly (Alborn *et al.*, 1992). Kumar and Singh (1996) reported negative correlation between HCN content and shoot fly deadhearts, and its antibiotic effects against sorghum shoot fly. Biotechnology offers the opportunity to increase the production of secondary metabolites in plants to increase the levels of resistance to

insect pests or inhibit the production of toxic metabolites such as HCN in sorghum crops meant for fodder.

2.4.5 Sugars

Swarup and Chaugale (1962) reported that low sugar content in sorghum was associated with susceptibility to *C. partellus*. Singh *et al.* (2004) reported that resistance to shoot fly is associated with low levels of reducing and total sugars in sorghum seedlings. Bhise *et al.* (1997) observed that reducing sugars increased slightly between 17 and 20 days after seedling emergence in shoot fly resistant sorghum genotypes, but decreased in susceptible varieties. Concentrations of reducing and total sugars influenced the resistance of little millet genotypes to *Atherigona pulla* (Wiedemann). Higher amounts of sugar in stem tissues of maize cultivar HY 4642 confers susceptibility to *C. partellus* (Arabjafari and Jalali, 2007).

Sekhon and Kanta (1994) observed that maize plants with resistance to spotted stem borer, *C. partellus* had low amounts of sugar. Development of sugarcane aphid, *Melanaphis sacchari* (Zhent.), and delphacid, *Peregrinus maidus* (Ashm.) populations were more pronounced in varieties with higher sugar content in leaves (Mote and Shahane, 1994). Soluble sugar content had little influence on midge resistance in wheat (Shi ZhongLiang *et al.*, 2002). Kabre and Ghorpade (1999) reported that total sugars and reducing sugars were positively correlated with stem borer susceptibility in maize. Total sugars, reducing and non-

reducing sugars, and amino acids are 2 times higher in midge susceptible than in the resistant genotypes (Naik *et al.*, 1994).

2.4.6 Proteins

Resistance to sorghum shoot fly in sorghum (Mote *et al.*, 1979; Kamatar *et al.*, 2002), and *C. partellus* and *Atherigona* in maize is associated with low levels of proteins (Rao and Panwar, 2002, 2001). Kabre and Ghorpade (1999) indicated that protein content was positively correlated with stem borer susceptibility in maize. Maiti *et al.* (1994c) isolated three polypeptides (106 kDa, 82 kDa, 54 kDa) from protein extracts of 6 glossy and 1 non-glossy sorghum leaves. The 54 kDa polypeptide was present in several glossy lines, while the non-glossy lines contained polypeptides of a higher molecular weight (106 kDa). Presence of 54 kDa band in the glossy lines may be related to *A. soccata* resistance in sorghum.

2.4.7 Nutritional elements

Several micronutrients play an important role in the host plant resistance to *A. soccata*. Low levels of nitrogen (Singh and Narayana, 1978; Singh and Jotwani, 1980; Khurana and Verma, 1983; Chavan *et al.*, 1990) and high levels of Ca (Chavan *et al.*, 1990) were earlier reported to be associated with the shoot fly resistance in sorghum. Higher amounts of Mg and Zn, and lower amounts of Fe were associated with the expression of resistance to shoot fly. Resistance to shoot fly in sorghum is associated with low levels of nitrogen, phosphorus, and

potassium (Bhaise *et al.*, 1997; (Rao and Panwar, 2002, 2001; Singh *et al.*, 2004).

Sorghum cultivars with low shoot fly infestation have low nitrogen and magnesium contents, and high silicon and calcium contents (Chavan *et al.*, 1990). Concentrations of silica and potassium also influence the resistance of little millets to *A. pulla* (Kadire *et al.*, 1996). High amounts of P, K, Fe and Si contribute to stem borer, *C. partellus* resistance in maize (Arabjafari and Jalali, 2007). The resistance of the wheat cultivars to *Rhopalosiphum padi* is associated with N and Zn contents (Li Sujuan *et al.*, 2001). Maize germplasm with high level of resistance to spotted stem borer, *C. partellus* had high contents of silica and iron, but low contents of nitrogen, phosphorus, and potassium (Sekhon and Kanta, 1994). However, varieties with high content of phosphorus and potassium were less preferred by delphacids and aphids in sorghum (Mote and Shahane, 1994). Higher concentration of silica, iron, zinc and manganese and lower concentration of nitrogen, phosphorus, potassium, calcium, magnesium and copper are associated with resistance to *Sogatella furcifera* (Horvth.) in rice (Mishra *et al.*, 1990; Mishra and Misra, 1993).

2.4.8 Volatiles

Green leaf volatiles (GLVs), generally occurring in C_6 alcohols, aldehydes, and acetates from plants, play an important role in plant-plant communication. These compounds induce intact plants to produce

jasmonic acid and defense-related gene expression, and the release of volatile compounds. Composition of young green leaves of barley and wheat analyzed for volatile compounds by GC-MS indicated that the barley extract had eight aliphatic alcohols, 18 aliphatic aldehydes, 17 aliphatic ketones, two aliphatic esters, one aliphatic acid, 20 heterocyclic compounds (furans, pyrroles, thiazoles, and pyrazines), 15 terpenes and related compounds, 12 aromatic compounds, and one sulfur containing compound. The main components of barley extract were: (E)-β-ionone, benzaldehyde, furfural, 5, 6-epoxy-β-ionone, and benzylaldehyde. Volatile compounds found in the wheat extract were similar to those found in the barley extract. The volatile compounds identified in the wheat extract were: 11 aliphatic alcohols, 20 aliphatic aldehydes, 16 aliphatic ketones, four aliphatic esters, five aliphatic acids, 10 heterocyclic compounds (furans, pyrroles, and pyrazines) 18 terpenes and related compounds, 14 aromatic compounds, five nitriles, and two sulfur containing compounds. The main components of wheat extract were 5-hexenenitrile, phytol, phenyl acetonitrile, 4-pentennitrile, (E)-β-ionone, 5, 6-epoxy-β-ionone, and β-cyclocitral (Shibamoto *et al.*, 2007).

Plant volatiles from resistant rice cultivars act as repellents or are toxic to brown plant hopper, *Nilaparvata lugens* (Stal.) (Reddy, 2003). Females of *A. soccata* are attracted to the volatiles emitted by the susceptible seedlings (Nwanze *et al.*, 1998a). Application of jasmonic acid (JA) in wheat seedlings induces the production of both direct and indirect

defences by emitting specific blends of volatiles that attract natural enemies (Yin Jiao, 2005). Linalool and 4, 8-dimethyl-1, 3, 7- nonatriene were the major volatiles induced by fall armyworm damage 6 h after initial damage in maize (Carroll *et al.*, 2005).

Maize releases specific volatiles in response to herbivory by lepidopterous larvae. These volatiles are known to serve as cues for parasitic wasps to locate the herbivores. Typical green leaf odours: (Z)-3-hexenal, (E)-2-hexenal, (Z)-hexen-1-ol, and (Z)-3-hexen-1-yl acetate were emitted upon damage, and their amounts dropped rapidly after the first collections. Several of the induced compounds were released within 2 h after treatment, while others (mainly sesquiterpenoids) were released after 4 h. The LG11 seedlings emitted several compounds (e.g., β-myrcene, (Z)-β-ocimene, benzyl acetate, β-caryophyllene, and (E, E)-α-farnesene) that were not detected for Ioana. (E, E)-α-farnesene was continuously emitted by LG11 seedlings, even in undamaged plants. Timing of the release of volatile compounds did not differ significantly, except for indole, for which the peak production was considerably earlier for LG11 (Turlings *et al.*, 1998).

2.5 Genetic diversity of sorghum in relation to expression of resistance to sorghum shoot fly, *Atherigona soccata*

2.5.1 DNA and SSR markers

The use of DNA-based markers for the genetic analysis and manipulation of important agronomic traits has become a useful tool in

crop improvement. DNA markers have the potential to enhance the operation of a plant breeding program through fingerprinting of elite genetic stocks, assessment of genetic diversity, increase the efficiency of selection for biotic and abiotic traits, and make environmemt-neutral selection. Earlier, morphological markers have been used as a valuable source in varietal identification and for assessing genetic diversity, but they had certain limitations. Later, protein based markers were used widely. Iso-electric variants of proteins, referred to as isozymes, were found to be important markers for specific chromosome/chromosome regions. Many studies have been aimed at assessing the genetic diversity of different crops using allozyme markers (Morden *et al.,* 1989).

Molecular biology has ushered in a new era involving direct DNA assay and overcame many of the problems that have previously limited the applied use of biochemical markers. However, the ultimate differences between individuals lie in the nucleotide sequences of their DNA. Detection of such differences employing various molecular techniques has led to development of DNA-based molecular markers. Molecular markers follow simple Mendelian patterns of inheritance. They are stable and not influenced by developmental or environmental factors. DNA-based molecular markers are based on two techniques: 1) hybridization (Southern, 1975), and 2) the polymerase chain reaction**.** Restriction fragment length polymorphisms (RFLP) were the first DNA-

based molecular marker system (Wyman and White, 1980), and were developed by Botstein *et al.* (1980). Later, various types of molecular markers were developed to assess the genetic diversity in crop plants. Mohan *et al.* (1997) and Kumar (1999) have described in detail the types of molecular markers used in crop improvement. Different marker systems are given in Table (2.4).

Recently, microsatellite, or SSR loci or STRs (simple tandem repeats), which correspond to tandemly repeated DNA with a very short repeat unit, have been identified as powerful genetic markers in plants (Morganate and Oliveri, 1993; Powell *et al.,* 1996a). They are found both in prokaryotes and eukaryotes. They appear scattered randomly throughout the genome. Comparative studies in crop plants have shown that microsatellite markers are more variable than most other molecular markers (Powell *et al.,* 1996b; Taramino and Tingey, 1996; Pejic *et al.,* 1998), and provide a powerful methodology for discriminating between genotypes (Yang *et al.,* 1994; Russell *et al.,* 1997; Bredemeijer *et al.,* 1998). To decide which marker system is best for a given application, several key factors should be considered. The information of the most widely used marker types was summarized in Table (2.3). SSRs have been developed and used for genome mapping and DNA fingerprinting in different plant species such as maize (Senior and Heun, 1993; Taramino and Tingey, 1996), rice (Wu and Tanksley, 1993), wheat (Roder *et al.*, 1998), barley (Saghai Maroof *et al.*, 1994),

and sorghum (Dean *et al.,* 1999; Dje *et al.,* 2000; Grenier *et al.,* 2000; Smith *et al.,* 2000, Deu *et al.*, 2008). Sorghum linkage maps using RFLP markers have been constructed (Hulbert *et al.*, 1990; Lin *et al.*, 1995; Subudhi *et al.*, 2000; Ventelon *et al.*, 2001).

The first application of microsatellite markers in plants has been in cultivar identification, and now these are markers of choice in cultivar finger printing (Weising *et al.,* 1991; Beyermann *et al.,* 1992). The informativeness of a polymorphic marker depends upon the number of alleles and their relative frequencies. Botstein *et al.* (1980) described Polymorphism Information Content (PIC), which provides statistical assessment of informativeness of a marker. The greater the number of alleles at a given locus, the more informative will be the marker for the purpose of discriminating between genotypes. However, for some purposes such as genetic diversity assessment, markers that have a large number of relatively rare alleles can be problematic, and for such uses, marker loci having a small number of relatively common alleles may be easier to use.

Haley *et al.* (1994) demonstrated how marker information content (or polymorphism) is directly related to mean maximum test statistic in quantitative trait loci (QTL) analysis. Microsatellite information has been found to be useful in assessing the genetic relationships, both within and between populations (Peelman *et al.,* 1998; Dhillon *et al.*, 2006). Dhillon *et al.* (2006) reported that SSR

markers linked with QTLs can be used to characterize the homologous traits in different sorghum mapping populations.

2.5.2 Applications of molecular markers in sorghum crop improvement

Molecular markers have proven to be robust and cost-effective for assessment of sorghum genetic diversity (Deu *et al.,* 1994; Oliveira *et al.,* 1996; Yang *et al.,* 1996). Genetic diversity in sorghum has been estimated utilizing several types of molecular markers (Tao *et al.,* 1993; Vierling *et al.,* 1994; Brown *et al.,* 1996; Taramino *et al.,* 1997; Uptmoor *et al.,* 2003). A set of 15 microsatellite or SSR markers has been developed for sorghum that allows a high rate of discrimination in sorghum genetic diversity assessment (Dean *et al.,* 1999; Dje *et al.,* 2000; Grenier *et al.,* 2000; Smith *et al.,* 2000; Deu *et al.*, 2008). Ahnert *et al.* (1996) used a set of 104 RFLP probes to evaluate the genetic diversity among a large set of elite proprietary sorghum inbred lines. Studies have shown that SSR loci give good discrimination between closely related individuals in some cases even when only a few loci were employed (Powell *et al.,* 1996a; Scotti *et al.,* 2000; Kong *et al.,* 2000). Analysis of SSRs has been automated (Saghai Maroof *et al.,* 1984; Powell *et al.,* 1996b), and high level of genetic variation has been detected among sorghum accessions, which was high in *bicolor* and *guinea* races, and low in *kafir* race (Ejeta *et al.*, 2000).

Table 2.3 Major classes of markers

1. Morphological markers: Leaf hairs, trichomes, leaf glossiness, leaf color and shape, plant architecture, and seed or flower color are used as morphological markers to select for resistance to insect pests.	
2. Biochemical markers: Seed storage proteins, isozymes, amino acids, secondary metabolites such as terpenoids, alkaloids, and flavonoids are used as biochemical markers.	
3. DNA hybridization based methods	
Restriction fragment length polymorphism (RFLP)	Botstein *et al.* (1980)
4. PCR-based methods	
Amplified fragment length polymorphism (AFLP)	Vos *et al.* (1995)
Amplicon length polymorphism (ALP)	Ghareyazie *et al.* (1995)
Arbitrarily primed PCR (AP-PCR)	Welsh and McClelland (1990)
Allele-specific PCR (AS-PCR)	Sarkar *et al.* (1990)
Cleaved amplified polymorphic sequence (CAPS)	Lyamichev *et al.* (1993)
DNA amplification fingerprinting (DAF)	Caetano-Anolles *et al.* (1991)
Inter-SSR amplification (ISA)	Zietkiewicz *et al.* (1994)
Randam-amplified polymorphic DNA (RAPD)	Williams *et al.* (1990)
Specific amplicon polymorphism (SAP)	Williams *et al.* (1991)
Sequence characterized amplified region (SCAR)	Williams *et al.* (1991)
Single-strand conformation polymorphism (SSCP)	Orita *et al.* (1989)
Microsatellite simple sequence length polymorphism (SSLP)	Saghai *et al.* (1994)
Minisatellite simple sequence length polymorphism (SSLP)	Jarman and Wells (1989)
Simple sequence repeats (SSR)	Hearne *et al.* (1992)
Sequence tagged sites (STS)	Fukuoka *et al.* (1994)
Diversity array technology (DArT)	Emmamace *et al.* (2008)

Table 2.4 Comparision of different marker systems

	RFLP	RAPD	Microsatillites	SCARS/CAPS	AFLP
Principle	Endonuclease restriction, Sourthern blotting, and Hybridization.	DNA amplification with random primers.	PCR of simple sequence repeats.	Polymorphic PCR products or endonuclease restriction of PCR products.	Endonuclease restriction, followed by selective PCR amplification.
Technology					
Type of polymorphism	Single base changes: insertions/ deletions	Single base changes: insertions/ deletions	Changes in repeat length	Single base changes: insertions/ deletions	Single base changes insertions, deletions
Genomic abundance	High	Very high	Medium	High	Very high
Level of polymorphism	Medium	Medium	High	Medium	Very high
Dominance	Co-dominant	Dominant	Co-dominant	Co-dominant	Dominant/Co-dominant
Amount of DNA required	2-10 µg	10-25 µg	50-100 µg	50-100 µg	500 µg
Sequence information required	No	No	Yes	Yes	No
Radiaoactive detection required	Yes/no	No	No/yes	No	Yes
Gel systems	Agarose	Agarose	Acrylamide/agarose	Agarose	Acrylamide
Implementation					
Development costs	Medium	Low	High	Medium/high	Medium/high
Start up costs	Medium/high	Low	High	High	Medium/high
Portability-lab/crop	High/high	Medium/nil	High/low	High/low	High/nil
Suitable applications	Comparative mapping; Framework mapping.	Varietal hybrid identification; Marker-assisted selection.	Framework/region specific mapping. Fingerprinting; Marker assisted selection.	Framework mapping. Marker assisted selection; Can be converted to allele specific probes.	Finger printing; Very fast mapping; Region-specific marker saturation.

The genotype, BTx 623 has been used as a reference genotype for genotyping. It has been used as a source of DNA to construct enriched libraries, and the two sorghum BAC libraries that are currently available (Bhattramakki *et al.*, 2000). The probability of alleles at a locus depends upon the working group to which the accessions belong. Kong *et al.* (2000) reported 0.88 to 0.67 alleles per locus, while Bhattramakki *et al.* (2000) reported 3.88 alleles per locus in their respective working materials. In addition, the number of alleles per locus is positively correlated with the number of repeated units at the loci in BTx 623, the strain from which the SSRs were originally isolated, confirms the usefulness of SSR loci in marker-assisted selection in sorghum (Kong *et al.*, 2000).

Pereira *et al.* (1994) performed segregation analysis on F_2 population using 7 SSR loci to verify the reliability of SSR-derived polymorphism for sorghum genetic mapping. Brown *et al.* (1996) developed 15 SSR markers, and identified polymorphic loci among 17 temperately and tropically adopted lines of sorghum. Fifteen SSR marker loci have been found to be widely spread in sorghum genome, and 14 of them have been mapped to nine of the 10 sorghum linkage groups (Dean *et al.*, 1999), those were 3 to 11 alleles per locus in 95 `Orange` accessions in USDA germplasm collection. Taramino *et al.* (1997), Tao *et al.* (1998), and Kong *et al.* (2000) reported 46 polymorphic SSR loci in sorghum. Kong *et al.* (2000) characterized 38 sorghum SSR

loci, while Bhattramakki *et al.* (2000) reported primer sequences for 147 sorghum SSR loci, and genetic linkage map locations for 113. Schloss *et al.* (2002) reported 70 additional sorghum SSR primer sequences derived from sorghum cDNA clones that had previously been mapped as RFLP markers.

Ghebru *et al.* (2002) assessed genetic diversity of 28 Eritrean landraces of sorghum, using high throughput SSR-based strategy (Dean *et al.,* 1999; Smith *et al.,* 2000). Uptmoor *et al.* (2003) carried out comparative analysis of the genetic relatedness of 46 sorghum accessions from Southern Africa using AFLPs, RAPDs, and SSRs indicated that these sorghum accessions were uniquely fingerprinted by all three marker systems. Casa *et al.* (2005) assayed 98 SSR distributed throughout the genome in 104 accessions comprising of 73 landraces and 31 wild sorghums. The results indicated that landraces retained 86% of the diversity observed in the wild sorghums. Statistical methods (Ewens-Watterson test) for identifying genomic regions with patterns of variation consistent with selection gave significant results for 11 loci, out of which seven loci mapped in or near genomic regions associated with domestication-related QTLs.

Folkertsma *et al.* (2005) used 21 SSR markers to assess genetic diversity in the *Guinea*-race of sorghum to develop F_1 hybrid cultivars, while Dhillon *et al.* (2006) used SSR markers linked to QTLS associated with resistance to sorghum shoot fly, *A. soccata* and characterized the

genetic diversity of 12 cytoplasmic male-sterile (CMS) and maintainers, 12 restorers, and 144 F_1 hybrids. The genetic diversity was quite high among the shoot fly-susceptible parents and the hybrids based on them, but limited genetic diversity was observed among the shoot fly-resistant lines.

3. MATERIALS AND METHODS

3.1 Experimental material

The experimental material consisted of a diverse array of 15 sorghum genotypes comprising of seven germplasm lines (IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, and IS 2205) and three breeding lines (SFCR 125, SFCR 151, and ICSV 700) with low to moderate levels of resistance to sorghum shoot fly (Sharma *et al.*, 2005), and five commercial cultivars (Swarna, CK 60B, ICSV 745, 296B, and ICSV 112) susceptible to shoot fly (Table 3.1). The experiments were conducted under field and greenhouse conditions. Biochemical and molecular diversity was studied under laboratory conditions at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, during the 2004-05 and 2006-07 rainy and post rainy cropping seasons.

3.2 Characterization of components of resistance to shoot fly, *Atherigona soccata*

3.2.1 Assessment of different components of resistance to shoot fly under multi-choice conditions in the field

The test material was planted in the field during the 2004-05 rainy and post-rainy seasons. Each genotype was sown in two row plots, 2 m row length. The rows were spaced 75 cm apart. There were three replications in a randomized complete block design (RCBD). The field was irrigated immediately after sowing during the post-rainy season, while the soil

moisture was optimum for crop sowing during the rainy season. One week after seedling emergence, thinning was carried out to maintain a spacing of 10 cm between the plants. Shoot fly infestation was optimized through the use of interlard fish-meal technique (Plate 3.1) (Soto, 1974; Sharma *et al.*, 1992). The infester rows were sown 20 days earlier than the test material.

Table 3.1 Pedigrees of 15 test genotypes of sorghum (ICRISAT, Patancheru, India)

Genotypes	**Pedigree**
Shoot fly- Resistant	
IS 1054	Maldandi 35-1, PI 248264 (Landrace, India)
IS 1057	Bird resistant, PI 248267 (Landrace, India)
IS 2146	Kaura, PI 221569 (Landrace, Nigeria)
IS 18551	Jijwejere 935 (Landrace, Ethiopia)
IS 4664	Dagri dahere (Landrace, India)
IS 2312	Safra shahadasal Q2-2-88 (Landrace, Sudan)
IS 2205	Jaglur (Landrace, India)
SFCR 125	(ICSV 705 × YT-3-47)-7-1-1-2
SFCR 151	(1011 E No 23-2 (PM 12645 × IS 2205))-5-1-2-2
ICSV 700	(IS 1082 × SC 108-3)-1-1-1-1-1
Shoot fly- Susceptible	
Swarna	Selection from IS 3924
CK 60 B	Day milo × Black hull kafir
ICSV 745	((IS 3443 × DJ 6514)-1-1-1-1-1) × (E35-1× US/B 487)-2-1-4-1-1-3)-4-1-1-1
296 B	IS 3922 × Karad local
ICSV 112	[(IS 12622C × 555) × ((IS 3612C × 2219 B)-5-1 × E 35-1)]-5-2

Plate 3.1 Interlard fish-meal technique used to screen the test material for resistance to sorghum shoot fly, *Atherigona soccata* under field conditions.

Moist fishmeal (placed in polythene bags) was placed in interlards to attract shoot flies, and to have uniform distribution of pest in the test material. The experimental plot was given a basal dose of ammonium phosphate at 150 kg ha^{-1}. Interculture and earthing up operations were carried out at 15 and 30 days after seedlings emergence (DAE). Top dressing was done with urea @ 100 kg ha^{-1} before earthing up at 30 DAE.

Observations

Observations were recorded on oviposition, deadheart formation due to shoot fly damage, and tiller production following shoot fly damage. Data were also recorded on morphological traits such as leaf glossiness, seedling vigor, trichomes, days to 50% flowering, plant height, plumule and leaf sheath pigmentation, and grain yield.

Deadhearts. Number of plants with deadhearts caused by shoot fly was recorded from the two rows in a 2-row plot at 14, 21, and 28 DAE, and was expressed as percentages.

Deadheart incidence (%) = (number of plants with deadheart symptoms/total number of plants) × 100

Number of eggs. The shoot fly females lay white, elongated, cigar shaped eggs singly on the undersurface of the leaves, parallel to the midrib. Total numbers of eggs laid were recorded from the two rows in a 2-row plot at 14 and 21 DAE. The data were expressed as number of eggs per 10 plants.

Seedlings with eggs. Seedlings with eggs laid by shoot fly adults were recorded from the two rows in a 2-row plot at 14 and 21 DAE, and expressed as percentage of the total number of seedlings with eggs.

Seedlings with eggs (%) = (number of seedlings with eggs/ total number of plants) × 100

Tiller deadhearts. After shoot fly damage, the main plants produce side tillers in sorghum. Later, the side tillers are also attacked by shoot fly. Tillers with deadheart symptoms following shoot fly damage at 28 DAE were recorded from the two rows in a 2-row plot, and computed as percentage of the total number of tillers with deadhearts.

Tiller deadhearts (%) = (number of tillers with deadhearts/ total number of tillers) × 100

Days to 50% flowering. The interval from the sowing to 50% anthesis was recorded as days to 50% flowering, and expressed as number of days to 50% flowering.

Plant height. Plant height was recorded in cm (from the base to the tip of the plant) at maturity.

Productive tillers. The healthy plants were tagged one month after seedling emergence. At crop maturity, total number of tillers and the tillers having panicles with grain were recorded from the two rows in a 2-row plot, and expressed as percentage productive tillers.

3.2.2 Maintenance of shoot fly culture for studies on antixenosis and antibiosis components of resistance under greenhouse conditions

To assess antixenosis and antibiosis components of resistance under controlled conditions in the greenhouse, shoot fly females were collected in fishmeal-baited traps (Plate 3.2) from the sorghum crop at the seedling stage in the field (Taneja and Leuschner, 1985; Sharma *et al.*, 1992). The shoot flies were collected in the morning between 0730 to 0900 h in 200 ml plastic bottles through an aspirator, and released inside the wire-mesh screened cages (30 × 30 × 30 cm) in the greenhouse (28 ± 2 ^{0}C and 75 ± 5% RH). The *A. soccata* females were separated from other flies and released in a separate cage. The shoot fly females were provided with 20% sucrose solution in a cotton swab, and a mixture of brewer's yeast and glucose (1: 1) in a petri dish. The sucrose solution was changed daily, while the yeast powder – glucose mixture was changed once in 3 days. After three days of conditioning, the shoot flies were used for studies on antixenosis and antibiosis components of resistance to this insect.

3.2.3 Antixenosis for oviposition under dual- and no-choice conditions in the greenhouse

Antixenosis for oviposition was studied under dual-choice and no-choice conditions in a wire-mesh screened cage (Plate 3.3).

Plate 3.2 Fish-meal baited trap used to collect *Atherigona soccata* females for screening sorghums in the greenhouse.

Plate 3.3 Cage technique used to screen sorghum lines for resistance to sorghum shoot fly, *Atherigona soccata* under multi-, dual-, and no-choice conditions in the greenhouse.

The screening system consisted of two plastic trays (40 × 30 × 14 cm) one for planting the test material, while the other, fitted with wire-mesh screen on the sides and at the top (10 ×15 cm), was clamped onto the tray with sorghum seedlings (Sharma *et al.*, 1992, Dhillon *et al.*, 2005a). The wire-mesh screen on the top of the plastic tray had a 5 cm diameter hole, which was blocked with a 20 ml plastic cup. The test genotypes were planted in plastic trays having a potting mixture of black soil and farm-yard manure (3: 1). Diammonium phosphate (20 g per tray) was mixed with the soil before sowing. Each genotype had four rows, and there were 40 seedlings in each tray. For no-choice tests, only one genotype was planted in each tray. For dual-choice tests, there were two rows of the test genotype and two rows of the susceptible check, Swarna. There were six replications for dual-choice tests and three replications for no-choice tests in a completely randomized design (CRD). The test genotypes were exposed to shoot fly females (12 flies seedlings^{-40}) at 9-days after seedling emergence (fifth leaf stage) for 24 h. After 24 h, the shoot fly females were removed from the trays. Data were recorded on the number of eggs, and plants with eggs. Five days after infestation, data were recorded on the number of seedlings showing deadheart symptoms, and was expressed as percentage of plants with deadhearts.

3.2.4 Expression of antibiosis to sorghum shoot fly, *Atherigona soccata*

The test genotypes exposed to shoot flies under no-choice conditions were further used to study survival and development of shoot fly on different genotypes. The plants were tagged for appearance of deadhearts at 12 h intervals to compute the larval period. Four days after deadheart formation, 25 seedlings with deadhearts were taken from each replication and placed in 20 ml glass vials individually. Observations were recorded on different life cycle (Plate 3.4) parameters, such as larval and pupal periods, larval and pupal survival, pupal weight, adult emergence, sex ratio, and fecundity (number of eggs laid per female). There were three replications in CRD.

Larval period. The deadhearts placed in glass vials were observed daily after 6 days of deadheart formation to record time to pupation. The days from deadheart appearance to pupation plus one day (because it takes one day for deadheart realization after egg hatching) was recorded as larval duration. The larval period was recorded separately for each larva, and the mean larval period per replication was calculated for the surviving larvae (out of 25 larvae).

Larval survival. The number of larvae survived out of 25 larvae were recorded, and expressed as percentage larval survival. Percentage larval mortality was calculated as 100 - percent larval survival.

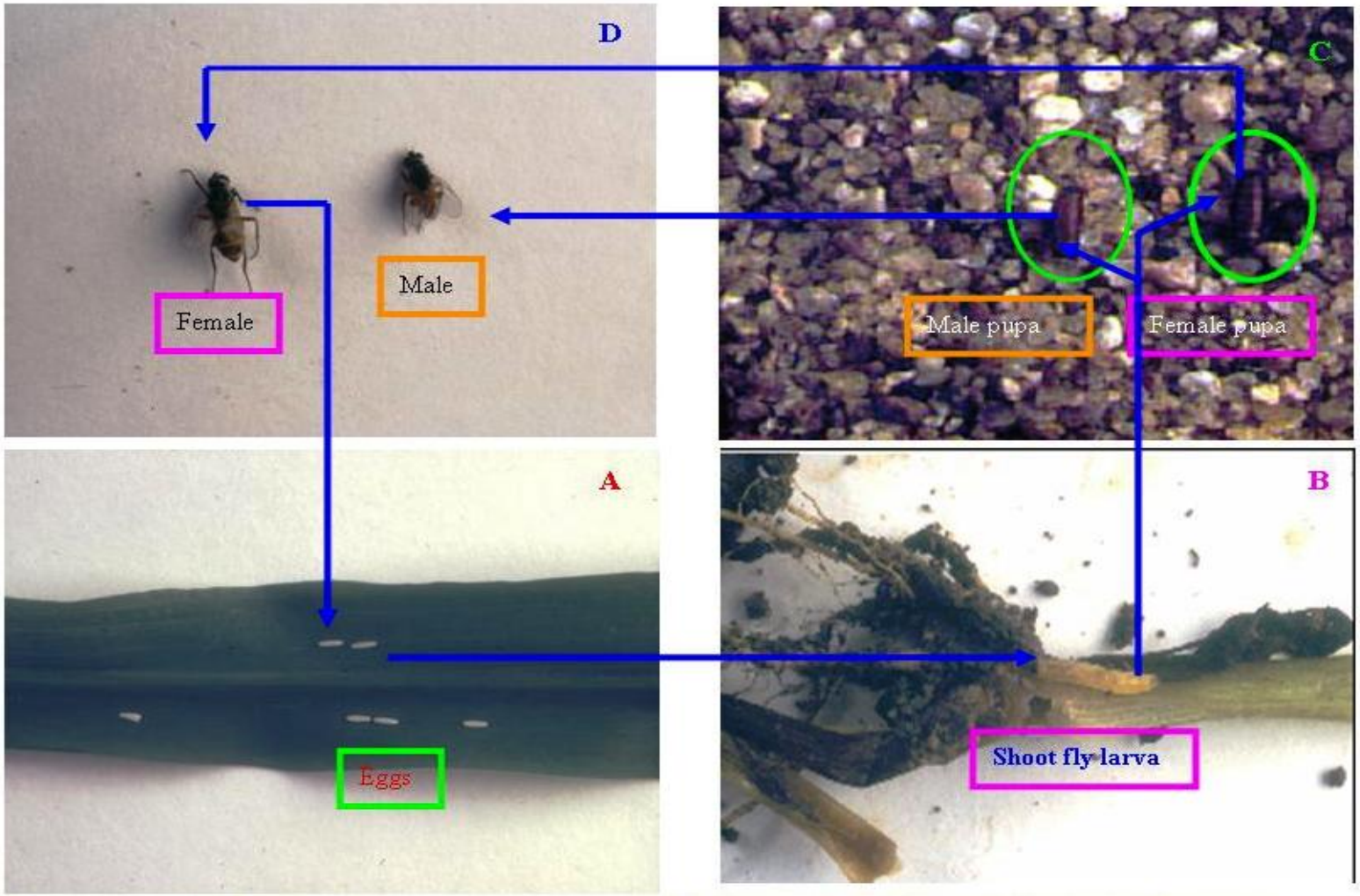


Plate 3.4 Various life stages of the sorghum shoot fly, *Atherigona soccata*. A = Eggs. B = Larva. C = Pupae. D = Adults.

Pupal period. Time taken from pupation to adult emergence was recorded as pupal period. The pupal period was recorded separately for each insect, and mean pupal period per replication was calculated for the surviving pupae.

Pupal weight. Pupal weight (mg) was measured for individual pupa on an electronic balance, within 24 h after pupation. The pupae were sorted into males and females, and their weights recorded separately in each replication. After weighing, the pupae were placed in respective vials on moist sand to avoid the water loss and pupal mortality because of desiccation.

Adult emergence and pupal mortality. The number of adults emerged from 25 insects were recorded, and expressed as percentage adult emergence. The mortality during the pupal stage was calculated by the following formula: Pupal mortality = (Number of dead pupae / Total number of pupae) x 100.

Sex ratio. The number of males and females emerged in each test genotype were recorded for computing the sex ratio (male: female).

Fecundity. Adults (five pairs in each of three replications) emerging from larvae reared on each genotype were released in a wire-framed cage with metal base (25 cm dia., 30 cm height) and covered with a white nylon-mesh (60 mesh) (Plate 3.5).

Plate 3.5 Wire-framed cages (25 cm dia, 30 cm height) covered with a nylon-mesh used for recording fecundity of *Atherigona soccata* females reared on different genotypes.

The adult flies were provided with 20% sucrose solution in a cotton swab, and brewer's yeast + glucose in the ratio of 1:1 in a Petridish. Ten sorghum seedlings (planted in pots of 10 cm dia.) of the same entry, on which the larvae were fed, were provided to the shoot flies as an oviposition substrate throughout its adult life. The seedlings were changed on alternate days, and data were recorded on number of eggs laid.

3.3 Evaluation of sorghum genotypes for physico-chemical traits

Data on seedling vigor, leaf glossiness, trichomes, and pigmentation was recorded on sorghum genotypes grown under field conditions, while the data on leaf surface wetness, retention of water droplets on the central leaf surface, and moisture content were recorded in seedlings grown under greenhouse conditions.

3.3.1 Seedling vigor. The seedling vigor (in terms of plant height, leaf expansion, plant growth, robustness and adaptation) was recorded at 10 DAE on a 1 to 5 rating scale.

1= highly vigorous (plants showing maximum height, more number of fully expanded leaves, good adaptation, and robust seedlings).

2 = vigorous (good plant height, good number of fully expanded leaves, and good adaptation and seedling growth).

3 = moderately vigorous (moderate plant height with moderate number of fully expanded leaves, and fairly good seedling growth).

4 = less vigorous (less plant height with poor leaf expansion, and poor adaptation).

5 = poor seedling vigor (plants showing poor growth, and weak seedlings).

3.3.2 Leaf glossiness. Leaf glossiness (plants with pale green, shiny, narrow and erect leaves) was evaluated on a 1 to 5 rating at 10 DAE in the early morning hours when there was maximum reflection of light from the leaf surfaces (Plate 3.6).

1= highly glossy (light green, shining, narrow and erect leaves).

2 = glossy (light green, less shining, narrow and erect leaves).

3 = moderate glossy (fair green, light shining, medium leaf width, and less drooping leaves).

4 = moderate non-glossy (green, pseudo-shine, broad, and drooping leaves).

5= non-glossy (dark green, dull, broad, and drooping leaves).

3.3.3 Trichome density. The presence and density of trichomes (Plate 3.7) was measured on the central portion of the 5th leaf (from the base) taken from three seedlings at random. For this purpose, leaf pieces (2 cm^2) taken from the central portion of the leaf were placed in acetic acid and alcohol (2: 1) in stoppered glass vials (10 ml capacity) for 24 h to clear the chlorophyll, and subsequently transferred into lactic acid (90%) as a preservative (Maiti and Bidinger, 1979). The leaf sections were

mounted on a glass slide in a drop of lactic acid, and magnified at 10_X under a stereo-microscope. The trichomes on leaf surfaces, both abaxial and adaxial surfaces, were expressed as number of trichomes/10_X microscopic field.

3.3.4 Pigmentation. Pink pigment on plumule and leaf sheath (Plate 3.8) was assessed at 5 DAE on a 1 - 5 rating scale (Dhillon *et al.*, 2005b).

1 = plumule or leaf sheath with dark pink pigment.

2 = plumule or leaf sheath with fair pink pigment.

3 = plumule or leaf sheath with light pink pigment.

4 = plumule or leaf sheath with very light pink pigment.

5 = plumule or leaf sheath with green color.

Plate 3.6 Glossy leaf trait associated with resistance to sorghum shoot fly, *Atherigona soccata*.

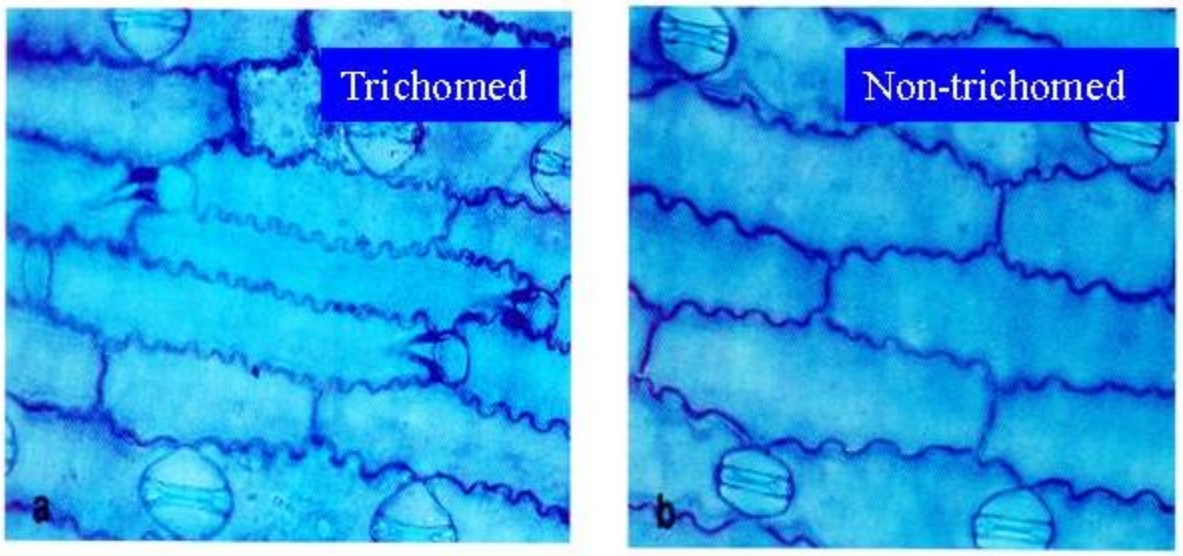


Plate 3.7 Trichomes on the leaf surface. a = Trichomed. b = Non-trichomed.

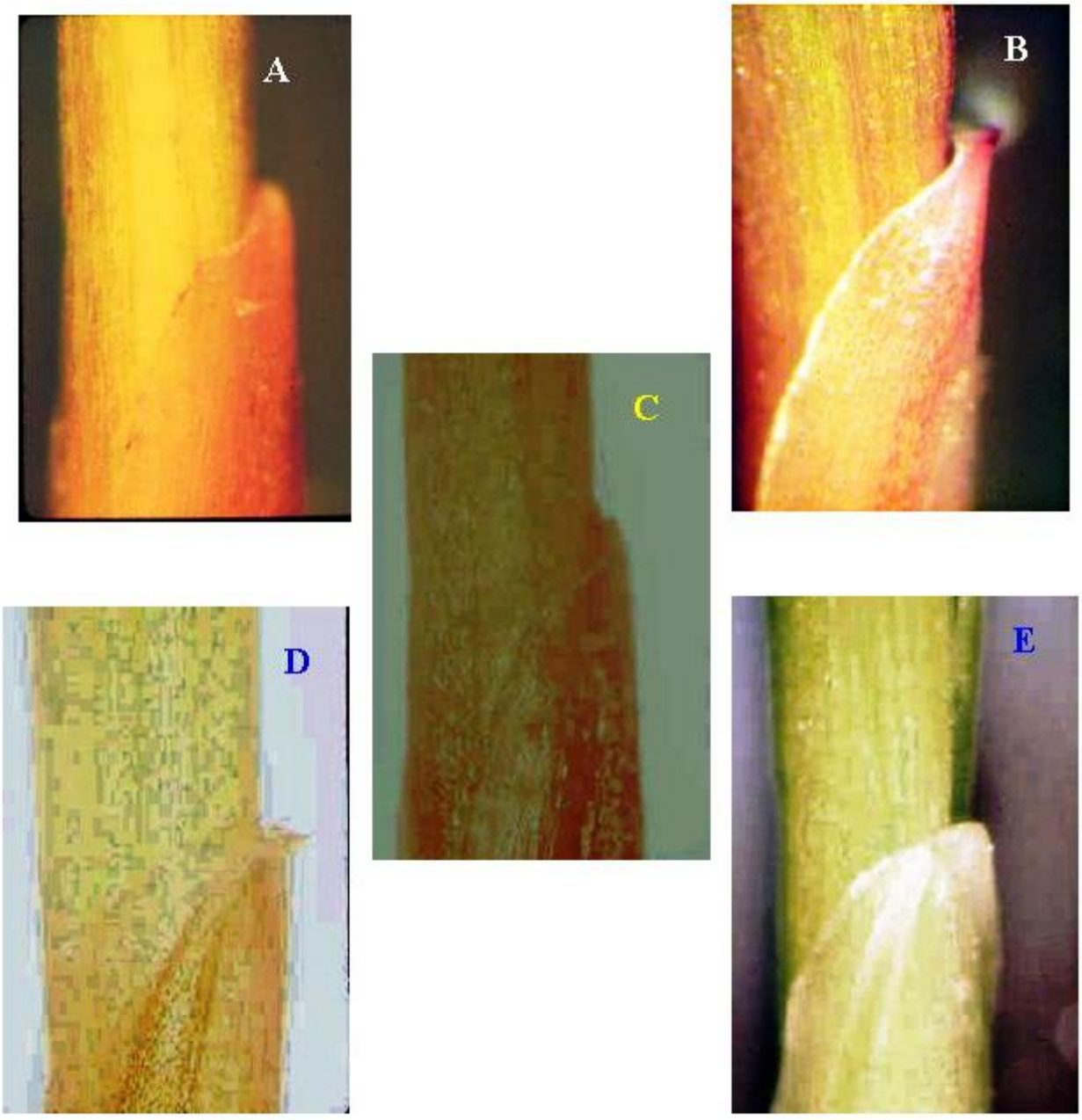


Plate 3.8 Plumule and leaf sheath purple pigmentation scores of sorghum genotypes at 5 days after seedling emergence. A = 1 (dark pink). B = 2 (fair pink). C = 3 (light pink). D = 4 (very light pink). E = 5 (green).

3.3.5 Leaf surface wetness. The test genotypes were planted in small cups (10 cm dia.) with 5 seedlings per cup in the open in greenhouse area. The observations on leaf surface wetness were recorded between 4:30 to 6:30 A.M. The seedlings at the 5th leaf stage (12 DAE) were brought to the laboratory, and the central whorl leaf was opened and mounted on a slide with a sticky tape. Water droplets on the leaf surface were observed under the microscope (10 x magnifications). Leaf surface wetness was rated on a 1 to 5 scale.

1 = leaf blade without water droplets.

2 = leaf blade with sparsely placed few water droplets.

3 = leaf blade near mid rib covered with water droplets.

4 = water droplets spread all over the leaf blade.

5= entire leaf blade densely covered with water droplets.

3.3.6 Moisture content. The test genotypes were planted in plastic trays in the greenhouse as described earlier. There were three replications for each genotype. At the 5th leaf stage (12 DAE), the seedlings were brought to the laboratory. Thirty seedlings were cut at the base and immediately weighed to record fresh weight of the seedlings. The seedlings were then kept in an oven at 50 ^{0}C for three days. Weight of the oven dried material was recorded after three days. The moisture percentage was calculated as follows.

Moister content (%) = [(fresh weight – dry weight) / (fresh weight)] × 100.

3.3.7 Retention of water on leaf surface. Water droplets retained on the leaf surface were recorded on the test genotypes grown in plastic trays outside the greenhouse. There were three replications for each genotype. The seedlings were exposed to fog during the pre-dawn morning hours and rated on a 1 -5 scale (1 = highly wet; and 5 = low /or no wetness) (Plate 3.9a, b). The seedlings were also exposed to rain water and retention of water droplets was rated on a 1 – 9 scale (1 = no water droplets retained on the leaf surface, but water stored in the whorl; and 9 = water droplets spread allover the leaf surface) (Plate 3.10a, b).

3.4 Inducible resistance to shoot fly, *Atherigona soccata* in sorghum

3.4.1 Effect of transplanting and clipping on shoot fly damage under field conditions

The test material was planted in the field as described above during the 2006-2007 rainy seasons. Fifteen genotypes were planted in three replications in a split plot design. The treatments consisted of transplanting and clipping (Plate 3.11), and an untreated control. Transplanting and clipping was carried out by uprooting 10 day-old seedlings, clipping the leaves above the leaf whorl, and transplanting them in the soil with enough moisture. While the untreated control plots were left undisturbed. Data on number of eggs seedling^{-1} and number of seedlings with eggs were recorded at 14 and 21 DAE. The percentage of plants with deadhearts was recorded at 14, 21, and 28 DAE. Recovery

resistance was assessed in terms of percentage tillers with deadhearts at 28 DAE.

3.4.2 Role of secondary metabolites on leaves of sorghum seedlings on host plant preference and damage by shoot fly, *Atherigona soccata*

The role of secondary metabolites in resistance to shoot fly was studied in a resistant (IS 18551) and susceptible (Swarna) genotypes during the 2006-2007 post rainy and rainy seasons. There were three treatments involving *p*-hydroxybenzaldehyde, *p*-hydroxy benzoic acid, and an untreated control. There were four concentrations (0.01%, 0.05%, 0.1%, 0.5%, and 0.5%, 1%, 2% and 5%, respectively) during the 2006 post rainy season. The phenolic acids were sprayed on sorghum seedlings in middle two rows of the four row plot at 7 DAE. There were three replications, in a split-split plot design for each treatment.

Observations

Data was recorded on oviposition, main plant deadhearts, and tiller deadhearts at 14, 21, and 28 DAE. Data was also recorded on days to 50 % flowering, plant height, and other agronomic traits.

Plate 3.9 Retention of fog water on leaf surface of the sorghum seedlings

a. Large sized globular droplets formed on the leaf surface of the non-glossy (shoot fly susceptible genotype of sorghum)

b. Smaller sized water droplets formed on the leaf surface of glossy (Shoot fly- resistant) genotype of sorghum.

Plate 3.10 Retention of rain water droplets on the leaf surface of the sorghum seedlings

a. No water droplets retained on the leaf surface (due to large size) and water stored in the whorl of non-glossy sorghum genotype.

b. Water droplets on the leaf surface of glossy genotype of sorghum.

Plate 3.11 Transplanted and clipped seedlings of the sorghum to induce the resistance to shoot fly, *Atherigona soccata* under field conditions.

3.4.3 Effect of KI, Cu_2So_4 and 2, 4-D on shoot fly damage

The effect of KI, Cu_2So_4 and 2, 4-D on shoot fly damage was studied on a resistant (IS 18551) and susceptible (Swarna) genotypes in a split-split plot design during the 2006-07 post rainy and rainy seasons, respectively. Each plot had four row plots 2 m long. The rows were 75 cm apart. There were three replications for each treatment, and the experiment was laid in split-split plot design. The test concentrations were 0.0, 0.05% and 0.1% for KI, Cu_2So_4, and 2, 4-D during the 2006 post rainy season, and 0.0, 0.01 %, 0.02 %, 0.05 %, and 0.1 % during the 2007rainy season. The test chemicals at the respective concentrations were sprayed on sorghum seedlings in middle two rows at 7 DAE.

Observations

Data was recorded on oviposition, and main plant and tiller deadhearts at 14, 21, and 28 DAE. Data were also recorded on days to 50 % flowering, and plant height and agronomic desirability.

3.5 Biochemical composition of sorghum seedlings in relation to resistance/susceptibility to shoot fly, *Atherigona socata*

3.5.1 Total soluble sugars

The 5^{th} leaf stage sorghum seedlings were collected from the field, and immediately lyophilized at -45 ^{0}C by using lyophylizer (Thermo savant) for three days. The lyophilized seedlings were powdered in a Willey mill using a 0.5 µ pore size blade to obtain a fine powder. Total soluble sugars

were extracted with hot aqueous-ethyl alcohol. On treatment with phenol sulphuric acid, the sugars produced golden yellow color (Dubois *et al.*, 1956). The absorbance of the golden yellow color was measured at 490 nm, which was used to estimate the percentage of total sugars present in the seedlings of sorghum. 80 % ethyl alcohol, 5 % phenol, 96 % sulphuric acid (specific gravity 1.84), glucose standard (stock solution: 1000 mg/1000ml), and glucose working standard (10 ml of stock standard pipetted in to 25 ml volumetric flask and volume made up to 100 ml, to have a final concentration of 100 µg/ml) were used for estimating total soluble sugars.

Sorghum seedlings leaf powder (100 mg) was weighed into a boiling test tube to which 25 ml of 80% hot ethanol was added. The mixture was shaken vigorously on a Vortex mixer. The material was allowed to settle for 30 minutes and the supernatant was filtered through Whatman No. 41 filter paper. This step was repeated thrice to complete extraction of sugars. Ethanol was completely evaporated by placing the extract on hot sand bath. After removal of ethanol, 3 ml of water was added to dissolve the contents. One ml of above solution was pipetted into a test tube, to which 1 ml of 5% phenol and 5 ml of 96% sulphuric acid were added. The mixture was shaken vigorously on a Vortex mixer. The tubes were allowed to cool in cold water. A blank was prepared by taking 1 ml of distilled water. Absorbance of the golden yellow color was read at 490 nm using Spectronic 21. Standards with concentrations of 10, 20, 40, 60,

80, and 100 μg of glucose were prepared from the working standard by taking 1 ml aliquots.

Total soluble sugars content was calculated as:

(Concentration of standard / Absorbance of standard) x Absorbance of 1 ml extract x (1 / 1,000,000) x (volume made up / sample weight) x 100

3.5.2 Total polyphenols

Total polyphenols were estimated by Folin Denis method (AOAC, 1984). Folin Denis reagent (100 gm of sodium tungstate (Na_2 Wo_4 $2H_2O$), 20 g phosphomolybdic acid, and 50 ml phosphoric acid) were dissolved in 750 ml of water. The mixture was refluxed for 2 h, and the final volume was made to 1 L by adding water. Saturated sodium carbonate solution (45 g anhydrous sodium carbonate) was dissolved in 100 ml of water at 70 – 80 ^{0}C, and allowed to cool overnight. The solution was supersaturated with Na_2CO_3 crystals filtered through glass wool after crystallization. Tannic acid standard solution was prepared by dissolving 100 mg tannic acid in 1 L water. Fresh solution was prepared for each determination. Methanol-HCL, 10 ml concentrated hydrochloric acid was added to methyl alcohol, and the final volume was made to 1 L and was used for estimating the phenols. For phenol estimation, 100 ml of methanol-HCL was added to 200 mg of sorghum seedling leaf powder in a round bottomed flask. This mixture was kept overnight for incubation at room temperature. After incubation, the extract was filtered through Whatman

No. 40 filter paper into 100 ml volumetric flask, and the volume was made to 100 ml with methanol-HCL by a few washings.

For estimation of polyphenols, 0.2 ml extract, 0.5 ml of Folin Denis reagent and 1 ml of saturated sodium carbonate solution were added in a test tube and the final volume was made to 10 ml with distilled water and Vortexed. After vortexing, the absorbance was read at 760 nm using Spectronic 21. A standard curve was prepared by pipetting 0 to 1 ml aliquots of standard tannic acid solution at intervals of 0.2 ml, and expressed as tannic acid equivalent mg /g sample.

3.5.3 Estimation of tannins

Tannins were estimated by Vanillin-Hydrochloric acid method (Price *et al.*, 1978). Vanillin-hydrochloric acid reagent was prepared by adding 4% hydrochloric acid in methanol (v/v), and 1% hydrochloric acid in methanol (v/v). A stock solution was prepared by dissolving 1 mg of catechin in 1 ml of methanol. Sorghum leaf powder (100 mg) was transferred to a centrifuge tube containing 2 ml of 1% acidic-methanol, centrifuged for 10 min, and the aliquot transferred to a 5 ml volumetric flask. This step was repeated by adding 1 ml of (1%) acidic-methanol. The aliquot was transferred to the first extraction and made the final volume to 4 ml with acidic-methanol (1%). Five ml of freshly prepared vanillin-HCL reagent was added slowly into the test tube containing 1 ml of extract. An individual blank was prepared for each extract by adding 5 ml of 4% HCL in methanol to 1 ml aliquot. Finally the absorbance was

recorded at 500 nm against the reagent blank in a spectrophotometer. Standard curve was prepared by plotting the average absorbance readings of the duplicate determinations of catechin concentrations. The catechin equivalents (CE) were calculated as:

CE (%) = (catechin mg ml $^{-1}$ / vol. of extract taken) x (volume made up / wt. of sample) x 100

3.5.4 Estimation of fat content

Fat content in the sorghum seedlings was estimated by Soxhlet extraction procedure (AOCS, 1981). The ground 3 g sample was placed in folded Whatman no. 2 filter paper, and placed in a Soxhlet extraction tube filled with 3/4 volume of hexane, and continued the extraction overnight. The contents from the extraction flask were transferred into a clean pre-weighed 250 ml beaker containing boiling beads, with three washings of hexane. Evaporated the hexane on the hot sand bath and then cooled in a desiccator to weigh the contents as oil (%) = (A - B) / C × 100, where A = weight of the beaker + oil; B = weight of the beaker; C = weight of the sample

3.5.5 Lignin estimation

Lignin was estimated following the procedure of Van Soest (1985). One gram seedling sample was transferred to the fiber estimation beaker containing 100 ml CTAB reagent and a few boiling chips. The mixture was pre-heated for 1 h on the plate of Labcano digestion apparatus, and rotated periodically to prevent the solids from adhering to sides of the

beaker. The extract was filtered through a pre-weighed (W1) sintered crucible using a vacuum pump, followed by washing the residue with hot water until the washings were free from acid, and finally washed the residue with acetone (25 ml). The crucible containing the extract was kept in an oven at 100 ^{0}C for 2 h and transfered the contents into a desiccator, cooled it to room temperature, and weighed (W2) immediately to prevent moisture absorption. A blank was run simultaneously.

Weight of the lignin = (W2 - W1) – blank

Lignin (%) = (Weight of acid detergent fiber / Weight of the sample taken) × 100.

Covered the contents of the crucible with cooled (15^o C) 72% H_2SO_4 and stirred with a glass rod to a smooth paste, and break all lumps, and kept the crucible at 20 – 23 ^{0}C. Filled the crucible about half full with acid and stirred. Refilled the crucible with 72% H_2SO_4 and stirred at hourly intervals as acid drained away. The step was repeated thrice. After 3 h filtered off as much acid as possible using vacuum. Washed the contents with hot water until free from acid (test with litmus paper), rinsed and removed stirring rod. The crucibles were dried at 105 ^{0}C, followed by cooling in desiccators and weighed (W4). Subsequently, the crucibles were ignited in a muffle furnace for 30 min at 600 ^{0}C, cooled them in desiccators, and weighed (W5). Recorded ADF-Ash as the difference between (W5) and the original weight (W2).

3.5.6 Determination of micronutrients

Estimation of N, P, K, and protein, was done from the sorghum seedling samples. The samples were lyophilized and powdered, and then oven dried at 60 ^{o}C for 48 h before use in estimations. The oven-dried sample (0.5 g) was transferred into 250 ml digestion tubes containing a mixture of 14 ml 0.5% concentrated H_2So_4 and Se (5 g) powder. The H_2So_4 and Se mixture was prepared by dissolving Se powder in concentrated H_2So_4 by heating on a hot plate with occasional stirring. The mixture was cooled and the volume made to one liter, and pre-heated to 37 ^{o}C for 2.5 h till the digestion became colorless. All the samples were analyzed twice. The digests were made to 250 ml with distilled water and suitable aliquots of digests were used to determine N by distillation with NaOH, P by phosphor-vanadomolybdeate colorimetric procedure, K using atomic absorption spectrophotometer (Jones *et al.*, 1991), and protein was calculated by multiplying the nitrogen content with factor 6.24.

Triacid digestion micronutrients such as Calcium (Ca), Magnesium (Mg), Iron (Fe), Zinc (Zn), Manganese (Mn), and Copper (Cu) were estimated by triacid digestion following the methodology given by Sahrawat *et al.* (2002). The seedling samples (0.5 g) were transferred into 125 ml conical digestion flasks containing 12 ml of triacid mixture of nitric acid, sulfuric acid, and perchloric acid [9: 2: 1 (v/v)] and digested in cold for 3 h, followed by another digestion for 2 - 3 h on a hot plate, until the digest became colorless. The flasks were allowed to cool and the

contents were diluted to an appropriate volume, and Ca, Mg, Mn, Fe, and Cu in the digests were determined by using atomic absorption spectrophotometry. All the samples were analysed twice.

3.6 HPLC analysis of sorghum phenols

Fifteen sorghum genotypes were grown in the greenhouse and infested with shoot fly adults using the cage screening technique as described above. Three days after infestation, the plants with deadhearts (leaf whorl portion) were collected, and the larvae were removed from the deadhearts. Central whorl leaves from un-infested plants were collected as controls. The samples were freeze dried in a lyophylizer at – 45 ^{0}C for 3 days. After freeze drying, the samples were ground in a mortar and pestle.

3.6.1 Extraction of phenols

Phenols or phenolic acids of test sorghum genotypes were extracted and analyzed by following the method described by Hahn *et al.* (1983), with a few modifications. Lyophilized sorghum leaf powder (100 mg) was extracted in 5 ml of 100% methanol by sonication for 30 minutes and centrifuged at 5000 rpm for 10 minutes. Supernatant was collected and partitioned with 5 ml of hexane in a separation funnel until the two phages separated clearly, and the process was repeated three times. Methanol extracts were reduced near to dryness in a vacuum rotavapor, and redissolved in 3 ml of HPLC grade methanol. The samples were filtered through 0.45 µm pore size Millipore filter. Available phenolic acid

standards such as gallic, protocatechuic, *p*-hydroxy benzoic, *p*-hydroxy benzaldehyde, vanillic, caffeic, *p*-coumaric, ferulic, and cinnamic acids were prepared at 100 ppm concentrations and filtered before analysis.

3.6.2 Separation procedure

The samples and standards (20 µl) were chromatographed singly and in mixtures directly on to a Waters Sunfire C_{18} column (4.6 × 250 mm) with 5 µm pore size. A Waters High Performance Liquid Chromatography (HPLC) 2695 separations module (alliance) system consisting of a PCM 11 reciprocating piston pump and a 2996 photodiode array detector in the range of 190 to 800 nm was used in a gradient elution mode. Multistep gradient solvent system of 2% acetic acid (A) and 2% acetic acid-acetonitril (B) was used for separation. The separation was programmed as follows:

Time (min)	A (%)	B (%)
0	95	5
10.00	95	5
17.50	85	15
31.00	85	15
41.00	50	50
45.00	50	50
50.00	85	15
55.00	95	5

Flow rate was 1 ml min^{-1}. The solvents were run at 6 curve (linear). The spectrum detection was made at 254 nm. The chromatographic data were recorded and processed by the Millennium^{32} software version 4.0. Phenols were identified and quantified by comparing the peak area obtained on similar retention time of the standard peak area with known concentrations.

3.7 HPLC finger printings of proteins from germinated seeds of sorghum

The seeds of 15 test sorghum genotypes were treated with fungicide thiram (@ 2 g a.i. /kg) uniformly by gently shaking them in a Petri plate to avoid the fungal contamination. The treated seeds were placed on three layers of moistened blotting paper in airtight plastic Petri plates. The plates were incubated at 20 ^{0}C for 7 days at 12 h light and darkness. After incubation, the germinated seeds were grounded in a motor and pestle to a fine seed powder for protein extraction.

To extract the proteins from the germinated seeds, 100 mg of defatted sample was weighed and mixed with 1: 16 ratio of 1% PVP solution in a micro centrifuge tube, Vortexed and incubated them at 4 ^{0}C for three hours. After incubation, centrifuged the contents at 10,000 rpm for 10 min and collected the supernatant. The supernatant was treated with SDS and β-mercapto-ethanol to denature the proteins at final concentrations of 10 mM and 15 mM, respectively, and boiled the samples for 5 seconds at 90 ^{0}C. Filtered the samples through 0.45 μm

pore size PVDF (polyvinylidene difluoride) memebrane filter, and refiltered through 0.2 μm pore size PVDF membrane filter.

The samples (20 μl) were chromatographed singly on a Waters Symmetry C_{18} column (4.6 × 250 mm) with 5 μm pore size. A Waters HPLC 2695 separations module (alliance) system consisting of a PCM 11 reciprocating piston pump and a 2996 photodiode array detector in the range of 190 to 800 nm was used in a gradient elution mode. Multistep gradient solvent system of the following solvents (A- 0.1% TFA in water, and B- 0.1% TFA in acetonitrile) was used to separate the proteins.

Time (min)	A (%)	B (%)	Curve
0.00	100	0.00	-
25.00	44.0	56.0	8
35.00	40.0	60.0	9
45.00	100	0.00	6
50.00	0.00	100	6
55.00	100	0.00	6

The flow rate was 1 ml min^{-1}. The spectrum detection was made at 215 nm. The chromatographic data were recorded and processed by the Millennium^{32} Software Version 4.0.

3.8 GC-MS analysis of the compounds on leaf surface of sorghum seedlings

To collect samples for GC-MS analysis of the compounds on leaf surface of sorghum seedlings, the sorghum seeds were sown in the greenhouse as described above. Ten days after seedling emergence, 3rd leaf from the seedlings was collected in a 25 ml centrifuge tube containing 10 ml HPLC grade hexane. After 1 min, the leaves were removed from the centrifuge tubes, and the leaf extract thus obtained was used for GC-MS analysis.

3.8.1 Analysis of volatiles by GC-MS. Samples of compounds extracted in hexane from the leaves of different sorghum genotypes were concentrated to 0.5 ml under a stream of nitrogen, and analyzed by GC-MS model (Agilent Technologies 6890 NGC) with 5973 inert mass selective detector. One µl of the sample was injected through the autosampler to the HP-5MS capillary column (30 m length ×0.25 mm i.d × 0.25 µm film thicknesses). The oven program was 50 ^{0}C (2 min) – 10 ^{0}C /min 280 ^{0} C (5 min) - (total run time 30 min). Injection temperature was 250 ^{0}C, and GC-MS interface temperature was 280 ^{0}C. Solvent delayed for 3 minutes. MS scan range was 30-600 Da. Compounds were identified by comparing their spectral data with those of the library of the mass spectrometer.

3.9 Genetic diversity of sorghum genotypes using SSR markers

Fifteen sorghum genotypes were grown in the greenhouse in plastic pots (20 cm dia.) as mentioned above. One- week- old seedlings were used for DNA extraction.

3.9.1 Genomic DNA isolation

Genomic DNA was isolated by CTAB method (Mace *et al.*, 2003). The method involved:

1. Lysis of the cell membrane,

2. Extraction of the genomic DNA, and

3. Precipitation of DNA

A. Preparation and processing

1. Steel balls (4-mm in size, two balls per extraction tube) pre-chilled at – 20 °C for about 30 minutes were added to the 12 × 8 well extraction tubes with strip caps, kept on ice.

2. 3% CTAB buffer (3% w/v CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl- pH 8.0, 0.17% β-mercaptoethanol) was pre-heated at 65°C on a water bath before start of the DNA extraction.

3. Fifteen cm long leaf strips were collected (final weight approximately 30 mg) from one-week-old seedlings, and cut into pieces (1 mm in length). These pieces were transferred to the extraction tubes.

B. Grinding and extraction

1. 450 µl of pre-heated 3% CTAB buffer was added to each extraction tube containing leaf sample.

2. Grinding was carried out using Sigma Geno/Grinder at 500 strokes/minute for 2 min.

3. Grinding was repeated until the color of solution became pale-green and leaf strips were sufficiently macerated.

4. After grinding, the box with the tubes was fixed in a locking device and incubated at 65 ^{0}C in a water bath for 10 min, with occasional manual shaking.

C. Solvent extraction

1. 450 µl of chloroform: iso-amylalcohol (24:1) mixture was added to each tube, tubes were inverted twice and the samples were centrifuged at 6,200 rpm for 10 minutes.

2. After centrifugation, the aqueous layer (approximately 300 µl) was transferred to a fresh tube.

D. Initial DNA precipitation

1. To each tube containing the aqueous layer, 0.7 volumes (approximately 210 µl) of cold (kept at –20 ^{0}C) isopropanol was added, then the solution was carefully mixed and the tubes were kept at –20 ^{0}C for 10 minutes.

2. The samples were centrifuged at 6,200 rpm for 15 minutes.

3. The supernatant was decanted under a fume-hood, and the pellets were allowed to air dry (approximately 30 min).

E. RNase treatment

1. To remove co-isolated RNA, 200 µl of low salt TE buffer and 30 µg of RNase (stock 10 mg/µl) were added to each tube containing the dry pellet and mixed properly.

2. The solution was incubated at 37 ^{0}C for 30 minutes or overnight at room temperature.

F. Solvent extraction

1. After incubation, 200 µl of phenol: chloroform: isoamylalcohol (25:24:1) mixture was added to each tube, mixed carefully and centrifuged at 5,000 rpm for 10 minutes.

2. The aqueous layer in each tube was transferred to a fresh tube and the step was repeated with the chloroform: isoamylalcohol (24:1) mixture. The aqueous layer was transferred to a fresh tube.

G. DNA precipitation

1. 15 µl (approximately 1/10th volume) 3 M sodium acetate (pH 5.2) and 300 µl (2 vol) 100% ethanol (kept at -20 ^{0}C) were added to each of the tubes, and the mixture was incubated in a freezer (-20 ^{0}C) for 5 minutes.

2. Following incubation, the tubes were centrifuged at 6,200 rpm for 15 minutes.

H. Ethanol wash

After centrifugation, the supernatant was carefully decanted and 200 µl of 70% ethanol was added to the tubes followed by centrifugation at 5,000 rpm for 5 min.

I. Final re-suspension

1. The supernatant was carefully decanted and the pellet was allowed to air dry for one hour.

2. Dried pellets were re-suspended in 100 µl of $T_{10}E_1$ buffer and kept overnight at room temperature to dissolve completely.

3. Dissolved DNA samples were kept in 4 ^{0}C.

3.9.2 Determination of quantity and quality of isolated genomic DNA

3.9.2.1 Ethidium bromide agarose gel electrophoresis

The DNA can be quantified in an agarose gel by comparing the intensity of the fluorescence emitted by an EtBr- stained DNA sample, relative to a dilution series of a DNA standard of known concentration. The DNA quality was checked using 0.8% agarose gel. One µl of DNA solution was mixed with 1 µl of orange dye and 4 µl of distilled water and loaded into wells on 0.8% agarose gel. The gel was run for 10 min, after which, the quality was checked under UV illumination. A smear of DNA indicated poor quality whereas a clear band indicated good quality. Samples of poor quality were re-extracted.

3.9.2.2 Flourimetry

The quantity of DNA was assessed using fluorescence spectrophotometer (Spectrafluor Plus, Tecan) by staining DNA with Picogreen™ (1/200 dilution). Based on relative fluorescence units (RFU) values, and using the standard graph, DNA concentrations were calculated (DNA concentration = -2.78273 + 0.002019* RFU).

3.9.2.3 Normalization of the DNA

The normalization of DNA was done robotically by using Tecan liquid Handling Robotic system, and the final concentration of DNA was adjusted to 2.5ng/µl, which was used for PCR reactions.

3.9.3 Selection of SSR markers

A total of 93 SSR primer pairs were tested, of which 79 SSR markers were found to be sufficiently polymorphic for assessment of genetic diversity and molecular characterization of 15 test sorghum genotypes. The primer details are given in Appendix 3.1

3.9.3.1 PCR amplification of SSR markers

PCR reactions were conducted in 384 well plates in a PE 9700 Perkin Elmer (Norwalk Conn.) DNA thermocycler. The reactions were performed in volumes of 5 µl using four different PCR protocols as shown in the Appendix 3.2. A touch down PCR program was used to amplify the DNA fragments. Reaction conditions were as follows: Initial denaturation for 15 min at 94 ^{0}C (to minimize primer-dimer formation and to activate the

Taq polymerase), subsequently 10 cycles of denaturation for 10 sec at 94 ^{0}C, annealing at 61-52 ^{0}C for 20 sec, the annealing temperature for each cycle was reduced with 1 ^{0}C, and extension at 72 ^{0}C for 30 sec and 35 cycles of denaturation for 10 sec at 94 ^{0}C, annealing at 54 ^{0}C for 20 sec and extension at 72 ^{0}C for 30 sec. The last PCR cycle was followed by a 20 min extension at 72 ^{0}C to ensure amplification to equal length of both DNA strands.

In case the observed polymorphism between the genotypes was less than 5 bp, then the PCR products were separated by capillary electrophoresis using ABI Prism 3700 (Perkin Elmer) DNA Sequencer. For this purpose, forward primers were labeled with 4, 7, 2`, 4`, 5`, 7`-hexachloro-6-carboxyfluorescein (HEX), 6-carboxyfluorescein (FAM), and 7`, 8`-benzo, 5`-fluoro-2`, 4, 7 trichloro-3-carboxyflourescein (NED) (Applied Biosystems). PCR products were pooled post-PCR, where 0.5 µl of the FAM-labeled product, 0.5 µl of HEX labeled product, and 1µl of the NED labeled product was mixed with 0.075 µl of the ROX-labeled 400 HD size standard (Applied Biosystems) and Formamide (Applied Biosystems) to a total volume of 15 µl. DNA fragments were denatured for 5 min at 94 ^{0}C (Perkin Elmer 9700, Applid Biosystems) and size fractioned using capillary electrophoresis. Data was subjected to Genescan 3.1 software (Applied Biosystems) to size the peaks patterns using the internal ROX 400 HD size standard. Genotyper 3.1 (Applied Biosystems) were used for allele definition.

3.10 Statistical analysis

3.10.1 Phenotypic and biochemical data

All field, greenhouse (except dual-choice tests) and biochemical data were subjected to analysis of variance (ANOVA), and the significance of differences between the genotypes was tested by *F*-test, while the treatment means were compared by least significant differences (LSD) at $P = 0.05$. For the dual-choice tests, paired *t*-test ($P = 0.05$) was used to test the significance of difference between the test genotype and the susceptible check at $P = 0.05$.

3.10.2 Antibiosis indices

Antibiosis indices were computed by following the methods given by Dhillon *et al.* (2005a, b).

Growth index = pupation (%) / larval period.

Relative growth index = growth index on the test genotype/ growth index on the susceptible check.

Developmental index = (larval + pupal periods on the test genotype) / (larval + pupal periods on the susceptible check).

Adult emergence index = adult emergence on the test genotype/ adult emergence on the susceptible check.

Fecundity index = total eggs laid by the insects reared on the test genotype/ total eggs laid by the insect reared on susceptible check.

3.10.3 Correlation, regression, path coefficient, and similarity matrix analysis

The relationship between different sorghum traits and shoot fly damage parameters, and the contribution of various parameters associated with shoot fly resistance, Pearson's correlation and regression coefficients were estimated for the parameters studied. Principle component and similarity matrix analysis was carried out to determine the variability between the genotypes studied. Path coefficient analysis was used to estimate the direct and indirect association of different traits with resistance /susceptibility to shoot fly. The relationship between physico-chemical characteristics of sorghum genotypes and shoot fly damage parameters was assessed through Pearson's correlation and regression analysis.

3.10.4 Factorial analysis

Factorial analysis was used to study the effects of genotypes, and different concentrations of phenolic acids; and 2, 4-D, Cu_2So_4, and KI on shoot fly damage. Interactions of genotypes × concentration × compounds were tested at $P = 0.05$.

3.10.5 HPLC, GC-MS and genetic diversity studies

The HPLC data were recorded and processed by the Millennium[32] software version 4.0., GC-MS analysis was carried with Chem Station software. For genetic diversity analysis, Genescan 3.1 software (Applied Biosystems) to size the peaks patterns using the internal ROX 400 HD

size standard and Genotyper 3.1 (Applied Biosystems) were used for allele definition. Allelobin, DARwin5, and power marker 3.25 version were used to generate diversity dendrograms.

4. RESULTS

Studies on biochemical mechanisms of resistance to shoot fly, *Atherigona soccata* (Rond.) in sorghum, *Sorghum bicolor* (L.) Moench were conducted at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. The experiments were carried out during the 2004-07 cropping seasons. The studies were carried out under field, greenhouse, and laboratory conditions. The results of these experiments are discussed here under.

4.1 Characterization of sorghum genotypes for different components of resistance to shoot fly, *Atherigona soccata*

4.1.1 Assessment of different components of resistance under multi-choice conditions in the field

4.1.1.1 Oviposition non-preference

Oviposition non-preference was one of the important components of resistance to shoot fly, *A. soccata*. The number of eggs per 10 seedlings ranged from 0.99 to 23.40 eggs at 14 days after seedling emergence (DAE), and 2.00 to 20.67 eggs at 21 DAE across seasons (Table 4.1). The genotypes Swarna, CK 60B, ICSV 745, 296B, and ICSV 112 had significantly more number of eggs as compared to resistant check, IS 18551 at 14 and 21 DAE, but were on par with the susceptible check, Swarna. IS 1054, IS 1057, IS 2146, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, and ICSV 700 had significantly less number of eggs

compared to the susceptible check, Swarna, but were on par with the resistant check, IS 18551. There were no significant differences between genotypes tested during the 2004 post-rainy season at 21 DAE. 296B had low oviposition during the 2004 post rainy season at 14 DAE; while IS 1057, SFCR 125, SFCR 151, and ICSV 700 had more number of eggs, but were on par with resistant check, IS 18551 during the 2005 rainy season at 21 DAE.

Mean number of eggs per 10 seedlings ranged from 3.2 to 16.8 and 5.4 to 15.1 eggs at 14 and 21 DAE, respectively, across seasons (Table 4.1). The genotypes IS 1054, IS 1057, IS 2146, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, and ICSV 700 had significantly lower numbers of eggs per 10 seedlings as compared to the susceptible check, Swarna, and were on par with resistant check, IS 18551 at 14 and 21 DAE. Genotypes CK 60B, ICSV 745, 296B and ICSV 112 had significantly more number of eggs as compared with that one the resistant check, IS 18551, and were on par with the susceptible check, Swarna at 14 and 21 DAE. However, IS 4664 exhibited moderate levels of oviposition non-preference at 21 DAE.

Seedlings with eggs ranged from 6.06 to 94.72%, and 21.35 to 100% at 14 and 21 DAE, respectively, across seasons (Table 4.2). Genotypes CK 60B, ICSV 745, 296B, and ICSV 112 had significantly more proportion of seedlings with eggs as compared to the resistant check, IS 18551, but were on par with the susceptible check, Swarna. IS

1054, IS 1057, IS 2146, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, and ICSV 700 were at par with the resistant check, IS 18551, but there were a few exceptions.

Percent of seedlings with eggs ranged from 29.1 to 87.3%, and 53.9 to 96.8% at 14 and 21 DAE, respectively (Table 4.2). The genotypes IS 1054, IS 1057, IS 2146, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, ICSV 700, and IS 18551 had significantly lower proportion of seedlings with eggs compared with Swarna, CK 60B, ICSV 745, 296B, and ICSV 112. However, moderate levels of oviposition non-preference were recorded in case of IS 1054 and IS 4664 at 14 and 21 DAE, respectively.

4.1.1.2 Deadheart incidence

Percentage plants with deadhearts ranged from 0.0 to 83.4% at 14 DAE across seasons (Table 4.3). Percentage plants with deadhearts were significantly greater in CK 60B, ICSV 745, 296B, and ISCV 112 as compared with resistant check, IS 18551. IS 4664 moderate levels of deadheart incidence during the 2004 rainy, and 2005 post rainy seasons. SFCR 125 had no deadhearts during the 2005 rainy season and moderate deadheart incidence during the 2005 post rainy season. However, CK 60B, ICSV 745, and ICSV 112 suffered comparatively lower deadheart incidence during the 2005 rainy season, and 296B during the 2004 post rainy season.

Percentage plants with deadhearts ranged from 10.5 to 99.21% at 21 DAE across seasons (Table 4.3). Percentage deadheart incidence at 21 DAE was significantly greater in CK 60B, ICSV 475, 296B, and ICSV 112 as compared to the resistant check, IS 18551. Deadheart incidence was greater during the 2005 rainy season as compared to other seasons.

At 28 DAE, the plants with deadhearts ranged from 12.32 to 99.21% across seasons (Table 4.3). The percentage deadhearts at 28 DAE were significantly greater in CK 60B, ICSV 475, 296B, and ICSV 112 as compared to the resistant check, IS 18551. IS 4664 had moderate levels of deadheart incidence in all the seasons. There was considerable variation in deadheart incidence among the genotypes at 28 DAE across seasons.

Percent deadhearts across seasons ranged from 6.8 to 48.6 % at 14 DAE, 31.1 to 84.1% at 21 DAE, and 42.2 to 92.6% at 28 DAE (Table 4.3). Genotypes IS 1054, IS 1057, IS 2146, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, and ICSV 700 exhibited significantly lower deadheart incidence as to compared the susceptible check, Swarna, but were on par with resistant check, IS 18551. However, CK 60B, ICSV 745, 296B, and ICSV 112 showed significantly more number of deadhearts as compared to the resistant check, IS 18551.

4.1.1.3 Recovery resistance

Tiller deadhearts, which is one of the measures of recovery resistance, ranged from 4.31 to 62.0% at 28 DAE during 2004-05 rainy and post

rainy seasons (Table 4.4). There were significant differences in tiller deadheart incidence between the resistant and susceptible genotypes, both during the rainy and the post rainy seasons.

Mean tiller deadheart incidence ranged from 20.6 to 45.7% at 28 DAE across seasons (Table 4.4). Tillers of the genotypes IS 1054, IS 1057, IS 2146, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151 and ICSV 700 suffered significantly lower deadheart incidence as compared to the tillers of the susceptible check, Swarna.

Plants with productive tillers ranged from 2.1 to 75.6% across seasons (Table 4.4). There were no significant differences in the number of productive tillers among the sorghum genotypes tested across seasons. In general, IS 2146, IS 4664, SFCR 125, and CK 60B during the 2004 post rainy season, ICSV 700 during the rainy and post rainy seasons, and SFCR 125 and Swarna during the 2005 rainy season had more number of productive tillers than in the other genotypes tested. Mean productive tillers on different sorghum genotypes ranged from 18.2 to 44.6 % across seasons (Table 4.4). Genotypes IS 1057, IS 2146, SFCR 125, SFCR 151, ICSV 700, CK 60B, ICSV 745, ICSV 112, and Swarna had more number of productive tillers than the other genotypes tested.

4.1.2 Non-preference for oviposition under greenhouse conditions

4.1.2.1 No-choice tests

Seedling with eggs, eggs per 10 plants, and deadhearts on different sorghum genotypes under no-choice tests in the greenhouse ranged from 98.3 - 100%, 57.6 - 88.1 eggs, and 62.2 - 91.6%, respectively (Table 4.5). There were no significant differences among the genotypes tested for seedling with eggs under no-choice conditions in the greenhouse, suggesting that non-preference for oviposition is not a strong component of resistance. Under no-choice conditions, the shoot fly females lay eggs both on resistant and susceptible genotypes, resulting in similar levels of damage on different genotypes. Genotypes IS 1054, IS 1057, IS 2146, IS 2205, SFCR 151, ICSV 700, CK 60B, and Swarna had more number of eggs seedlings^{-10} than the resistant check, IS 18551. In case of deadhearts, genotypes IS 1054, IS 1057, IS 2312, SFCR 125, and SFCR 151 were on par with the resistant check, IS 18551, while CK 60B, ICSV 745, 296B, ICSV 112, and Swarna had more deadhearts as compared to the resistant check, IS 18551 under no-choice conditions (Table 4.5).

4.1.2.2 Dual-choice test

Under dual-choice conditions in the greenhouse, seedlings with eggs, eggs per 10 seedlings, and deadhearts ranged from 63.1 - 89.0%, 7 - 24 eggs, and 42.5 - 89.7% in test entries; and 88.7 - 97.3%, 18 - 30 eggs, and 78.5 - 89.7% in Swarna (Table 4.6).

Table 4.1 Antixenosis for oviposition on 15 genotypes of sorghum by shoot fly, *A. soccata* under multi-choice conditions in the field (ICRISAT, Patancheru, India 2004-05)

Genotypes	Eggs seedlings^{-10}									
	14 DAE					21 DAE				
	I	II	III	IV	Mean	I	II	III	IV	Mean
IS 1054	1.7	2.1	6.6	7.5	4.5	2.0	3.0	11.0	10.0	6.5
IS 1057	3.4	2.7	10.6	6.3	5.7	7.0	4.9	14.4	8.6	8.7
IS 2146	2.8	1.6	5.1	3.8	3.3	4.2	2.4	10.2	5.4	5.6
IS 4664	9.5	2.7	6.2	7.1	6.4	13.5	5.4	12.4	8.4	9.9
IS 2312	2.0	2.4	5.1	5.2	3.7	3.1	3.4	8.6	7.7	5.7
IS 2205	2.4	1.1	7.1	5.7	4.1	3.4	3.5	9.7	7.4	6.0
SFCR 125	4.2	2.3	5.9	7.2	4.9	6.7	5.4	14.7	9.1	9.0
SFCR 151	3.5	1.5	7.6	4.7	4.3	5.4	4.0	15.2	9.3	8.5
ICSV 700	4.2	3.2	8.5	7.2	5.8	4.3	4.4	14.6	9.9	8.3
CK 60B	18.6	7.8	15.8	13.4	13.9	11.9	7.2	18.2	18.1	13.9
ICSV 745	21.9	8.3	15.9	15.6	15.4	14.2	4.4	18.5	16.6	13.4
296B	14.5	3.1	19.6	13.3	12.6	13.7	6.2	17.6	15.4	13.2
ICSV 112	22.5	9.5	19.6	15.6	16.8	14.7	10.0	17.0	14.4	14.0
IS 18551(R)	2.5	1.0	5.3	4.1	3.2	3.6	2.1	9.2	6.7	5.4
Swarna (S)	23.4	6.5	16.5	14.8	15.3	14.4	7.9	20.7	17.6	15.1
F probanility	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.296	< 0.001	< 0.001	< 0.001
SE ±	2.3	0.9	1.1	1.4	1.4	1.5	1.9	1.3	1.0	1.0
LSD (p = 0.05)	6.6	2.5	3.2	4.0	4.1	4.2	NS	3.7	2.9	2.9
CV (%)	43.1	40.5	18.5	27.0	35.7	30.8	67.3	15.6	15.8	21.2

I = Kharif 2004, II = Rabi 2004, III = Kharif 2005, IV = Rabi 2005. Mean across the four seasons. DAE = Days after seedling emergence. NS = Nonsignificant.

Table 4.2 Oviposition preference by shoot fly, *A. soccata* among 15 sorghum genotypes under multi-choice conditions in the field (ICRISAT, Patancheru, India 2004-05)

	Seedlings with eggs (%)								
	14 DAE					21 DAE			
Genotypes	I	II	III	IV	Mean	I	III	IV	Mean
IS 1054	18.4	24.5	56.0	71.1	42.5	22.5	86.0	80.9	63.1
IS 1057	28.4	28.6	78.7	54.0	47.4	40.6	92.8	71.4	68.3
IS 2146	24.2	19.0	41.9	43.6	32.2	39.5	75.5	51.5	55.5
IS 4664	46.7	28.8	49.9	44.9	42.6	78.3	84.3	69.5	77.4
IS 2312	17.8	22.4	58.0	39.8	34.5	21.4	66.0	74.4	53.9
IS 2205	20.1	8.7	54.0	45.6	32.1	28.9	78.5	55.2	54.2
SFCR 125	37.3	24.7	48.8	57.3	42.0	42.2	90.0	69.7	67.3
SFCR 151	28.7	17.5	60.5	34.6	35.3	43.7	86.8	69.8	66.8
ICSV 700	33.0	24.2	58.9	52.6	42.2	42.0	91.6	71.8	68.5
CK 60B	89.2	62.6	88.2	81.2	80.3	85.1	98.6	94.7	92.8
ICSV 745	84.9	67.9	89.0	86.2	82.0	90.8	99.2	92.8	94.3
296B	82.5	30.1	92.0	84.2	72.2	84.0	98.3	96.2	92.9
ICSV 112	89.3	72.1	92.9	94.7	87.3	91.5	98.2	91.5	93.8
IS 18551 (R)	21.2	6.1	51.5	37.8	29.1	31.6	79.2	62.0	57.6
Swarna (S)	88.5	56.7	92.0	84.5	80.4	94.8	100.0	95.7	96.8
F-probability	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
SE ±	6.6	5.7	8.4	7.6	4.9	8.0	2.9	5.1	6.1
LSD (p = 0.05)	19.0	16.4	24.2	21.9	13.9	23.3	8.4	14.7	17.7
CV (%)	24.0	29.7	21.5	21.5	18.7	24.9	5.7	11.5	16.6

I = Kharif 2004, II = Rabi 2004, III = Kharif 2005, IV = Rabi 2005. Mean across the seasons.
DAE = Days after seedling emergence.

Table 4.3 Deadheart formation in 15 sorghum genotypes due to sorghum shoot fly, *A. soccata* under multi-choice conditions in the field (ICRISAT, Patancheru, India 2004-05)

	Deadhearts (%)														
	14 DAE					21 DAE					28 DAE				
Genotypes	I	II	III	IV	Mean	I	II	III	IV	Mean	I	II	III	IV	Mean
IS 1054	5.4	11.1	5.4	16.1	9.5	10.5	18.1	78.2	50.4	39.3	12.3	24.9	82.8	74.0	48.5
IS 1057	13.9	9.9	9.0	16.1	12.2	24.5	35.6	77.7	38.3	44.0	33.5	46.2	86.6	51.9	54.6
IS 2146	11.5	5.3	4.2	13.4	8.6	22.9	15.7	65.4	25.8	32.5	25.6	24.2	71.5	47.3	42.2
IS 4664	46.7	4.4	1.0	21.0	18.3	65.0	15.1	72.6	52.1	51.2	71.7	39.1	80.2	66.6	64.4
IS 2312	9.3	7.9	1.7	14.9	8.4	12.9	21.6	55.9	28.0	29.6	15.2	33.5	71.1	51.4	42.8
IS 2205	11.2	5.3	5.2	11.7	8.4	19.1	13.4	63.1	28.8	31.1	23.6	31.4	75.2	51.6	45.5
SFCR 125	23.3	7.3	0.0	28.0	14.7	32.0	27.8	81.0	50.8	47.9	41.5	43.1	89.1	68.5	60.6
SFCR 151	23.3	2.5	5.2	14.3	11.3	29.6	24.9	80.6	36.1	42.8	37.4	30.0	87.7	61.2	54.1
ICSV 700	23.9	7.2	1.9	18.5	12.9	32.8	27.5	75.1	45.1	45.1	34.8	41.1	86.3	66.7	57.2
CK 60B	74.4	21.3	9.1	37.4	35.6	75.2	58.5	97.7	74.9	76.6	81.4	74.1	96.6	91.6	85.9
ICSV 745	81.9	36.0	10.9	52.3	45.3	87.8	80.3	94.1	74.1	84.1	90.9	90.9	94.9	90.2	91.7
296B	68.3	5.7	19.5	36.8	32.6	70.4	49.6	97.6	76.5	73.5	85.1	55.8	99.2	93.7	83.5
ICSV 112	78.0	44.5	13.6	41.4	44.4	86.4	57.1	94.7	77.5	78.9	84.2	59.6	97.3	86.1	81.8
IS 18551 (R)	16.3	3.9	0.8	6.1	6.8	21.5	11.2	71.3	28.5	33.1	25.6	18.8	78.3	49.2	43.0
Swarna (S)	83.4	33.8	29.4	47.6	48.6	85.5	58.9	99.2	77.0	80.1	98.7	87.4	97.7	86.6	92.6
F-probability	<0.001	<0.001	0.031	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SE ±	5.7	5.4	5.3	5.0	6.0	7.0	8.9	4.5	7.3	5.0	8.2	8.7	3.4	3.9	5.9
LSD (*p*= 0.05)	16.5	15.6	15.5	14.3	17.0	20.3	26.0	13.0	21.2	14.2	23.6	25.2	9.8	11.3	16.8
CV (%)	25.8	67.5	118.5	34.2	56.4	26.9	45.0	9.7	24.8	18.8	27.8	32.2	6.8	9.8	18.6

I = Kharif 2004. II = Rabi 2004. III = Kharif 2005. IV = Rabi 2005. Mean across the four seasons. DAE = Days after seedling emergence.

Table 4.4 Tiller deadhearts and productive tillers in 15 sorghum genotypes in response to damage by shoot fly, *A. soccata* under multi-choice conditions in the field (ICRISAT, Patancheru, India, 2004-05)

	Tiller deadhearts (%) (28 DAE)					Productive tillers (%)			
Genotypes	I	II	III	IV	Mean	I	II	III	**Mean**
IS 1054	39.5	16.9	15.0	12.9	21.1	9.7	58.7	14.9	27.8
IS 1057	38.4	26.0	20.9	15.1	25.1	13.0	68.2	20.7	34.0
IS 2146	40.0	9.3	27.8	5.1	20.6	14.6	75.6	16.4	35.5
IS 4664	26.7	17.7	35.3	24.9	26.2	8.3	71.4	2.1	27.3
IS 2312	51.4	19.4	28.1	11.2	27.5	8.9	62.0	10.4	27.1
IS 2205	38.8	22.8	28.0	12.9	25.6	6.9	51.4	7.8	22.0
SFCR 125	51.9	13.6	38.8	17.7	30.5	16.8	75.6	41.3	44.6
SFCR 151	45.5	4.3	37.0	21.1	27.0	8.9	59.4	27.3	31.9
ICSV 700	38.9	11.6	32.8	21.4	26.2	20.5	78.4	13.7	37.5
CK 60B	58.7	42.3	40.1	24.8	41.5	5.5	72.6	37.6	38.6
ICSV 745	61.7	39.8	48.9	32.3	45.7	18.3	56.3	21.1	31.9
296B	50.9	47.7	40.3	17.9	39.2	14.3	54.3	10.4	26.4
ICSV 112	62.0	47.6	57.3	39.8	51.7	16.5	61.2	35.3	37.6
IS 18551 (R)	58.4	13.3	35.2	15.7	30.7	11.2	41.0	2.6	18.2
Swarna (S)	43.2	35.6	48.6	21.6	37.3	17.2	56.6	46.1	40.0
F-probability	0.080	0.003	< 0.001	0.391	< 0.001	0.394	0.465	< 0.001	0.091
SE ±	7.6	7.7	5.6	8.2	3.8	4.4	10.5	6.7	5.4
LSD (*p*= 0.05)	NS	22.3	16.2	NS	10.8	NS	NS	19.3	NS
CV (%)	27.8	54.2	27.3	72.4	23.8	59.5	28.8	56.2	29.1

I = Kharif 2004. II = Rabi 2004. III = Kharif 2005. IV = Rabi 2005. Mean across the four seasons. DAE = Days after seedling emergence. NS = Nonsignificant.

Table 4.5 Expression of antixenosis for oviposition to sorghum shoot fly, *A. soccata* under no-choice conditions in the greenhouse (ICRISAT, Patancheru, 2005)

Genotypes	Seedlings with eggs (%)	Eggs seedlings^{-10}	Deadhearts (%)
IS 1054	99.2	88.1	66.3
IS 1057	100.0	73.3	69.1
IS 2146	100.0	86.0	80.8
IS 4664	98.3	57.6	73.6
IS 2312	100.0	66.3	67.5
IS 2205	100.0	86.6	76.7
SFCR 125	100.0	63.6	63.8
SFCR 151	100.0	86.8	68.5
ICSV 700	100.0	77.5	75.8
CK 60 B	100.0	83.5	86.7
ICSV 745	98.3	57.8	88.3
296 B	99.0	67.2	90.4
ICSV 112	99.2	66.5	85.0
IS 18551 (R)	100.0	65.3	62.2
Swarna (S)	100.0	70.5	91.6
F-probability	0.479	0.025	<0.001
SE ±	0.63	7.03	4.90
LSD (p = 0.05)	NS	20.36	14.18
CV (%)	1.10	16.70	11.10

R = Resistant check. S = Susceptible check. NS = Nonsignificant.

Table 4.6 Expression of antixenosis for oviposition to sorghum shoot fly, *A. soccata* under dual-choice conditions in the greenhouse (ICRISAT, Patancheru, 2005)

	Seedlings with eggs (%)			Eggs seedlings^{-10}			Deadhearts (%)		
Genotypes	Test entry	Swarna	t-value	Test entry	Swarna	t-value	Test entry	Swarna	t-value
IS 1054	67.4	93.6	-5.7**	10	21	-2.8*	46.1	84.7	-7.6**
IS 1057	76.5	94.0	-2.5*	13	25	-4.5**	42.5	78.5	-12.5**
IS 2146	78.9	94.6	-2.7*	17	27	-8.8**	55.9	87.7	-7.1**
IS 18551	76.6	94.0	-1.9	14	24	-2.9*	36.5	81.9	-5.7**
IS 4664	63.1	97.3	-5.7**	7	24	-2.6*	42.7	89.7	-7.3**
IS 2312	72.6	91.2	-3.5*	11	24	-3.0*	52.9	85.3	-11.6**
IS 2205	69.0	88.7	-4.1*	10	18	-3.6*	50.0	80.2	-8.6**
SFCR 125	73.1	92.9	-5.1**	11	19	-3.7*	46.8	82.1	-5.4**
SFCR 151	76.9	92.5	-2.2*	15	21	-5.3**	49.2	85.5	-3.2*
ICSV 700	81.7	97.3	-5.8**	16	30	-4.6**	67.9	83.0	-2.6*
CK 60 B	88.1	92.6	-1.0	19	25	-5.8**	84.6	83.7	0.1
ICSV 745	89.0	92.3	-0.9	18	20	-3.1*	77.6	85.4	-1.9
296 B	88.1	92.0	-0.5	13	18	-2.6*	73.8	87.4	-3.6*
ICSV 112	89.0	93.1	-1.1	24	24	0.02	81.0	85.2	-0.7

*, ** t-test significant at $P = 0.05$ and 0.01, respectively.

Genotypes IS 1054, IS 1057, IS 2146, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151 and ICSV 700 had lower percentage of seedlings with eggs as compared to the susceptible check, Swarna. In case of deadhearts, the genotypes CK 60B, ICSV 745, 296B, and ICSV 112 did not differ significantly from the susceptible check, Swarna.

4.1.3 Expression of antibiosis component of resistance to shoot fly, *Atherigona soccata* under greenhouse conditions

The larval and pupal periods, pupation, adult emergence, male and female pupal weights, and fecundity on 15 sorghum genotypes ranged from 9.1 - 10.7 days, 7.2 - 8.1 days, 58.2 - 90.5 %, 33.2 - 71.2 %, 3.2 - 4.0 mg, 4.6 - 5.5 mg, and 136.9 - 191.6 eggs per female, respectively (Table 4. 7). The larval period was prolonged by one day on IS 1054, IS 1057, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, ICSV 700, and IS 18551 (10.0 to 10.7 days) as compared to that on the susceptible check, Swarna (9.1 days), while the pupal period was extended by nearly one day on IS 18551, IS 2146, and IS 2312, than that on the susceptible check, Swarna. Pupation was significantly lower on IS 2146, IS 4664, IS 2312, SFCR 125, ICSV 700, and IS 18551 (58.2 to 65.2%) as compared to that on the susceptible check, Swarna (77.1%), while adult emergence was lower on IS 2146, IS 4664, IS 2312, IS 2205, SFCR 125, ICSV 700, and IS 18551 (33.2 to 42.3%) as compared to the susceptible check, Swarna (55.3 %). There was little variation in pupal weights, but the female pupae were heavier than the male pupae. More numbers of eggs

were laid by insects reared on IS 1054, IS 1057, IS 4664, CK 60B, ICSV 745 and IS 18551 (172.0 to 191.6 eggs female^{-1}) than the insects reared on the susceptible check, Swarna (149.6 eggs female^{-1}) (Table 4.7).

Growth, relative growth, developmental, adult emergence, and fecundity indices on 15 sorghum genotypes ranged from 5.3 - 9.7, 0.6 - 1.1, 0. 9 - 1.0, 0.6 - 1.3, and 1.0 - 1.3, respectively (Table 4.8). Growth index was significantly lower on IS 4664, IS 2312, IS 2205, SFCR 125, ICSV 700, and IS 18551 (5.3 to 6.7) than on the susceptible check, Swarna (8.5). Differences were also significant in terms of relative growth and developmental indices between the resistant and susceptible genotypes. Adult emergence index was better on the susceptible genotypes as compared to that on the other genotypes tested. Fecundity did not differ significantly between the resistant and susceptible genotypes (Table 4.8).

4.2 Variation in morphological traits in relation to expression of resistance to shoot fly, *Atherigona soccata*

4.2.1 Leaf glossiness

Leaf glossiness score ranged from 1.0 to 5.0 at 10 DAE across seasons (Table 4.9). The shoot fly susceptible genotypes Swarna, CK 60B, ICSV 745, 296B, and ICSV 112 were non-glossy; while the shoot fly resistant genotypes IS 1054, IS 2146, IS 18551, IS 2312, IS 2205, SFCR 125, and SFCR 151 were glossy. IS 1057, IS 4664, IS 1054, SFCR 151, and ICSV 700 exhibited intermediate levels of glossiness across seasons.

4.2.2 Seedling vigor

Seedling vigor scores ranged from 1.0 to 5.0 at 10 DAE across seasons (Table 4.9). Genotypes IS 1054, IS 1057, IS 2146 and IS 2312 were more vigorous than the susceptible check, Swarna, but were on par with the resistant check, IS 18551. While CK 60B, ICSV 745, 296B, and ICSV 112 were significantly less vigorous than the resistant check, IS 18551. However, there were few exceptions. SFCR 151 and ICSV 700 exhibited moderate vigor across seasons.

4.2.3 Pigmentation

Plumule pigmentation scores ranged from 1.0 to 5.0 at 10 DAE during the 2004 post-rainy and 2005 rainy seasons, while leaf sheath and bottom leaf pigmentation scores ranged from 2.0 to 5.0 and 2.5 to 5.0 at 10 DAE, respectively, during the 2005 rainy season (Table 4.9). CK 60B, ICSV 745, and ICSV 112 were non-pigmented (green colored), while the rest of the test genotypes were highly pigmented.

4.2.4 Trichome density

Trichome density varied from 0.2 to 231.0 and 0.0 to 146.0 trichomes in a 10x microscopic field on the abaxial and adaxial leaf surfaces, respectively, at 10 DAE across seasons (Table 4.10). Genotypes IS 1054, IS 1057, IS 18551, IS 2146, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151 and ICSV 700 were trichomed and relatively resistant to shoot fly damage, whereas, Swarna, CK 60B, ICSV 745, 296B, and ICSV 112 were non-trichomed and susceptible to shoot fly.

Table 4.7 Expression of antibiosis to shoot fly, *A. soccata* in 15 genotypes of sorghum under greenhouse conditions (ICRISAT, Patancheru, 2005)

Genotypes	Larval period (days)	Pupal period (days)	Pupation (%)	Adult emergence (%)	Pupal weight (mg)		Fecundity female^{-1}
					Male	Female	
IS 1054	10.7	7.4	78.7	53.0	3.5	5.0	173.1
IS 1057	10.5	7.5	83.7	48.2	3.6	5.2	159.9
IS 2146	9.8	8.1	65.1	42.3	3.8	5.2	148.1
IS 2205	10.0	7.9	70.7	40.3	3.7	5.3	151.0
IS 2312	10.0	8.0	62.2	39.3	3.2	5.5	155.7
IS 4664	10.0	7.6	59.3	35.2	3.5	5.0	172.0
SFCR 125	10.2	7.8	65.2	38.9	3.7	4.9	136.9
SFCR 151	10.1	7.6	74.0	47.9	3.2	5.3	154.7
ICSV 700	10.6	7.8	59.8	33.2	3.5	5.1	155.4
CK 60B	9.9	7.2	76.6	52.8	3.4	4.7	172.6
ICSV 745	9.4	7.3	90.5	69.4	3.7	5.0	191.6
296B	9.5	7.5	87.5	68.0	3.9	4.9	154.5
ICSV 112	9.4	7.8	86.6	71.2	3.7	5.3	146.4
IS 18551 (R)	10.4	8.0	58.2	38.8	3.6	4.9	166.2
Swarna (S)	9.1	7.3	77.1	55.3	4.0	4.6	149.6
F-probability	0.002	0.022	< 0.001	< 0.001	0.474	0.148	0.830
SE ±	0.3	0.2	4.1	4.6	0.2	0.2	17.7
LSD (*P* = 0.05)	0.7	0.5	12.0	13.4	NS	NS	NS
CV (%)	5.0	4.8	11.3	18.8	12.3	7.6	22.2

R = Resistant check, and S = Susceptible check. NS = Nonsignificant.

Table 4.8 Growth indices of sorghum shoot fly, *A. soccata* on 15 sorghum genotypes (ICRISAT, Patancheru, 2005)

Genotypes	Growth index	Relative growth index	Developmental index	Adult emergence index	Fecundity index
IS 1054	7.4	0.9	0.9	1.0	1.2
IS 1057	7.7	0.9	0.9	0.9	1.2
IS 2146	7.0	0.8	0.9	0.7	1.1
IS 2205	6.7	0.8	0.9	0.8	1.1
IS 2312	6.1	0.7	0.9	0.7	1.1
IS 4664	5.3	0.6	0.9	0.6	1.2
SFCR 125	6.6	0.8	0.9	0.7	1.0
SFCR 151	7.7	0.9	0.9	0.9	1.1
ICSV 700	5.8	0.7	0.9	0.6	1.1
CK 60 B	8.2	1.0	1.0	1.0	1.2
ICSV 745	9.7	1.1	1.0	1.2	1.3
296 B	9.2	1.1	1.0	1.2	1.1
ICSV 112	9.4	1.1	1.0	1.3	1.1
IS 18551 (R)	5.8	0.7	0.9	0.7	1.2
Swarna (S)	8.5	1.0	1.0	1.0	1.0
F-probability	< 0.001	< 0.001	< 0.001	< 0.001	0.840
SE ±	0.5	0.1	0.0	0.1	0.1
LSD (P < 0.05)	1.6	0.2	0.04	0.3	NS
CV (%)	14.6	14.8	2.8	19.6	20.5

= Resistant check, and S = Susceptible check. NS = Nonsignificant.

Table 4.9 Variation in morphological traits of 15 sorghum genotypes in relation to expression of resistance to shoot fly, *A. soccata* under field conditions (ICRISAT, Patancheru 2004 - 05)

	Leaf glossiness (10 DAE)					Seedling vigor (10 DAE)					Pigmentation (10 DAE)			
Genotypes	I	II	III	IV	Mean	I	II	III	IV	Mean	A (II)	B (III)	C (III)	D (III)
IS 1054	2.2	3.0	1.7	2.3	2.3	1.7	2.3	1.7	3.0	2.2	2.0	1.0	4.3	5.0
IS 1057	3.0	3.0	2.0	3.0	2.8	2.0	3.2	1.0	1.0	1.8	1.5	1.3	2.0	2.5
IS 2146	2.2	2.3	1.0	1.0	1.6	2.0	4.0	1.7	1.5	2.3	2.7	1.0	2.8	3.2
IS 4664	3.2	3.2	1.7	2.7	2.7	3.7	4.9	1.0	2.0	2.9	2.1	1.7	2.3	3.0
IS 2312	2.7	2.7	1.0	1.7	2.0	2.0	2.5	1.0	1.0	1.6	1.7	2.2	2.8	3.7
IS 2205	2.0	1.5	1.0	1.7	1.5	3.0	3.5	1.7	2.0	2.5	1.0	1.3	2.2	3.3
SFCR 125	2.8	2.7	1.0	2.0	2.1	3.3	4.0	3.0	1.0	2.8	1.7	1.5	2.5	4.7
SFCR 151	2.5	3.0	1.0	2.3	2.2	3.3	3.7	2.3	2.0	2.8	1.0	1.7	2.3	4.0
ICSV 700	2.0	2.8	1.3	2.3	2.1	3.7	3.0	2.3	2.5	2.9	1.0	1.0	2.2	2.7
CK 60B	4.7	5.0	5.0	4.3	4.8	3.7	3.0	3.0	3.0	3.2	5.0	5.0	5.0	5.0
ICSV 745	4.7	5.0	5.0	4.7	4.8	4.0	3.0	2.3	3.5	3.2	5.0	5.0	5.0	5.0
296B	4.3	5.0	5.0	4.7	4.7	4.0	5.0	2.7	4.5	4.0	3.1	2.0	3.2	4.0
ICSV 112	3.8	5.0	5.0	4.7	4.6	4.0	1.3	2.3	1.5	2.3	5.0	5.0	5.0	5.0
IS 18551 (R)	1.7	1.7	1.0	1.7	1.5	2.3	3.0	1.8	2.0	2.3	3.5	1.2	2.0	3.2
Swarna (S)	5.0	5.0	5.0	4.3	4.8	2.7	4.3	1.7	3.0	2.9	3.0	1.0	3.5	4.7
F-probability	< 0.001	< 0.001	< 0.001	< 0.001	<0.001	< 0.001	< 0.001	0.005	0.026	0.007	< 0.001	< 0.001	< 0.001	< 0.001
SE ±	0.3	0.4	0.2	0.3	0.2	0.4	0.4	0.4	0.6	0.4	0.6	0.2	0.3	0.3
LSD (*P* = 0.05)	0.9	1.0	0.4	0.9	0.7	1.1	1.3	1.1	1.8	1.1	1.6	0.7	0.8	0.8
CV %	17.4	18.5	10.5	19.5	15.5	22.6	22.4	33.5	37.5	28.0	36.6	18.7	14.7	11.8

I = Kharif 2004, II = Rabi 2004, III = Kharif 2005, IV = Rabi 2005. Mean across the seasons. DAE = Days after seedling emergence. A = Pigmentation, B = Plumule, C = Plumule leaf sheath color, D = Bottom leaf. Leaf glossiness (1 = Highly glossy, 5 = Non-glossy). Seedling vigor (1 = Highly vigor, 5 = Poor vigor). Pigmentation (1 = Highly pigmented, and 5 = Non pigmented-green)

Table 4.10 Trichome density on the leaves of 15 genotypes of sorghum at the seedling stage (ICRISAT, Patancheru, India 2004- 05)

Genotypes	No. of trichomes /10 X microscopic field (10 DAE)									
	Abaxial leaf surface					Adaxial leaf surface				
	I	II	III	IV	Mean	I	II	III	IV	Mean
IS 1054	105.9	154.2	89.6	126.0	118.9	47.7	79.1	72.2	71.8	67.7
IS 1057	88.2	134.7	98.5	127.5	112.2	55.8	64.8	77.8	77.3	68.9
IS 2146	126.8	192.2	131.4	145.5	149.0	86.8	121.3	106.8	103.8	104.7
IS 4664	92.9	108.9	63.8	144.7	102.6	72.8	78.8	51.6	100.4	75.9
IS 2312	112.1	145.1	101.8	113.7	118.2	63.9	89.1	85.1	71.9	77.5
IS 2205	131.7	180.7	136.6	153.8	150.7	67.4	115.9	109.3	118.2	102.7
SFCR 125	163.7	211.8	181.8	154.6	178.0	107.0	125.8	146.0	118.1	124.2
SFCR 151	87.5	228.9	57.3	178.4	138.0	82.8	129.0	53.5	119.6	96.2
ICSV 700	155.9	214.8	96.9	231.0	174.6	73.7	129.9	70.4	133.9	102.0
CK 60B	0.4	8.4	0.6	3.1	3.1	0.2	1.7	0.3	1.1	0.8
ICSV 745	0.3	1.6	0.2	1.8	1.0	0.1	1.0	0.1	0.6	0.4
296 B	0.2	3.8	0.3	0.9	1.3	0.0	1.0	0.2	0.6	0.4
ICSV 112	0.4	2.4	0.7	1.9	1.4	0.1	0.9	0.4	0.8	0.6
IS 18551(R)	146.9	191.4	124.5	175.7	159.6	75.2	123.8	104.4	115.5	104.7
Swarna (S)	21.3	39.9	6.2	31.3	24.7	8.4	26.1	4.2	18.2	14.2
F-probability	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	<0.001	< 0.001	< 0.001	< 0.001
SE ±	8.4	9.2	8.1	5.8	12.4	6.1	6.4	4.4	2.9	7.5
LSD (p = 0.05)	23.6	25.9	22.8	16.2	35.4	17.2	18.1	12.5	8.2	21.3
CV (%)	24.9	18.5	27.2	13.3	25.9	30.2	21.6	18.4	10.2	23.8

I = Kharif 2004, II = Rabi 2004, III = Kharif 2005, IV = Rabi 2005. DAE = Days after seedling emergence. Mean across the four seasons.

Trichome density on the abaxial leaf surface was greater compared to that on the adaxial leaf surface.

4.2.5 Days to 50% flowering

Days to 50% flowering ranged from 56.0 to 82.3 days across seasons (Table 4.11). Swarna, CK 60B, ICSV 745, and ICSV 112 flowered early during the rainy season; while 296B took more number of days to 50% flowering during the post rainy season. IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, and ICSV 700 took more number of days to 50% flowering as compared to the susceptible check, Swarna.

4.2.6 Plant height at maturity (cm)

Plant height at maturity ranged from 81.8 to 304.4 cm across seasons (Table 4.11). All the test genotypes were taller during the rainy season than in the post-rainy season. Genotypes SFCR 151, CK 60B, ICSV 745, 296B, ICSV 112 and Swarna were shorter than the other genotypes tested across seasons. Mean of plant height ranged from 109.3 to 262.4 cm during the rainy and post rainy 2004 and rainy 2005 seasons (Table 4.11). Genotypes SFCR 151, CK 60B, ICSV 745, 296B, ICSV 112, and Swarna were shorter height than other genotypes tested (Table 4.11).

4.2.7 Moisture content, leaf surface wetness, and retention of water droplets in the leaves

Moisture content and surface wetness scores inside and outside the greenhouse, and retention of rain water droplets on the leaf surface and

foggy conditions at 6 and 10 AM ranged from 91.04 - 92.43%, 1.0 - 4.8, 1.0 - 4.8, 1 - 9, 1- 5, and 1- 5, respectively (Table 4.12). There were significant differences between the genotypes in moisture content of the seedlings under greenhouse conditions. However, moisture content of the genotypes IS 1054, IS 4664, and SFCR 125 was on par with the resistant check, IS 18551. The genotypes CK 60B, ICSV 745, 296B, ICSV 112, and Swarna had high leaf surface wetness, both in and outside the glasshouse. Retention of water droplets on the leaf surface on these genotypes was poor as compared to that on the resistant check, IS 18551.

4.3 Inducible resistances to shoot fly, *Atherigona soccata* in sorghum

4.3.1 Effect of transplanting sorghum seedlings on shoot fly oviposition and deadheart incidence

Eggs per 10 seedlings ranged from 0.4 to 3.4 and 4.3 to 13.4 at 14 DAE, and 2.4 to 7.4 eggs and 8.2 to 15.5 eggs at 21 DAE in the transplanted and control seedlings, respectively (Table 4.13). There was a significant reduction in oviposition on transplanted seedlings as compared that on the normal seedlings of all the genotypes tested at 14 and 21 DAE. However, the differences between the transplanted and normal seedlings of IS 4664 and IS 2312 were nonsignificant at 21 DAE. Across treatments, the genotypes IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, and ICSV 700 had significantly

lower numbers of eggs than on Swarna, CK 60B, ICSV 745, 296B and ICSV 112 both at 14 and 21 DAE.

Seedlings with eggs ranged from 3.3 to 31.8% and 40.0 to 89.7% at 14 DAE, 23.9 to 62.0% and 63.4 to 99.5% at 21 DAE in transplanted and normal control seedlings, respectively (Table 4. 14). Significantly lower numbers of eggs were laid on the transplanted seedlings as compared to the non-transplanted ones at 14 and 21 DAE. However, the differences between the transplanted and normal seedlings of IS 2146, IS 18551, IS 4664 and IS 2312 were nonsignificant at 21 DAE. The genotypes IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151 and ICSV 700 had significantly lower numbers of seedling with eggs than Swarna, CK 60B, ICSV 745, 296B and ICSV 112 at 14 and 21 DAE.

Deadheart incidence ranged from 0.0 to 17.5% and 7.2 to 53.5% at 14 DAE, 11.9 to 37.5% and 34.2 to 93.0% at 21 DAE, and 23.6 to 55.5% and 64.7 to 96.7% at 28 DAE in transplanted and control seedlings, respectively (Table 4.15). All the test genotypes had significantly lower deadheart incidence in transplanted seedlings as compared to the normal seedlings at 14, 21 and 28 DAE. However, the differences between transplanted and normal seedlings of IS 4664, IS 2312 and ICSV 112 at 14 DAE, IS 2312 and ICSV 700 at 21 DAE, and IS 2146, IS 4664, IS 2312 and ICSV 700 at 28 DAE were nonsignificant.

Table 4.11 Days to 50% flowering, and plant height of 15 sorghum genotypes under field conditions (ICRISAT, Patancheru, India, 2004- 05)

	Days to 50% flowering				Plant height at maturity (cm)			
Genotypes	I	II	III	Mean	I	II	III	Mean
IS 1054	65.7	62.7	67.0	65.1	229.4	187.8	238.9	218.7
IS 1057	68.3	65.3	72.7	68.8	258.9	188.9	253.3	233.7
IS 2146	66.7	67.0	69.3	67.7	239.4	177.8	258.3	225.2
IS 4664	65.9	64.3	72.7	67.6	246.1	158.9	271.7	225.6
IS 2312	68.0	66.3	69.3	67.9	256.7	201.1	276.7	244.8
IS 2205	71.0	69.0	72.7	70.9	276.1	206.7	304.4	262.4
SFCR 125	67.7	64.3	67.3	66.4	206.7	161.1	226.1	198.0
SFCR 151	66.0	60.7	64.3	63.7	140.0	117.2	136.7	131.3
ICSV 700	75.0	82.3	80.0	79.1	287.2	201.7	283.3	257.4
CK 60 B	56.0	63.3	57.3	58.9	124.0	105.0	113.3	114.1
ICSV 745	56.0	65.7	61.7	61.1	148.9	110.6	149.2	136.2
296 B	63.3	77.0	69.3	69.9	137.2	81.8	108.9	109.3
ICSV 112	63.7	66.7	67.3	65.9	147.2	120.6	164.4	144.1
IS 18551 (R)	72.3	71.0	71.3	71.6	278.9	202.2	294.4	258.5
Swarna (S)	62.3	62.0	62.0	62.1	143.3	122.8	143.9	136.7
F-probability	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
SE ±	1.6	1.1	1.1	1.8	5.1	7.8	10.5	10.0
LSD (*P* = 0.05)	4.6	3.1	3.3	5.3	14.8	22.6	30.4	29.0
CV (%)	4.2	2.7	2.9	4.7	4.2	8.6	8.5	9.0

I = Kharif 2004, II = Rabi 2004, III = Kharif 2005. Mean across the four seasons.

Table 4.12 Moisture content, leaf surface wetness, and retention of water droplets on seedlings of 15 sorghum genotypes under greenhouse conditions (ICRISAT, Patancheru, India, 2005)

Genotypes	Moisture content (%)	LSW		Retention of water droplets		
		Inside GH	Outside GH	Rain water	Fog water 6AM	Fog water 10AM
IS 1054	91.9	1.0	1.0	8.5	3.0	4.0
IS 1057	91.7	1.0	1.1	8.5	3.5	5.0
IS 2146	91.5	1.0	1.0	9.0	1.0	3.0
IS 4664	92.0	1.0	1.0	9.0	2.5	2.0
IS 2312	91.3	1.7	1.5	9.0	2.0	3.0
IS 2205	91.3	1.2	1.2	9.0	2.5	3.0
SFCR 125	92.4	1.0	1.2	8.5	3.5	4.0
SFCR 151	91.7	1.1	1.3	8.5	2.5	4.0
ICSV 700	91.4	1.3	1.1	9.0	3.0	4.0
CK 60B	91.2	2.8	3.2	1.0	4.5	1.0
ICSV 745	91.2	3.2	3.8	1.0	4.5	2.0
296B	91.6	4.2	4.2	1.5	5.0	2.0
ICSV 112	91.2	4.8	4.5	1.0	4.5	1.0
IS 18551 (R)	91.6	1.1	1.2	8.5	2.5	4.0
Swarna (S)	91.0	4.1	4.8	1.0	4.0	2.0
F-probability	0.01	< 0.001	< 0.001	< 0.001	0.029	*
SE ±	0.23	0.1	0.1	0.3	0.7	*
LSD (*P* = 0.05)	0.67	0.4	0.4	0.8	2.0	*
CV (%)	0.40	13.6	14.0	5.8	28.9	*

LSW = Leaf surface wetness, 1 = No wet, and 5 = Highly wet.

Rain water, 1 = No water droplets retained on the leaf surface and water stored in the whorls, and 9 = Water spread on leaf surface and little water stored in the whorl.

Fog water 6 AM, 1 = Highly wet, and 5 = Low wet.

Fog water 10 AM, 1 = low wet, and 5 = high wet.

Table 4.13 Effect of transplanting on oviposition by the sorghum shoot fly, *A. soccata* on 15 sorghum genotypes under field conditions (ICRISAT, Patancheru, India, 2006-07 rainy seasons)

	Eggs seedlings^{-10}					
	14 DAE			21 DAE		
Genotypes	Transplanted	Control	t-test	Transplanted	Control	t-test
IS 1054	0.7	7.1	4.16*	3.3	9.7	5.55**
IS 1057	1.0	8.8	7.68**	2.4	11.8	6.16**
IS 2146	0.3	6.1	8.29**	3.6	8.6	2.21*
IS 18551	0.7	5.5	3.42*	3.0	8.2	2.16*
IS 4664	0.9	5.7	3.63*	3.1	8.9	1.58
IS 2312	1.4	5.3	2.64*	4.3	9.1	1.48
IS 2205	0.4	4.3	5.65**	2.5	9.6	2.96*
SFCR 125	0.8	6.4	4.9**	2.5	9.2	2.85*
SFCR 151	1.5	7.1	5.56**	3.9	10.5	4.52**
ICSV 700	1.2	6.5	4.57**	3.8	11.3	2.42*
Swarna	3.0	13.0	5.54**	7.4	15.5	3.94*
CK 60 B	3.4	11.4	5.57**	4.8	14.2	5.32**
ICSV 745	2.4	13.4	12.16**	5.2	14.1	5.79**
296 B	2.1	12.3	4.35**	4.9	14.8	3.06*
ICSV 112	2.8	12.6	7.92**	4.7	12.6	4.11*

*, ** t-test significant at p = 0.05 and .001, respectively. DAE = Days after seedling emergence.

Table 4.14 Effect of transplanting sorghum seedlings on oviposition by shoot fly, *A. soccata* under field conditions (ICRISAT, Patancheru, India, 2006-07 rainy season).

	Seedlings with eggs (%)					
	14 DAE			21 DAE		
Genotypes	Transplanted	Control	t-test	Transplanted	Control	t-test
IS 1054	7.4	58.8	4.63**	28.5	74.6	4.92**
IS 1057	9.9	73.4	6.64**	24.0	87.5	6.13**
IS 2146	3.5	48.1	6.02**	32.3	69.0	1.72
IS 18551	5.8	43.7	3.15*	33.6	63.0	1.45
IS 4664	7.4	45.7	4.17**	25.4	68.7	1.84
IS 2312	13.6	44.6	2.6*	30.9	63.4	1.75
IS 2205	3.3	40.0	5.29**	28.8	76.8	2.58*
SFCR 125	8.4	53.0	4.85**	26.2	76.5	2.38*
SFCR 151	12.3	60.5	6.05**	38.7	80.8	3.33*
ICSV 700	12.2	54.8	4.41**	32.7	75.3	2.15*
Swarna	28.2	89.7	4.87**	62.0	99.3	3.04*
CK 60 B	31.8	82.8	2.84*	46.0	96.3	2.84*
ICSV 745	19.9	86.4	7.52**	47.9	96.2	2.82*
296 B	17.1	72.7	4.61**	45.5	99.5	3.13*
ICSV 112	31.3	84.0	3.36*	46.1	97.9	2.98*

*, ** t-test significant at p= 0.05 and .001, respectively. DAE = Days after seedling emergence.

Table 4.15 Effect of transplanting sorghum seedlings on shoot fly, *A. soccata* damage in 15 sorghum genotypes under field conditions (ICRISAT, Patancheru, India, 2006-07 rainy season)

Genotypes	Deadhearts (%)									Tiller deadhearts (%)		
	14 DAE			21 DAE			28 DAE			28 DAE		
	Transplanted	Control	t-test	Transplanted	Control	t-test	Transplanted	Control	t-test	Transplanted	Control	t-test
IS 1054	3.4	27.6	6.94**	21.9	62.7	5.23**	36.3	78.3	2.91*	7.3	31.5	7.85**
IS 1057	5.0	34.4	2.91*	16.1	64.9	3.8*	24.1	68.7	2.95*	18.0	22.2	0.50
IS 2146	0.5	16.6	4.58**	11.9	50.7	3.43*	26.8	74.3	2.02	17.6	23.7	0.54
IS 18551	1.1	7.2	4.75**	17.5	46.9	2.56*	24.5	71.0	2.08*	9.3	22.9	3.27*
IS 4664	4.4	10.9	1.27	12.1	49.2	2.62*	31.8	64.7	1.51	13.2	24.9	2.57*
IS 2312	4.6	11.6	1.50	22.6	34.2	1.00	31.6	67.7	1.34	9.3	24.0	3.4*
IS 2205	0.0	8.8	3.27*	13.0	51.1	3.6*	26.9	76.1	2.15*	9.6	21.2	2.66*
SFCR 125	4.4	18.0	4.91**	17.6	58.5	2.8*	23.6	77.4	3.27*	3.3	30.3	2.69*
SFCR 151	3.9	20.4	5.23**	17.8	58.7	4.19**	26.6	77.0	3.00*	21.4	24.3	0.33
ICSV 700	2.6	16.8	3.01*	22.7	42.0	1.96	32.0	76.6	1.99	11.9	25.2	2.37*
Swarna	11.8	53.5	5.89**	36.7	93.0	5.59**	54.7	91.1	2.35*	23.8	50.7	2.99*
CK 60 B	13.2	44.6	2.36*	37.5	88.4	2.96*	48.3	94.2	2.64*	25.3	40.6	1.13
ICSV 745	11.3	48.4	11.29**	33.4	85.7	3.58*	55.5	83.7	3.12*	18.7	51.9	3.8*
296 B	9.0	43.1	7.07**	31.4	82.2	2.97*	54.0	96.7	2.59*	40.9	53.1	0.65
ICSV 112	17.5	41.7	1.98	32.1	90.9	4.04*	55.1	93.0	2.37*	21.0	53.9	3.26*

*, ** t-test significant at p = 0.05 and .001, respectively. DAE = Days after seedling emergence.

Genotypes IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, and ICSV 700 had significantly lower percentage of plants with deadhearts than Swarna, CK 60B, ICSV 745, 296B and ICSV 112 across treatments and observation dates.

Tiller deadhearts ranged from 3.3 to 40.9% and 21.2 to 53.9% at 28 DAE in transplanted and normal seedlings, respectively (Table 4.15). All the test genotypes had significantly lower percentage of tillers with deadhearts in the transplanted seedlings at 28 DAE, except in IS 1057, IS 2146, SFCR 151, CK 60B and 296B. Tiller deadhearts in IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, and ICSV 700 was significantly lower than in Swarna, CK 60B, ICSV 745, 296B, and ICSV 112.

4.3.2 Effect of 2, 4-D, Cu_2So_4 and KI on shoot fly damage in sorghum

The effect of copper sulphate (Cu_2So_4), potassium iodide (KI), and 2,4-dichlorophenoxy acetic acid (2,4-D) on shoot fly damage was studied on IS 18551 (resistant), and Swarna (susceptible) under field conditions. There were no effects of application of 2, 4-D, copper sulphate and potassium iodide sprays on oviposition and deadheart formation by the sorghum shoot fly, *A. soccata* during the 2006 post-rainy season (Table 4.16). Similar results were obtained during the 2007 rainy season (Table 4.17). The results suggested that these compounds did induce resistance to shoot fly, *A. soccata*.

4.4 Biochemical composition of sorghum seedlings in relation to expression of resistance to shoot fly, *Atherigona soccata*

4.4.1 Sugars

The amount of total soluble sugars in the seedlings ranged from 2.70 - 3.16% (Table 4.18). The genotypes CK 60B, ICSV 745, 296B, ICSV 112, and Swarna had significantly more amounts of soluble sugars as compared with the resistant check, IS 18551. The genotypes IS 1054, IS 2146, IS 4464, IS 2312, IS 2205, SFCR 125, SFCR 151 and ICSV 700 which are less susceptible to shoot fly damage, had low amounts of soluble sugars as compared to the susceptible check, Swarna, but were on par with the resistant check, IS 18551.

4.4.2 Polyphenols

The amounts of polyphenols ranged from 26.06 - 36.15 mg g^{-1} (Table 4.18). However, the differences between the genotypes tested were nonsignificant.

4.4.3 Tannins

The amount of tannins ranged from 0.08 - 0.21% (Table 4.18). The genotypes IS 1054, IS 1057, IS 2146, IS 18551, IS 4464, IS 2312, IS 2205, SFCR 125, SFCR 151 and ICSV 700 had significantly more amounts of tannins than CK 60B, ICSV 745, ICSV 112, and Swarna. However, 296B, which is susceptible to shoot fly, had high amounts of tannins.

Table 4.16 Effect of KI, Cu_2SO_4 and 2,4-D sprays on damage by sorghum shoot fly, *A. soccata* (ICRISAT, Patancheru, post-rainy season- 2006)

Genotype	Compound	Concentration (%)	Deadhearts (%)			Seedlings with eggs (%)		Eggs seedlings^{-10}		Tiller dead hearts (%)
			14 DAE	21 DAE	28 DAE	14 DAE	21 DAE	14 DAE	21 DAE	
IS 18551	2,4-D	0.05	0.9	30.5	36.6	27.9	38.3	3.0	4.2	3.0
		0.1	0.0	21.3	26.7	16.2	38.3	1.6	4.1	4.1
		0	0.0	23.4	30.4	18.6	27.9	1.9	3.1	0.9
	Cu_2So_4	0.05	1.0	27.1	30.8	21.4	38.5	2.1	4.1	1.0
		0.1	0.8	16.6	29.7	19.3	35.6	2.1	3.5	8.5
		0	0.8	21.1	25.5	14.1	38.0	1.4	3.3	5.1
	KI	0.05	0.9	21.5	33.2	28.4	35.7	2.9	4.1	4.5
		0.1	2.1	22.0	28.5	22.7	32.5	2.3	3.3	3.4
		0	0.9	22.1	24.8	23.0	26.6	2.6	2.8	6.5
Swarna	2,4-D	0.05	11.0	89.7	96.2	85.6	97.1	13.7	18.6	38.6
		0.1	29.4	96.6	99.5	91.4	98.1	17.6	24.1	33.4
		0	29.4	95.8	96.7	89.4	99.2	13.5	18.4	40.0
	Cu_2So_4	0.05	13.9	87.7	95.3	85.7	97.2	13.5	17.5	38.4
		0.1	23.6	88.7	95.1	87.0	96.8	14.7	20.3	40.8
		0	19.1	90.0	93.8	79.2	99.1	12.1	16.7	35.8
	KI	0.05	12.0	91.9	96.3	85.6	97.4	13.1	17.8	37.4
		0.1	30.1	91.6	96.3	80.3	98.1	11.5	17.7	29.5
		0	22.6	82.1	85.1	72.7	91.4	10.4	16.4	35.4
	F-probability		0.93	0.50	0.85	0.77	0.85	0.61	0.79	0.82
	SE ±		5.5	4.9	4.9	5.9	4.8	1.5	1.4	3.9
	LSD (P = 0.05)		15.7	14.0	14.1	17.0	14.0	4.4	4.1	11.3
	CV (%)		89.5	14.9	13.5	19.7	11.8	29.6	22.2	31.1

d = Days. DAE = Days after seedling emergence.

Table 4.17 Effect of 2, 4-D, Cu_2So_4, and KI on damage by sorghum shoot fly, *A. soccata* (ICRISAT, Patancheru, India, rainy season- 2007)

Genotype	Compound	Concentration (%)	Deadhearts (%)			Seedlings with eggs (%)		Eggs seedlings^{-10}		Tiller dead hearts (%)
			14 DAE	21 DAE	28 DAE	14 DAE	21 DAE	14 DAE	21 DAE	
IS 18551	2,4-D	0.01	19.9	47.6	54.5	55.4	63.9	7.4	8.7	51.3
		0.02	22.7	45.0	48.9	54.2	64.3	6.2	8.6	52.1
		0.05	30.9	42.6	51.5	41.7	64.3	5.0	6.9	34.7
		0.1	17.7	44.5	54.3	39.6	49.0	6.3	6.9	42.7
		0	13.9	38.9	49.9	49.2	62.0	6.0	7.4	44.6
	Cu_2SO_4	0.01	15.0	37.5	40.8	50.6	55.6	4.7	5.1	56.5
		0.02	11.2	40.9	53.5	42.3	52.0	5.3	6.4	48.2
		0.05	23.0	41.7	45.8	41.1	50.7	5.9	7.3	41.4
		0.1	24.7	41.9	46.9	36.3	46.4	4.1	5.3	38.6
		0	15.8	31.0	37.6	40.8	51.1	4.5	5.9	53.1
	KI	0.01	28.9	42.6	53.3	48.6	54.8	5.8	6.3	46.6
		0.02	14.9	48.8	60.7	50.3	61.1	6.8	7.5	55.6
		0.05	15.2	41.1	46.3	35.3	44.4	4.6	5.1	49.1
		0.1	13.4	50.7	55.7	50.8	67.1	7.1	7.6	44.9
		0	19.1	37.6	46.9	41.7	53.6	4.7	6.0	33.1
Swarna	2,4-D	0.01	75.0	86.7	88.6	79.4	91.1	13.8	16.4	64.8
		0.02	70.4	79.6	93.1	69.9	94.0	12.7	13.6	60.0
		0.05	68.7	88.7	96.5	90.4	93.1	13.8	15.5	61.9
		0.1	63.7	86.6	90.9	77.6	92.0	11.6	14.2	50.4
		0	64.9	90.2	94.5	86.8	95.5	13.4	15.0	56.9
	Cu_2SO_4	0.01	67.7	83.2	87.7	89.7	93.3	13.5	17.1	60.0
		0.02	65.4	78.8	81.7	81.7	86.3	13.9	16.2	60.9
		0.05	70.2	87.5	89.3	93.3	94.3	17.5	18.4	52.4
		0.1	63.3	90.1	90.1	89.4	92.8	13.3	16.7	60.4
		0	74.0	84.4	90.3	89.1	93.6	12.3	15.5	61.4
	KI	0.01	65.9	83.2	94.3	90.4	92.8	13.0	13.1	68.5
		0.02	66.3	90.6	93.3	83.7	93.2	14.9	17.8	64.9
		0.05	58.7	84.5	90.8	93.7	95.5	12.5	15.7	48.7
		0.1	78.0	82.1	92.3	93.9	96.5	15.1	17.9	67.7
		0	66.2	92.1	93.0	91.3	98.2	13.9	16.3	44.0
	F-probability		0.2	0.8	0.7	1.0	0.3	0.8	0.6	0.8
	SE ±		5.0	4.7	5.0	7.7	5.4	1.2	1.2	7.7
	LSD (P = 0.05)		14.1	13.6	14.4	22.0	15.5	3.5	3.5	21.9
	CV %		21.1	11.2	11.6	17.3	10.0	19.3	17.4	27.7

Table 4.18 Biochemical composition of seedlings of 15 sorghum genotypes in relation to expression of resistance to shoot fly, *A. soccata* (ICRISAT, Patancheru, 2006)

Genotypes	Soluble sugars (%)	Polyphenols (mg g^{-1})	Tannins (%)	Fats (%)	Lignins (%)	Proteins (%)
IS 1054	2.86	34.64	0.19	5.49	1.27	24.08
IS 1057	-	32.77	0.21	5.64	1.18	25.06
IS 2146	2.82	29.40	0.21	4.64	1.65	24.23
IS 4664	2.80	31.19	0.20	5.10	1.41	24.17
IS 2312	2.88	30.74	0.18	5.83	1.13	23.75
IS 2205	2.83	33.29	0.18	5.17	1.20	23.22
SFCR 125	2.80	33.70	0.17	5.40	1.25	23.38
SFCR 151	2.90	36.11	0.16	4.82	1.19	23.14
ICSV 700	2.99	35.64	0.14	5.29	1.21	23.19
CK 60 B	2.97	35.48	0.10	5.55	1.42	23.40
ICSV 745	2.99	26.06	0.10	6.44	1.19	24.51
296 B	3.16	36.15	0.18	6.89	1.10	25.44
ICSV 112	3.14	32.50	0.10	7.30	1.23	24.58
IS 18551(R)	2.70	32.09	0.16	5.76	1.67	23.99
Swarna (S)	3.10	31.82	0.08	6.40	1.43	23.24
F-probability	< 0.001	0.13	< 0.001	< 0.001	< 0.001	< 0.001
SE ±	0.03	2.23	0.02	0.12	0.09	0.09
LSD (P = 0.05)	0.09	NS	0.06	0.34	0.25	0.27
CV (%)	1.80	11.80	34.60	3.60	11.50	0.70

R = Resistant check. S = Susceptible check. - = Not studied. NS = Nonsignificant.

4.4.4 Fats

The fat content ranged from 4.64 - 7.30% (Table 4.18). Genotypes ICSV 745, 296B, ICSV 112, and Swarna had significantly more amount of fats than the resistant check, IS 18551. IS 1054, IS 1057, IS 4664, IS 2312, IS 2205, SFCR 125, ICSV 700, and CK 60B were on par with the resistant check, IS 18551; while IS 2146 and SFCR 151 had lower amounts of fats than the resistant check, IS 18551.

4.4.5 Lignins

The lignin content ranged from 1.10 - 1.67% (Table 4.18). Lignin content was significantly lower in IS 1057, IS 2312, SFCR 151, ICSV 745, and 296B as compared to the resistant check, IS 18551. Genotypes IS 2146 and IS 18551 had higher lignin content than the susceptible check, Swarna.

4.4.6 Proteins

The amounts of proteins ranged from 23.14 - 25.44% (Table 4.18). IS 2312, IS 2205, SFCR 125, SFCR 151, ICSV 700, CK 60B and Swarna had <24 % proteins compared to 25.44 % in 296B. However, there was no trend in protein content between the shoot fly-resistant and –susceptible genotypes.

4.4.7 Micronutrients profile of 15 sorghum genotypes in relation to expression of resistance to sorghum shoot fly, *Atherigona soccata*

The amounts of N, P, K, Mg, Ca, Zn, Fe, Cu, and Mn on seedlings of 15 test sorghum genotypes ranged from 3.70 - 4.07%, 0.37 - 0.55%, 2.83 -

3.69%, 0.24 - 0.42%, 0.42 - 0.66%, 29.00 - 64.33 ppm, 1556.00 - 2934.00 ppm, 15.67 - 10.00 ppm, and 104.70 - 155.50 ppm, respectively (Table 4.19). The seedlings of genotypes IS 1057, ICSV 745, 296B, and ICSV 112 had more nitrogen content than the resistant check, IS 18551. The genotypes IS 1057, IS 2146, IS 4664, IS 2312, SFCR 125, ICSV 745, 296B and IS 18551 had high P and K contents than the susceptible check, Swarna. The Mg content was greater in the resistant genotypes than in the susceptible ones, except in IS 2205. Ca content was more in the resistant check, IS 18551 than in the susceptible check, Swarna. The Zn content was significantly lower in the susceptible genotypes as compared to the resistant check, IS 18551. However, there were no significant differences in Cu content among the genotypes tested. There were significant differences in Fe content among the resistant and susceptible genotypes. The Mn content was significantly greater in IS 1057, SFCR 125, IS 4664, ICSV 700, CK 60B, and ICSV 745 than in the other genotypes tested (Table 4.19).

4.5 Association of physico-chemical characteristics of sorghum genotypes with expression of resistance to shoot fly, *Atherigona soccata*

Soluble sugars, leaf surface wetness, and fat contents were positively and significantly associated with susceptibility to shoot fly, *A. soccata*; while leaf glossiness, leaf sheath and plumule pigmentation, plant height, trichome density (adaxial and abaxial leaf surfaces), days to 50%

flowering, tannins, Mg and Zn were significantly and negatively associated with deadhearts, seedlings with eggs, and percent tillers with deadhearts. Seedling vigor was significantly and positively associated with percent seedlings with eggs and deadhearts. The N, K and Ca contents showed a positive association; while moisture content, soluble polyphenols, lignins, P, Mn, Cu and Fe showed a negative association with shoot fly damage, but the correlation coefficients were nonsignificant (Table 4. 20). Multiple linear and stepwise regressions indicated that leaf surface wetness, Mg, soluble sugars, tannins, Zn, fats, leaf glossiness, leaf sheath and plumule pigmentation, and trichome density on abaxial and adaxial leaf surfaces explained 99.8% variation for plants with deadhearts (Table 4.20).

Path coefficient analysis revealed that correlation coefficients and direct effects of leaf glossiness, plumule pigmentation, trichomes on adaxial leaf surface, Mg and fat contents were in the same direction, and these traits can be used to select for shoot fly resistance. The correlation and path coefficients for leaf pigmentation, trichomes on abaxial leaf surface, and tannins were in the opposite direction, and selection for these traits may not be effective in screening and selecting sorghum genotypes for resistance to shoot fly, *A. soccata* (Table 4.21).

Table 4.19 Micronutrient profile of 15 sorghum genotypes evaluated for resistance to sorghum shoot fly, *A. soccata* (ICRISAT, Patancheru, India 2005)

Genotype	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	Zn (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)
IS 1054	3.85	0.41	3.12	0.38	0.45	53.50	1984.00	13.50	113.50
IS 1057	4.01	0.55	3.15	0.42	0.66	55.67	1866.00	14.33	130.00
IS 2146	3.88	0.51	3.23	0.35	0.52	64.33	1972.00	14.50	126.30
IS 4664	3.87	0.49	3.37	0.34	0.52	44.83	2653.00	15.00	137.70
IS 2312	3.80	0.49	3.69	0.38	0.53	39.50	2180.00	15.00	106.00
IS 2205	3.72	0.45	2.94	0.28	0.49	38.00	2257.00	14.50	115.00
SFCR 125	3.74	0.46	3.32	0.32	0.50	38.50	2113.00	15.00	155.50
SFCR 151	3.70	0.44	3.21	0.31	0.52	41.50	2110.00	12.50	108.50
ICSV 700	3.71	0.47	3.03	0.33	0.55	39.17	2313.00	15.67	138.30
CK 60B	3.74	0.46	2.86	0.30	0.43	35.67	2934.00	15.67	137.30
ICSV 745	3.92	0.49	3.41	0.28	0.51	31.50	2443.00	15.00	137.50
296B	4.07	0.50	3.32	0.32	0.54	29.00	2248.00	10.00	115.50
ICSV 112	3.93	0.45	3.54	0.27	0.53	31.50	2055.00	14.00	117.00
IS 18551 (R)	3.84	0.46	3.02	0.32	0.51	45.50	1556.00	14.00	118.00
Swarna (S)	3.72	0.37	2.83	0.24	0.42	39.00	1994.00	12.67	104.70
F-probability	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.151	< 0.001
SE ±	0.01	0.01	0.03	0.01	0.02	0.91	17.96	1.18	1.16
LSD (*P* = 0.05)	0.04	0.02	0.09	0.03	0.06	2.62	52.02	NS	3.35
CV %	0.70	2.30	1.70	4.80	7.00	3.80	1.40	14.50	1.60

R = Resistant check. S = Susceptible check. NS = Nonsignificant.

Table 4.20 Correlation coefficients of shoot fly damage parameters under field conditions with morphological, biochemical, and nutritional traits (ICRISAT, Patancheru, India)

Morphological traits	Deadhearts (%)			Seedlings with eggs (%)		Eggs seedlings^{-10}		Tiller deadhearts (%)
	14 DAE	21 DAE	28 DAE	14 DAE	21 DAE	21 DAE	14 DAE	
Morphological traits								
Leaf glossiness (GS)	0.96**	0.98**	0.97**	0.98**	0.97**	0.96**	0.98**	0.86**
Bottom leaf pigmentation (BLP)	0.64**	0.65**	0.61*	0.70**	0.59*	0.61*	0.64**	0.61*
Leaf sheath pigmentation (LSP)	0.72**	0.72**	0.66**	0.81**	0.67**	0.64**	0.76**	0.69**
Plumule pigmentation (PP)	0.66**	0.68**	0.63*	0.73**	0.63*	0.62*	0.72**	0.83**
Plant height (PH)	-0.78**	-0.84**	-0.82**	-0.80**	-0.83**	-0.84**	-0.78**	-0.71**
Productive tillers (PT)	0.43	0.44	0.45	0.43	0.42	0.49	0.39	0.29
Seedling vigor (SV)	0.50	0.63*	0.67**	0.51*	0.66**	0.64**	0.51*	0.39
Trichome density (Adaxial)	-0.90**	-0.90**	-0.87**	-0.94**	-0.89**	-0.87**	-0.93**	-0.83**
Trichome density (Abaxial)	-0.91**	-0.90**	-0.88**	-0.94**	-0.90**	-0.88**	-0.93**	-0.84**
Days to 50% flowering (DF)	-0.54*	-0.53*	-0.52*	-0.53*	-0.50	-0.52*	-0.50	-0.42
Biochemical traits								
Moisture content (MC)	-0.50	-0.38	-0.37	-0.48	-0.35	-0.36	-0.53*	-0.46
Protein	0.21	0.22	0.17	0.23	0.24	0.15	0.23	0.22
Total soluble polyphenols (TSP)	-0.33	-0.25	-0.24	-0.23	-0.17	-0.14	-0.27	-0.28
Tannins (T)	-0.87**	-0.84**	-0.85**	-0.84**	-0.82**	-0.86**	-0.86**	-0.75**
Total soluble sugars (TSS)	0.76**	0.74**	0.72**	0.80**	0.73**	0.74**	0.80**	0.72**
Fat (F)	0.80**	0.78**	0.75**	0.82**	0.78**	0.76**	0.83**	0.81**
Lignin (L)	-0.06	-0.09	-0.08	-0.14	-0.04	-0.07	-0.12	-0.27
Leaf surface wetness (LSW)	0.95**	0.91**	0.90**	0.94**	0.89**	0.90**	0.95**	0.88**
Nutritional traits								
Nitrogen (N)	0.21	0.22	0.17	0.23	0.24	0.15	0.23	0.22
Phosphorus (P)	-0.28	-0.21	-0.23	-0.24	-0.22	-0.26	-0.23	-0.08
Potassium (K)	0.12	0.11	0.09	0.12	0.12	0.12	0.12	0.19
Calcium (Ca)	0.03	0.08	0.03	0.18	0.06	0.03	0.13	0.18
Magnesium (Mg)	-0.77**	-0.73**	-0.69**	-0.75**	-0.69**	-0.71**	-0.76**	-0.73**
Manganese (Mn)	-0.07	0.05	0.06	-0.03	0.04	0.03	-0.06	-0.06
Copper (Cu)	-0.31	-0.33	-0.33	-0.33	-0.36	-0.36	-0.32	-0.25
Iron (Fe)	-0.14	-0.03	-0.002	-0.09	0.01	-0.02	-0.09	-0.10
Zinc (Zn)	-0.63*	-0.67**	-0.70**	-0.66**	-0.66**	-0.70**	-0.67**	-0.77**

Percentage deadhearts with morpho-, chemo-, and nutritional traits:

Multiple linear regression equation:

Deadhearts (%) = 102.3 - 0.49 LSW -108.5 Mg -37.62 TSS+15.7 T + 0.167 Zn + 2.418 F+ 24.75 GS - 1.88 BLP -3.31 LSP + 2.775 PP - 0.318 Adaxial + 0.3856 Abaxial (R^2 = 99.8%)

Stepwise regression equation:

Deadherats (%) = 108.7 - 108.5 Mg - 37.88 TSS + 23.2 T +1.817 F + 24.689 GS - 3.784 BLP + 1.612 PP - 0.1778 Adaxial + 0.3152 Abaxial (R^2 = 99.8 %)

*, ** Correlation coefficients significant at P = 0.05 and 0.01, respectively.

Table 4.21 Direct and indirect path coefficients for deadheart formation due to sorghum shoot fly, *A. soccata* and physico-chemical traits of 15 sorghum genotypes (ICRISAT, Patancheru, India)

Traits	X1	X2	X3	X4	X5	X6	X7	X8	X9	r
Leaf glossiness (X1)	**1.75**	0.08	-0.09	-1.13	0.45	0.13	0.03	-0.06	-0.19	0.96**
Plumule pigmentation (X2)	1.19	**0.12**	-0.09	-0.86	0.33	0.10	0.02	-0.05	-0.10	0.67**
Bottom leaf pigmentation (X3)	1.07	0.07	**-0.15**	-0.70	0.27	0.14	0.02	-0.05	-0.11	0.56*
Trichome (Abaxial) (X4)	-1.68	-0.08	0.09	**1.17**	-0.47	-0.12	-0.03	0.06	0.18	-0.89**
Trichome (Adaxial) (X5)	-1.68	-0.08	0.09	1.16	**-0.47**	-0.12	-0.03	0.06	0.19	-0.88**
Magnesium (X6)	-1.18	-0.06	0.11	0.74	-0.29	**-0.19**	-0.02	0.06	0.15	-0.69**
Fat (X7)	1.41	0.06	-0.07	-0.95	0.39	0.11	**0.04**	-0.05	-0.18	0.77**
Tannin (X8)	-1.34	-0.07	0.09	0.79	-0.32	-0.13	-0.02	**0.08**	0.14	-0.77**
Total soluble sugars (X9)	1.38	0.05	-0.07	-0.87	0.37	0.12	0.03	-0.05	**-0.24**	0.72**

Path coefficients equation :

Deadhearts (%) = 83.87 + 1.75 X1 + 0.12 X2 - 0.15 X3 + 1.17 X4 - 0.47 X5 - 0.19 X6 + 0.04 X7 + 0.08 X8 - 0.24 X9 (Residual variance = 0.02).

*, ** Correlation coefficients (r) significant at *P* = 0.05 and 0.01, respectively.

4.6 Associations of physico-chemical characteristics of sorghum genotypes with expression of antibiosis to shoot fly, *Atherigona soccata*

4.6.1 Morphological traits

Leaf glossiness, bottom leaf, leaf sheath, and plumule pigmentation were negatively associated with larval survival and adult emergence, but positively associated with developmental period. Plant pigmentation was negatively correlated with pupal mortality, female pupal weight, and fecundity. Trichomes on the adaxial and abaxial surface of leaves showed significant and negative association with larval survival, and adult emergence, but positive association with developmental period. The results suggested that the genotypes with high leaf glossiness, trichomes and pigmentation contributed to antibiosis component of resistance to sorghum shoot fly, *A. soccata* (Table 4.22).

4.6.2 Biochemical traits

Total soluble sugars, fats, and leaf surface wetness showed significant and positive association with larval survival and adult emergence, but negative association with developmental period. Protein content was significantly and negatively correlated with the pupal mortality, but positively correlated with the adult emergence. Soluble sugars, proteins, fats, and leaf surface wetness contributed to shoot fly susceptibility. Tannins showed a significant and positive correlation with developmental

period. Tannins, moisture content, total soluble polyphenols, and lignins were responsible for antibiosis to shoot fly (Table 4.22).

4.6.3 Nutritional traits

Nitrogen content showed a significant and negative correlation with pupal mortality, and positive correlation with adult emergence. Potassium content was positively correlated with female pupal weight; while magnesium content was significantly and positively correlated with developmental period, but negatively correlated with larval survival, adult emergence, and male pupal weight. Zinc content showed a significant and negative correlation with larval survival. Phosphorus, calcium, manganese, copper and iron amounts were also associated with susceptibility to shoot fly, but the correlation coefficients were nonsignificant (Table 4.22).

Multiple linear regressions indicated that leaf surface wetness, Mg, total soluble sugars, tannins, fats, leaf glossiness, bottom leaf pigmentation, leaf sheath pigmentation, plumule pigmentation, and trichomes on abaxial and adaxial leaf surfaces explained 76.7% variation in developmental period. Leaf surface wetness, Mg, total soluble sugars, Zn, fats, leaf glossiness, bottom leaf pigmentation, leaf sheath pigmentation, plumule pigmentation, and trichomes on abaxial and adaxial leaf surfaces explained 19% of the total variability for larval survival; while leaf surface wetness, Mg, N, P, total soluble sugars, fats, leaf glossiness, bottom leaf, leaf sheath and plumule pigmentation, and

trichomes on abaxial and adaxial leaf surfaces explained 87.6% of the total variability for adult emergence (Table 4.22). Stepwise regression indicated that tannins, plumule pigmentation, and trichomes on abaxial and adaxial leaf surfaces explained 92.1% of the total variability for developmental period. Trichomes on abaxial leaf surfaces explained 51% of the total variability for larval survival; while Mg, N, leaf sheath pigmentation, and trichomes on abaxial and adaxial leaf surfaces explained 96.5% of the total variability for adult emergence (Table 4.22).

Table 4.22 Correlations between antibiosis to shoot fly and morphological, chemical, and nutritional traits (ICRISAT, Patancheru, India)

	Developmental period (d)	Larval survival (%)	Pupal mortality (%)	Adult emergence (%)	Male pupal weight (mg)	Female pupal weight (mg)	Fecundity female^{-1}	Shoot fly deadhearts (GH)
Deadhearts (%) (GH)	-0.87**	0.60*	-0.55*	0.77**	0.59*	-0.33	-0.29	*
Morphological traits								
Leaf glossiness (GS)	-0.91**	0.73**	-0.36	0.79**	0.41	-0.32	-0.29	0.83**
Bottom leaf pigmentation (BLP)	-0.57*	0.55*	-0.18	0.55*	0.15	-0.08	-0.40	0.32
Leaf sheath pigmentation (LSP)	-0.61*	0.54*	-0.46	0.71**	0.15	-0.10	-0.33	0.56*
Plumule pigmentation (PP)	-0.52*	0.60*	-0.30	0.66**	-0.06	0.12	-0.19	0.49
Trichome density (Adaxial)	0.85**	-0.69*	0.46	-0.83**	-0.34	0.24	0.20	-0.81**
Trichome density (Abaxial)	.88**	-0.74*	0.45	-0.86**	-0.34	0.16	0.21	-0.82**
Biochemical traits								
Moisture content (MC)	0.48	-0.13	0.44	-0.39	-0.17	0.13	-0.28	-0.66**
Protein	-0.29	0.47	-0.53*	0.70**	0.41	0.10	-0.40	0.35
Total soluble polyphenols (TSP)	0.35	-0.28	0.29	-0.40	-0.22	-0.30	-0.12	-0.25
Tannins (T)	0.75**	-0.50	0.13	-0.47	-0.20	0.35	0.00	-0.67**
Total soluble sugars (TSS)	-0.65**	0.53*	-0.32	0.61*	0.44	-0.40	-0.35	0.74**
Fat (F)	-0.67**	0.64**	-0.43	0.76**	0.48	-0.26	-0.30	0.61*
Lignin (L)	-0.02	-0.24	-0.21	-0.05	0.11	0.08	0.14	0.03
Leaf surface wetness (LSW)	-0.89**	0.67**	-0.46	0.81**	0.54*	-0.29	-0.27	0.83**
Nutritional traits								
Nitrogen (N)	-0.29	0.47	-0.52*	0.70**	0.41	0.10	-0.40	0.35
Phosphorus (P)	0.23	0.00	-0.06	0.04	-0.14	0.44	-0.19	-0.05
Potassium (K)	0.05	0.27	0.06	0.18	-0.17	0.59*	-0.21	-0.15
Calcium (Ca)	0.40	0.00	0.21	-0.13	-0.15	0.36	-0.06	-0.24
Magnesium (Mg)	0.69**	-0.52*	0.25	-0.56*	-0.52*	0.31	0.04	-0.66**
Manganese (Mn)	0.12	-0.03	0.05	-0.05	0.04	-0.19	-0.40	-0.07
Copper (Cu)	0.34	-0.44	0.01	-0.35	-0.37	0.36	-0.06	-0.26
Iron (Fe)	-0.36	0.33	0.13	0.18	-0.22	-0.14	-0.09	0.40
Zinc (Zn)	0.48	-0.55*	-0.01	-0.43	-0.13	0.29	0.09	-0.41

Multiple linear regression equation

Developmental period: 16.30 - 0.075 LSW + 0.47 Mg - 0.12 TSS + 4.20 T + 0.142 F - 0.133 GS + 0.026 BLP - 0.109 LSP + 0.182 PP + 0.0271 Abaxial - 0.0336 Adaxial (R^2 = 76.7%).

Larval survival (%): - 118.0 - 18.5 LSW out - 149.0 Mg + 76.1 TSS + 0.29 Zn + 8.07 F+ 3.44 GS + 6.7 BLP - 5.6 LSP + 0.49 PP - 0.610 Abaxial + 0.63 (R^2 = 19.0%).

Adult emergence (%): - 140 - 2.95 LSW out - 170.8 Mg + 226 N - 27 Protein + 9.4 TSS + 1.33 F - 1.95 GS - 0.54 BLP + 5.39 LSP - 1.35 PP - 0.272 Abaxial + 0.315 Adaxial (R^2 = 87.6%).

Stepwise regression equation

Developmental period: 15.496 + 6.23 T + 0.1743 PP + 0.02776 Abaxial - 0.02973 Adaxial (R^2 = 92.1%).

Larval survival (%): 84.58 - 0.0894 Abaxial (R^2 = 51.0%).

Adult emergence (%): - 152.2 - 133.3 Mg + 62.39 N + 3.780 LSP - 0.1510 Abaxial - 0.231 adaxial (R^2 = 96.5%).

*, ** Correlation coefficients significant at *P* = 0.05 and 0.01, respectively.

4.7 HPLC fingerprints of phenolic compounds in damaged and undamaged seedlings of sorghum

The High Performance Liquid Chromatographic (HPLC) profiles of phenolic compounds in the seedlings revealed considerable differences between the damaged and undamaged sorghum seedlings (Tables 4.23 and 4.24; Fig. 4.1) *p*-hydroxy benzoic acid (RT 18.63) was present in both damaged and undamaged seedlings of all the genotypes, except in SFCR 151, while *p*-hydroxybenzoic acid amounts were more in undamaged seedlings of shoot fly- resistant genotypes. However, amounts of this compound were low in damaged seedlings of shoot fly- resistant genotypes (IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, IS 2205, SFCR 151 and ICSV 700), indicating an important role of this compound in host plant resistance to *A. soccata*. Amounts of *p*-hydroxy benzoic acid were greater in damaged seedlings of the shoot fly- susceptible genotypes.

Amounts of *p*-hydroxy benzoic acid were greater (> 0.35-0.80 mg g^{-1}) in Swarna, CK 60B, ICSV 745, 296B, and ICSV 112 as compared to the resistant check, IS 18551 (0.06mg g^{-1}). However, amounts of *p*-hydroxy benzoic acid in IS 4664 and ICSV 700 (which are resistant to shoot fly damage) were on par with the resistant check, IS 18551.

p-hydroxy benzaldehyde (RT 23.41) was present in undamaged seedlings of all the test genotypes, except in IS 2312, SFCR 125, SFCR 151 and 296B. However, in the shoot fly damaged seedlings, it was

present only in the shoot fly susceptible genotypes. In the undamaged seedlings, *p*-hydroxy benzaldehyde amounts were more in the susceptible genotypes [Swarna (0.16 mg g^{-1}), CK 60B (0.18 mg g^{-1}), ICSV 745 (0.12 mg g^{-1}) and ICSV 112 (0.25 mg g^{-1})] as compared to the resistant check, IS 18551 (0.08 mg g^{-1}). The results suggested that the amounts of *p*-hydroxy benzoic acid and *p*-hydroxy benzaldehyde were greater in the shoot fly susceptible genotypes, and their concentrations declined in the shoot fly damaged seedlings. This may be one of the reasons for non-preference of damaged seedlings for oviposition by the shoot fly females.

Protocatechuic acid (RT 11.46), *p*-coumaric acid (RT 29.33), and cinnamic acid (RT 43.24) were absent in all the genotypes in damaged and undamaged seedlings, except protocatechuic acid, which was present in IS 1054, and *p*-coumaric acid, which was present in undamaged seedlings of IS 1054 and ICSV 745. Small amounts of cinnamic acid were detected in damaged seedlings of IS 2146, IS 4664, and IS 2205. Small quantities of luteolin (RT 41.93) and apigenin (RT 43.58) were present in damaged and undamaged seedlings of most of the test genotypes. However, apigenin was present in resistant check, IS 18551, but absent in undamaged seedlings of Swarna and SFCR 125. Apigenin was absent in damaged seedlings of IS 18551, SFCR 151 and 296B, but present in the susceptible check, Swarna (Tables 4.23 and 4.24).

The compounds at peaks RT 21.44 and RT 40.66 in the undamaged seedlings were present in shoot fly resistant genotypes, except RT 21.44 in IS 1054 and RT 40.66 in SFCR 125, but absent in the susceptible genotypes (except ICSV 745). The compound at peak RT 24.38 was present only in undamaged seedlings of the susceptible genotypes, but absent in the resistant genotypes, except in IS 1057 and IS 4664. However, this compound was absent in damaged seedlings of all the genotypes. These compounds probably play an important role in expression of resistance/susceptibility to sorghum shoot fly, *A. soccata* (Table 4.23 and 4.24).

The compounds at peaks RT 2.34 and RT 4.15 were absent in undamaged seedlings of all the genotypes, but present in the damaged seedlings, except the compound at RT 4.15 which was absent in IS 18551, IS 2312 and SFCR 151. Compounds with peaks at RTs 2.13, 20.30, 36.51, 38.88, and 39.56 were present in undamaged seedlings of all the genotypes, but absent in the damaged seedlings, although, there were some exceptions (Tables 4.23 and 4.24), indicating that resistance to shoot fly is mediated through a complex interaction of secondary metabolites, and shoot fly response to these compounds.

Compounds with peaks at RTs 2.76 and 3.70 were in greater concentrations in damaged seedlings than in the undamaged seedlings. There were significant differences in the amounts of these compounds between shoot fly resistant and susceptible genotypes. The compound at

RT 2.76 was absent in damaged seedlings of IS 2205 and 296B. Greater amounts of the compound at RT 37.08 were recorded in undamaged seedlings as compared to damaged seedlings of different genotypes. Its amounts were greater in the resistant genotypes than in the susceptible check. The compound at RT 38.88 was present in both damaged and undamaged seedlings of different genotypes, except in damaged seedlings of IS 2205, SFCR 151, ICSV 700 and Swarna (Tables 4.23 and 4.24).

4.7.1 Association of phenolic compounds with expression of resistance to *Atherigona soccata*

p-hydroxy benzaldehyde and the compound at peak RT 24.38 were significantly and positively associated with percent deadhearts at 14, 21 and 28 DAE, seedlings with eggs, eggs per 10 seedlings at 14 and 21 DAE, and tiller deadhearts at 28 DAE. However, the compound at RT 24.38 was not significantly associated with deadhearts at 14 DAE and tiller deadhearts at 28 DAE. The compound at RT 3.70 was also significantly and positively associated with deadhearts at 21 and 28 DAE, eggs per 10 seedlings at 14 DAE, but the correlation coefficients were nonsignificant. Amounts of *p*-hydroxy benzoic acid and luteolin were positively correlated with shoot fly damage, but the correlation coefficients were nonsignificant. Amounts of protocatechuic acid, *p*-coumaric acid, cinnamic acid and apigenin were negatively associated with shoot fly damage, but the correlation coefficients were nonsignificant. The results suggested that *p*-hydroxybenzaldehyde, *p*-

hydroxy benzoic acid, luteolin, and the compounds at RTs 24.38 and 3.70 were associated with susceptibility to shoot fly, while protocatechuic acid, *p*-coumaric acid, cinnamic acid, and apigenin were associated with resistance to shoot fly, *A. soccata* (Table 4.25).

Compounds at RTs 2.34, 2.76, 4.15, 20.30, 22.61 and 36.51 were associated positively, while those at RTs 2.13, 21.44, 37.08, 38.63, 38.88, 39.56 and 40.66 were associated negatively with shoot fly damage, but the correlation coefficients were nonsignificant (except the compound at RT 39.56 with tiller deadhearts) (Table 4.25).

4.7.2 Association of biochemical constituents with expression of antibiosis to shoot fly, *Atherigona soccata*

There was no significant association between phenol content and expression of antibiosis to shoot fly in the greenhouse. Amounts of *p*-hydroxy benzoic acid and *p*-hydroxy benzaldehyde were negatively associated with larval period, pupal period, pupal mortality, female pupal weight, and fecundity; but positively correlated with larval survival, adult emergence, and male pupal weight.

Protocatechuic acid showed a negative correlation with biological parameters, except the larval period and pupal mortality. *p*-coumaric acid was negatively correlated with pupal period, pupal mortality, pupal weights, and fecundity, but positively correlated with larval period and adult emergence. Cinnamic acid amounts were negatively correlated with larval period, larval survival, pupal mortality, and adult emergence; but

positively correlated with the pupal period, pupal weights, and fecundity. Luteolin amounts were negatively correlated with larval survival, pupal mortality, pupal weights, and fecundity, but positively correlated with larval and pupal periods, adult emergence, and male pupal weight. Apigenin content was negatively correlated with larval survival, pupal mortality, adult emergence, and male pupal weight, but positively correlated with larval and pupal periods, pupal weights, and fecundity (Table 4.26).

4.7.3 Effect of *p*-hydroxy benzaldehyde and *p*-hydroxy benzoic acid on oviposition and damage by sorghum shoot fly, *Atherigona soccata*

There was no significant effect of spraying *p*-hydroxy benzaldehyde on oviposition and damage by the sorghum shoot fly, *A. soccata*. However, deadheart incidence in the shoot fly resistant genotype, IS 18551 was greater in plots sprayed with 0.01% of *p*-hydroxy benzaldehyde than in the unsprayed plots at 14 and 21 DAE. In the susceptible genotype, Swarna, the deadheart incidence increased with an increase concentration of *p*-hydroxy benzoic acid at 14 DAE during the 2006 post rainy season. However, such a trend was not apparent at 21 DAE as over all shoot fly damage was very high. However, a slight increase in oviposition was observed with an increase in concentration of *p*-hydroxy benzoic acid (Fig. 4.12 and 4.13).

Table 4.23 Qualitative and quantitative analysis of the flavonoids in damaged seedlings of 15 sorghum genotypes (ICRISAT, Patancheru, India).

		Deadheart seedlings (areas)						
RT (min)	Phenol (mg g-1)	IS 1057	IS 2146	IS 18551(R)	IS 4664	IS 2312	IS 2205	SFCR 151
2.13	Unknown	*	*	*	*	*	*	*
2.34	Unknown	66028	54654	41594	40510	44536	101017	37238
2.76	Unknown	975269	1020405	713069	966412	839340	*	665055
3.70	Unknown	381085	383588	298204	365632	365116	517845	290851
4.15	Unknown	60558	34787	*	31865	*	83750	*
11.46	Protocatechuic acid #	*	*	*	*	*	*	*
18.63	p-hydroxybenzoic acid #	0.19	0.11	0.06	0.37	0.11	0.23	*
20.30	Unknown	*	*	*	*	*	*	*
21.44	Unknown	*	*	*	*	*	*	*
22.61	Unknown	*	*	*	*	*	*	*
23.41	p-hydroxybenzaldehyde #	*	*	*	*	*	*	*
24.38	Unknown	*	*	*	*	*	*	*
29.33	p-coumaric acid #	*	*	*	*	*	*	*
36.51	Unknown	*	*	*	*	*	*	*
37.08	Unknown	1486458	2491009	2465953	2084342	3769277	2147155	2122041
37.50	Unknown	*	*	*	*	*	*	*
38.07	Unknown	28466	118316	79678	44328	74392	277685	*
38.63	Unknown	*	*	*	*	*	*	*
38.88	Unknown	155675	200151	207773	121519	530356	*	*
38.96	Unknown	240823	*	*	*	*	*	259315
39.56	Unknown	*	*	*	*	*	*	*
40.66	Unknown	*	*	*	*	*	*	*
41.93	Luteolin #	0.02	0.01	0.01	0.02	0.02	0.03	0.01
43.24	Cinnamic acid #	*	0.01	*	0.01	*	0.01	*
43.58	Apigenin #	0.04	0.02	*	0.03	0.02	0.03	*

Continued…….Table 4.23.

Continued.....Table 4.23

		Deadheart seedlings (areas)					
RT (min)	Phenol (mg g-1)	ICSV 700	Swarna (S)	CK 60B	ICSV 745	296B	ICSV 112
2.13	Unknown	*	*	*	*	*	*
2.34	Unknown	151825	25302	56218	145780	38515	56548
2.76	Unknown	1393730	1105621	1186186	1573181	*	1446664
3.70	Unknown	580811	460390	581455	786214	540660	578366
4.15	Unknown	101684	58858	49956	179537	158929	145105
11.46	Protocatechuic acid #	*	*	*	*	*	*
18.63	p-hydroxybenzoic acid #	0.68	0.36	0.35	0.80	0.60	0.37
20.30	Unknown	*	*	*	*	*	*
21.44	Unknown	*	*	*	*	*	*
22.61	Unknown	*	*	*	*	*	*
23.41	p-hydroxybenzaldehyde #	*	0.17	0.10	0.14	0.11	0.06
24.38	Unknown	*	*	*	*	*	*
29.33	p-coumaric acid #	*	*	*	*	*	*
36.51	Unknown	*	*	*	*	*	*
37.08	Unknown	2587979	1211683	2042730	546532	2028982	1562783
37.50	Unknown	*	*	*	*	*	*
38.07	Unknown	43252	74046	52058	*	27401	32952
38.63	Unknown	*	*	*	*	*	*
38.88	Unknown	*	*	33294	64250	82066	98113
38.96	Unknown	177965	*	*	*	*	*
39.56	Unknown	*	*	*	*	*	*
40.66	Unknown	*	*	*	*	*	*
41.93	Luteolin #	0.03	0.01	0.02	0.01	0.03	0.02
43.24	Cinnamic acid #	*	*	*	*	*	*
43.58	Apigenin #	0.03	0.02	0.01	0.01	*	0.02

Concentrations of identified phenols were calculated by comparing the mean peak area of samples with peak area of standards at known concentrations.

Note: IS 1054 and SFCR 125 genotypes were missied while experimenting.

Table 4.24 Qualitative and quantitative analysis of flavonoids in undamaged seedlings of 15 sorghum genotypes (ICRISAT, Patancheru, India)

		Control seedlings (areas)							
RT (min)	Phenol (mg g-1)	IS 1054	IS 1057	IS 2146	IS 18551 (R)	IS 4664	IS 2312	IS 2205	SFCR 125
2.13	Unknown	87034	67700	69211	29724	53742	36481	63270	*
2.34	Unknown	*	*	*	*	*	*	*	*
2.76	Unknown	558442	566594	682367	510036	621913	439085	425634	500763
3.70	Unknown	121275	181126	245190	188957	241301	171374	182416	243796
4.15	Unknown	*	*	*	*	*	*	*	*
11.46	Protocatechuic acid#	0.16	*	*	*	*	*	*	*
18.63	p-hydroxybenzoic acid #	0.04	0.30	0.31	0.56	0.32	0.43	0.23	0.33
20.30	Unknown	243132	1697681	1758269	3170205	1822104	2424210	1277352	1844643
21.44	Unknown	*	72474	564450	709793	683995	453825	342665	616512
22.61	Unknown	*	*	139252	*	*	*	*	*
23.41	p-hydroxybenzaldehyde #	0.08	0.08	0.08	0.08	0.18	*	0.06	*
24.38	Unknown	*	111356	*	*	173872	*	*	*
29.33	p-coumaric acid #	1.55	*	*	*	*	*	*	*
36.51	Unknown	*	*	*	86352	203160	69925	97423	66310
37.08	Unknown	12228713	14473261	13511694	14135761	19966377	10714825	12101385	8633995
37.50	Unknown	63777	*	*	*	*	*	*	*
38.07	Unknown	88692	106162	89250	89995	31280	91645	111425	42443
38.63	Unknown	618200	628000	919730	564843	*	*	*	*
38.88	Unknown	557901	527849	617074	698718	907949	425474	484684	499910
38.96	Unknown	*	*	*	*	*	*	*	*
39.56	Unknown	*	79267	124526	159013	36403	48527	143206	*
40.66	Unknown	44183	25210	46047	35858	35629	31283	26741	*
41.93	Luteolin #	0.02	0.02	0.01	0.02	0.02	0.01	0.02	0.01
43.24	Cinnamic acid #	*	*	*	*	*	*	*	*
43.58	Apigenin #	0.03	0.02	0.02	0.03	0.03	0.03	0.02	*

Concentrations of identified phenols was calculated by comparing the mean peak area of samples with peak area of standards at known concentrations. * Peak area was absent.

Continued..... 4.24

Continued ……Table 4.24

		Control seedlings (areas)						
RT (min)	Phenol (mg g-1)	SFCR 151	ICSV 700	Swarna (S)	CK 60B	ICSV 745	296B	ICSV 112
2.13	Unknown	59175	18492	25239	40983	16177	*	*
2.34	Unknown	*	*	*	*	*	*	*
2.76	Unknown	621906	624937	619835	860778	880488	678874	656295
3.70	Unknown	249008	271207	280042	324170	398208	313924	303289
4.15	Unknown	*	*	*	*	*	*	*
11.46	Protocatechuic acid#	*	*	*	*	*	*	*
18.63	p-hydroxybenzoic acid #	0.39	0.35	0.34	0.33	0.42	0.22	0.74
20.30	Unknown	2227047	1972889	1917151	1885961	2376145	1227571	4169274
21.44	Unknown	884863	651141	*	*	*	*	*
22.61	Unknown	*	*	497855	*	256633	*	*
23.41	p-hydroxybenzaldehyde #	0.25	0.12	0.16	0.18	0.12	*	0.25
24.38	Unknown	*	*	353151	925240	409544	383361	441620
29.33	p-coumaric acid #	*	*	*	*	0.40	*	*
36.51	Unknown	*	*	80713	141844	194252	176425	274861
37.08	Unknown	11978606	16002339	9405818	13139772	11714644	11158047	16798867
37.50	Unknown	*	*	20739	480130	54314	*	*
38.07	Unknown	26249	92370	76701	78964	*	75235	60244
38.63	Unknown	77169	755418	889553	*	82040	*	61220
38.88	Unknown	835256	404745	160673	802461	447477	289137	353071
38.96	Unknown	*	*	*	*	*	*	*
39.56	Unknown	*	*	*	86037	48608	52925	149367
40.66	Unknown	47999	28078	*	*	54579	*	*
41.93	Luteolin #	0.01	0.02	0.01	0.02	0.02	0.02	0.02
43.24	Cinnamic acid #	*	*	*	*	*	*	*
43.58	Apigenin #	0.02	0.02	*	0.01	0.03	0.02	0.02

Concentrations of identified phenols was calculated by comparing the mean peak area of samples with peak area of standards at known concentrations.

Table 4.25 Associations of phenolic compounds with expression of resistance to sorghum shoot fly, *A. soccata* (ICRISAT, Patancheru, India)

RT (min)	Phenol	Deadhearts (%)			Seedlings with eggs (%)		Eggs seedlings^{-10}		Tiller deadhearts (%)
		14 DAE	21 DAE	28 DAE	14 DAE	21 DAE	14 DAE	21 DAE	
2.13	Unknown	-0.32	-0.32	-0.32	-0.30	-0.31	-0.33	-0.32	-0.41
2.34	Unknown	0.04	0.05	0.05	0.04	0.02	0.06	0.01	0.06
2.76	Unknown	0.39	0.39	0.36	0.39	0.37	0.38	0.35	0.34
3.70	Unknown	0.50	0.51*	0.51*	0.50	0.48	0.52*	0.47	0.50
4.15	Unknown	0.37	0.38	0.36	0.38	0.36	0.39	0.34	0.37
11.46	Protocatechuic acid	-0.16	-0.14	-0.16	-0.09	-0.13	-0.14	-0.18	-0.24
18.63	p-hydroxybenzoic acid	0.47	0.47	0.46	0.45	0.45	0.48	0.43	0.52*
20.30	Unknown	0.08	0.06	0.04	0.07	0.05	0.08	0.05	0.18
21.44	Unknown	-0.42	-0.40	-0.39	-0.47	-0.39	-0.45	-0.39	-0.36
22.61	Unknown	0.39	0.32	0.34	0.29	0.29	0.32	0.31	0.17
23.41	p-hydroxybenzaldehyde	0.52*	0.52*	0.51*	0.49	0.52*	0.51*	0.52*	0.43
24.38	Unknown	0.49	0.52*	0.51*	0.53*	0.52*	0.52*	0.52*	0.48
29.33	p-coumaric acid	-0.08	-0.06	-0.08	-0.02	-0.07	-0.07	-0.12	-0.16
36.51	Unknown	0.36	0.36	0.35	0.36	0.36	0.38	0.34	0.44
37.08	Unknown	-0.11	-0.09	-0.10	-0.09	-0.07	-0.09	-0.09	-0.09
37.50	Unknown	0.20	0.25	0.25	0.28	0.24	0.24	0.24	0.20
38.07	Unknown	-0.37	-0.45	-0.43	-0.36	-0.46	-0.35	-0.43	-0.36
38.63	Unknown	-0.11	-0.14	-0.15	-0.13	-0.14	-0.14	-0.15	-0.27
38.88	Unknown	-0.30	-0.27	-0.28	-0.28	-0.26	-0.30	-0.28	-0.23
38.96	Unknown	-0.22	-0.16	-0.16	-0.18	-0.14	-0.20	-0.12	-0.23
39.56	Unknown	-0.06	-0.09	-0.13	-0.03	-0.11	-0.02	-0.12	0.09
40.66	Unknown	-0.29	-0.28	-0.30	-0.31	-0.30	-0.31	-0.34	-0.31
41.93	Luteolin	-0.05	0.01	0.01	0.04	0.05	0.04	0.03	0.06
43.24	Cinnamic acid	-0.22	-0.25	-0.23	-0.27	-0.24	-0.24	-0.24	-0.30
43.58	Apigenin	-0.23	-0.24	-0.25	-0.19	-0.23	-0.20	-0.25	-0.26

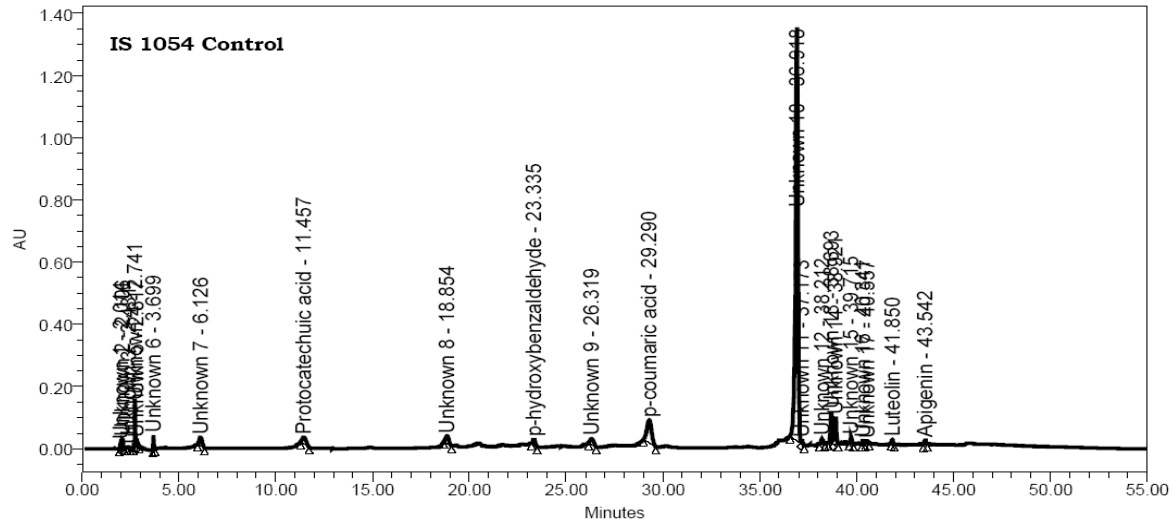
* Correlation coefficients significant at $P = 0.05$.

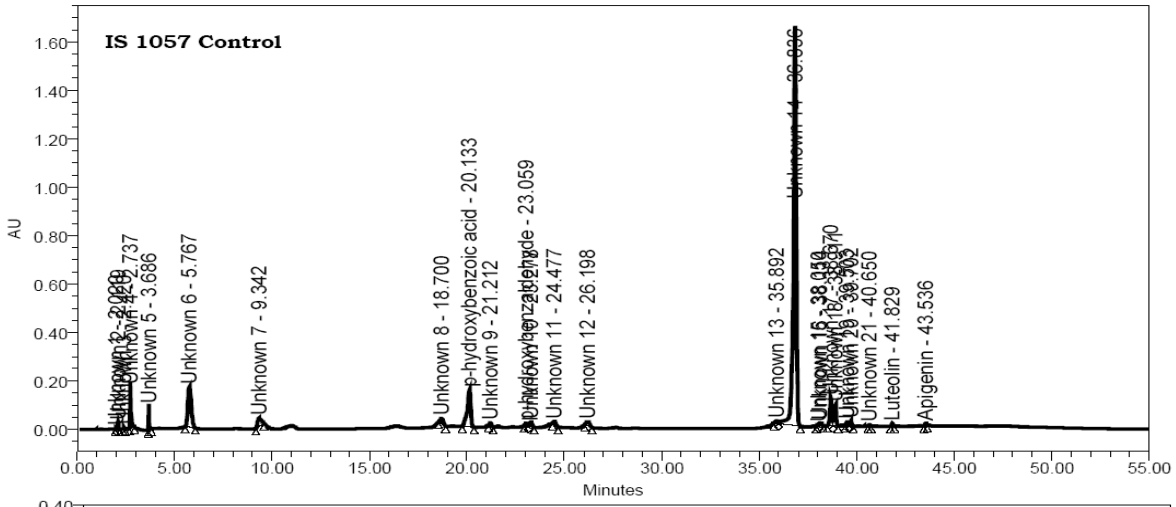
Table 4.26 Association of phenolic compounds in sorghum seedlings with expression of antibiosis to sorghum shoot fly, *A. soccata* (ICRISAT, Patancheru, India)

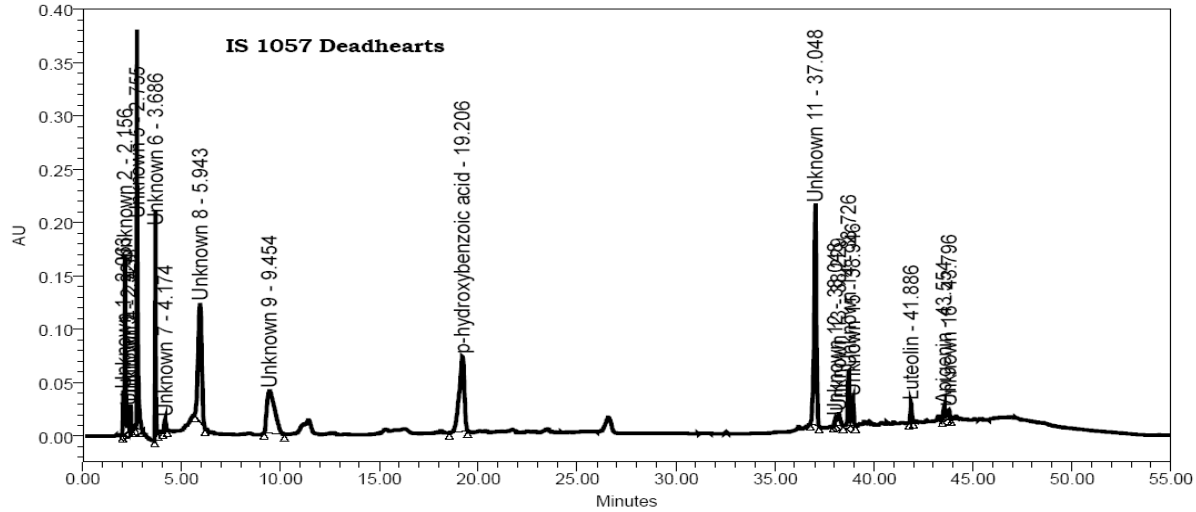
RT (min)	Phenolic compound	Larval period (d)	Larval survival (%)	Pupal period (d)	Pupal mortality (%)	Adult emergence (%)	Puapal weight (mg)			Fecundity female^{-1}
							Male	Female	Mean	
2.13	Unknown	0.30	-0.20	0.00	0.11	-0.23	-0.20	0.14	0.07	0.18
2.34	Unknown	0.02	0.01	-0.01	-0.02	0.02	0.02	-0.12	-0.09	0.05
2.76	Unknown	-0.18	0.14	-0.26	-0.11	0.18	-0.02	-0.04	0.01	-0.02
3.70	Unknown	-0.41	0.39	-0.29	-0.21	0.44	0.25	-0.22	-0.04	-0.22
4.15	Unknown	-0.29	0.35	-0.18	-0.24	0.42	0.32	-0.21	0.02	-0.24
11.46	Protocatechuic acid	0.33	-0.09	-0.15	0.00	-0.07	-0.06	-0.14	-0.22	-0.08
18.63	p-hydroxybenzoic acid	-0.30	0.31	-0.16	-0.19	0.36	0.24	-0.25	-0.14	-0.18
20.30	Unknown	-0.06	0.03	0.11	-0.04	0.05	-0.02	0.08	0.02	-0.03
21.44	Unknown	0.31	-0.25	0.36	0.38	-0.44	-0.27	0.19	-0.15	0.11
22.61	Unknown	-0.44	0.15	-0.27	-0.15	0.21	0.37	-0.16	0.04	0.01
23.41	p-hydroxybenzaldehyde	-0.37	0.42	-0.43	-0.01	0.34	0.10	-0.15	-0.08	-0.04
24.38	Unknown	-0.34	0.32	-0.43	-0.24	0.41	0.10	-0.18	0.02	-0.20
29.33	p-coumaric acid	0.26	0.00	-0.21	-0.02	0.01	-0.04	-0.12	-0.20	-0.08
36.51	Unknown	-0.33	0.35	-0.10	-0.22	0.42	0.18	-0.06	0.00	-0.19
37.08	Unknown	0.16	-0.08	0.07	0.02	-0.07	-0.07	-0.01	-0.06	-0.01
37.50	Unknown	-0.02	0.04	-0.36	-0.10	0.10	-0.18	-0.14	-0.08	-0.08
38.07	Unknown	0.14	-0.46	0.41	-0.27	-0.19	0.15	-0.12	-0.02	0.10
38.63	Unknown	0.16	-0.33	0.02	-0.13	-0.18	0.17	-0.18	-0.02	0.06
38.88	Unknown	0.28	-0.17	0.14	0.15	-0.22	-0.33	0.28	0.05	0.10
38.96	Unknown	0.33	-0.07	-0.07	0.42	-0.32	-0.29	0.09	0.06	0.23
39.56	Unknown	-0.04	-0.07	0.26	-0.36	0.17	0.09	0.13	0.22	0.00
40.66	Unknown	0.25	-0.09	0.10	0.18	-0.18	-0.21	0.23	0.00	0.20
41.93	Luteolin	0.18	-0.05	0.11	-0.17	0.07	0.08	-0.34	-0.23	-0.16
43.24	Cinnamic acid	-0.02	-0.07	0.26	-0.04	-0.03	0.09	0.12	0.08	0.03
43.58	Apigenin	0.29	-0.24	0.10	-0.04	-0.17	-0.13	0.05	0.05	0.29

*, ** Correlation coefficients significant at $P = 0.05$ and 0.01, respectively.

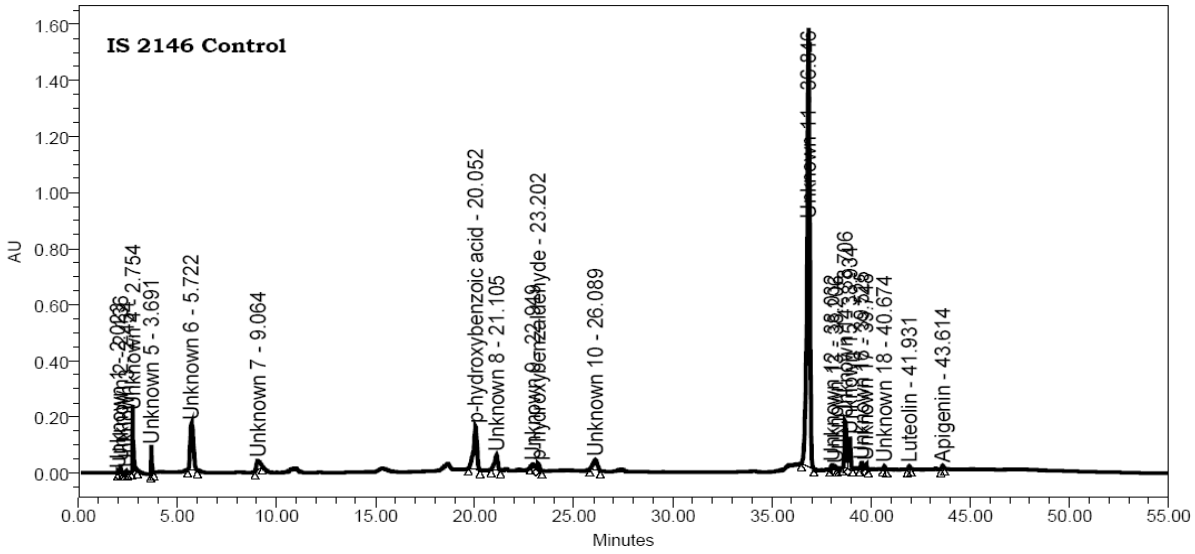
Fig. 4.1 HPLC fingerprints of flavonoids in damaged and undamaged seedlings of different sorghum genotypes.

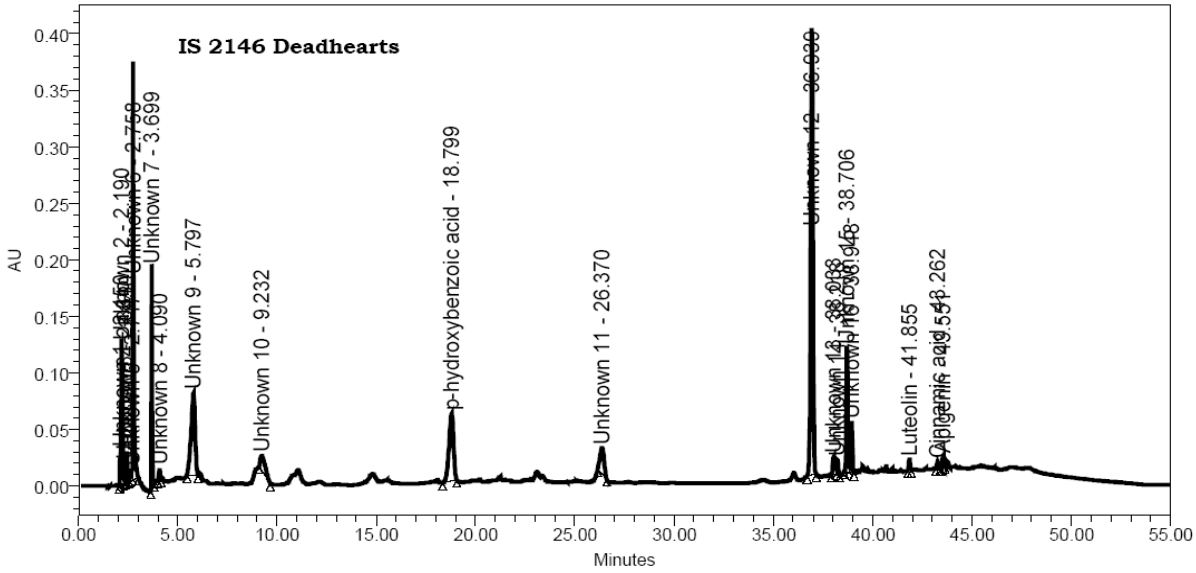


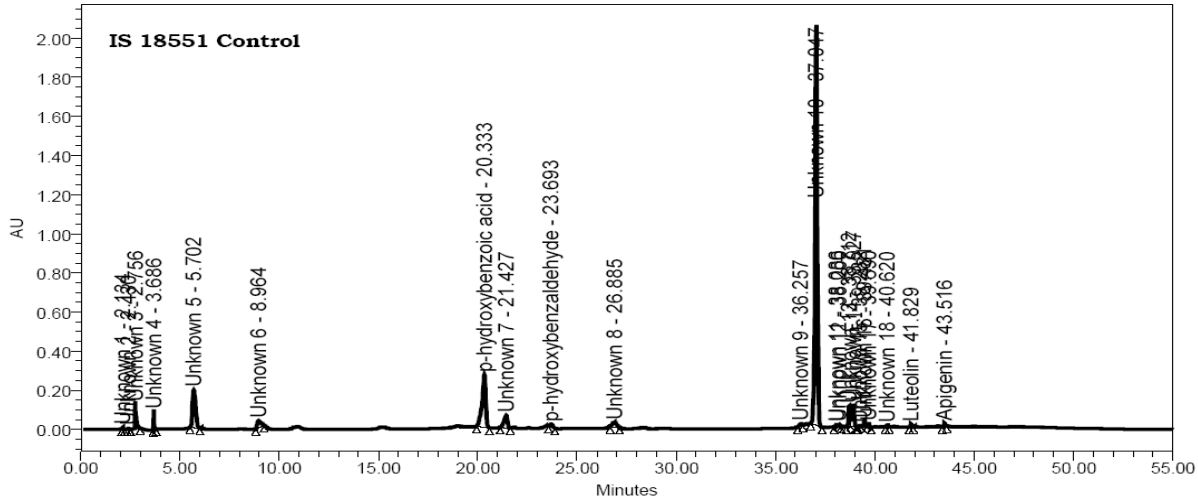




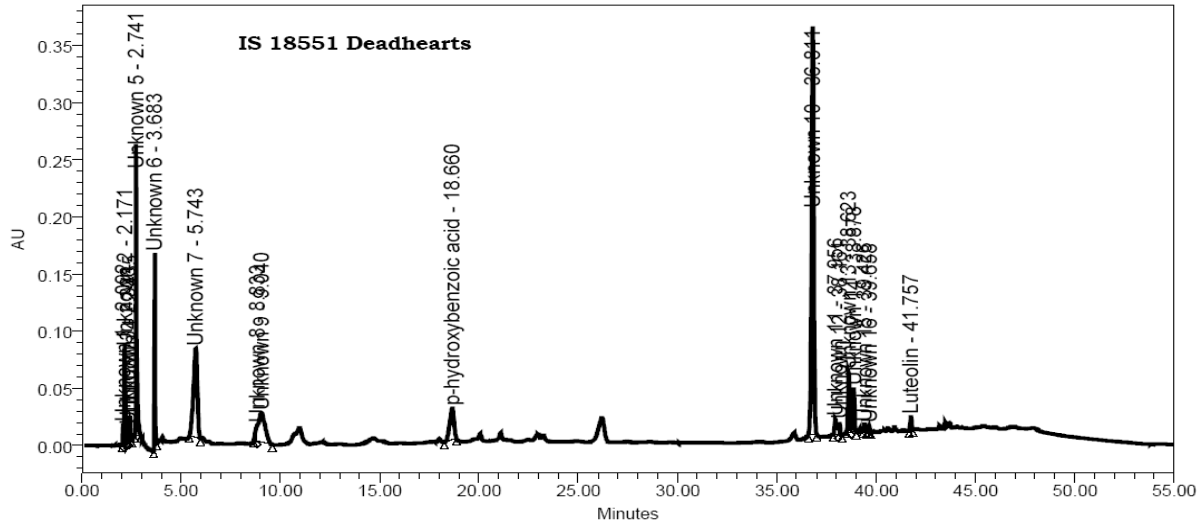
Continued……

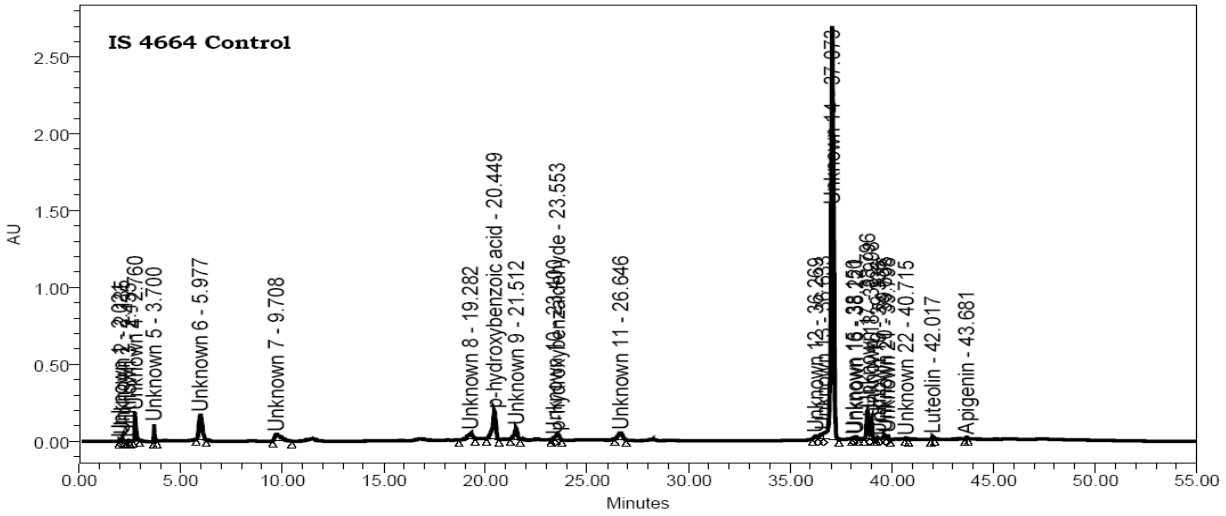

IS 2146 Control
AU
Minutes
Unknown 5 - 3.691
Unknown 6 - 5.722
Unknown 7 - 9.064
p-hydroxybenzoic acid - 20.052
Unknown 8 - 21.105
p-hydroxybenzaldehyde - 23.202
Unknown 10 - 26.089
Unknown 11 - 36.946
Unknown 18 - 40.674
Luteolin - 41.931
Apigenin - 43.614

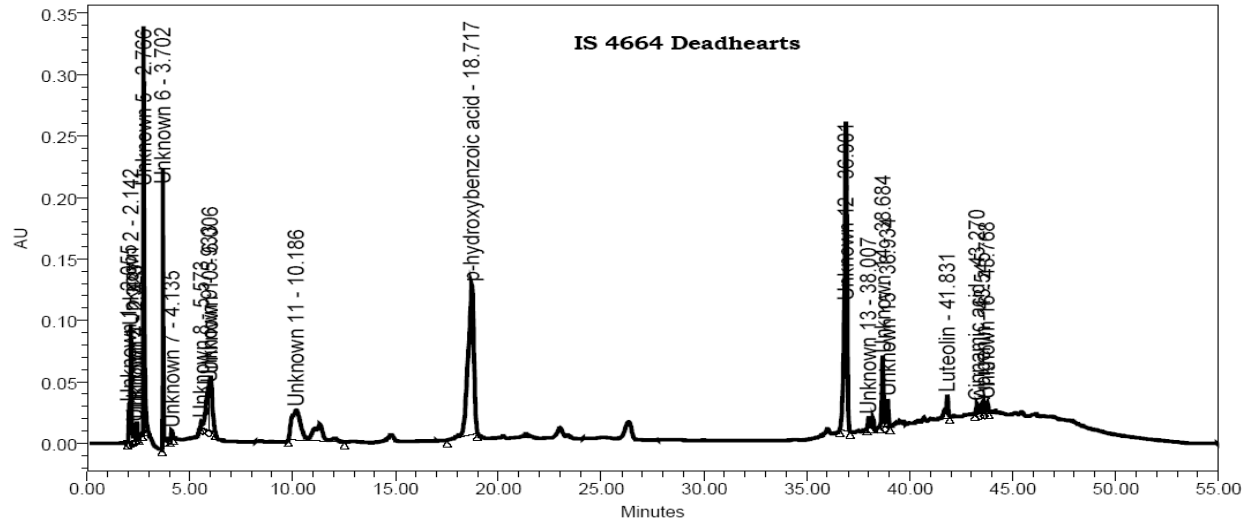

IS 2146 Deadhearts
AU
Minutes
Unknown 7 - 3.699
Unknown 8 - 4.090
Unknown 9 - 5.797
Unknown 10 - 9.232
p-hydroxybenzoic acid - 18.799
Unknown 11 - 26.370
Unknown 12 - 36.999
Unknown 16 - 38.706
Luteolin - 41.855


IS 18551 Control
AU
Minutes
Unknown 4 - 3.686
Unknown 5 - 5.702
Unknown 6 - 8.964
p-hydroxybenzoic acid - 20.333
Unknown 7 - 21.427
p-hydroxybenzaldehyde - 23.693
Unknown 8 - 26.885
Unknown 9 - 36.257
Unknown 10 - 37.047
Unknown 18 - 40.620
Luteolin - 41.829
Apigenin - 43.516

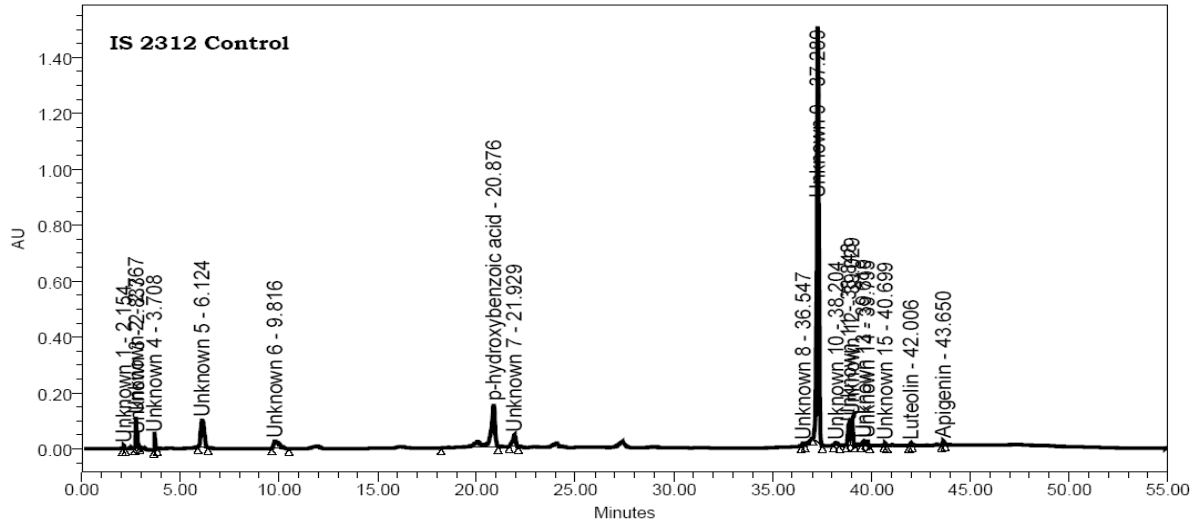
Continued.....

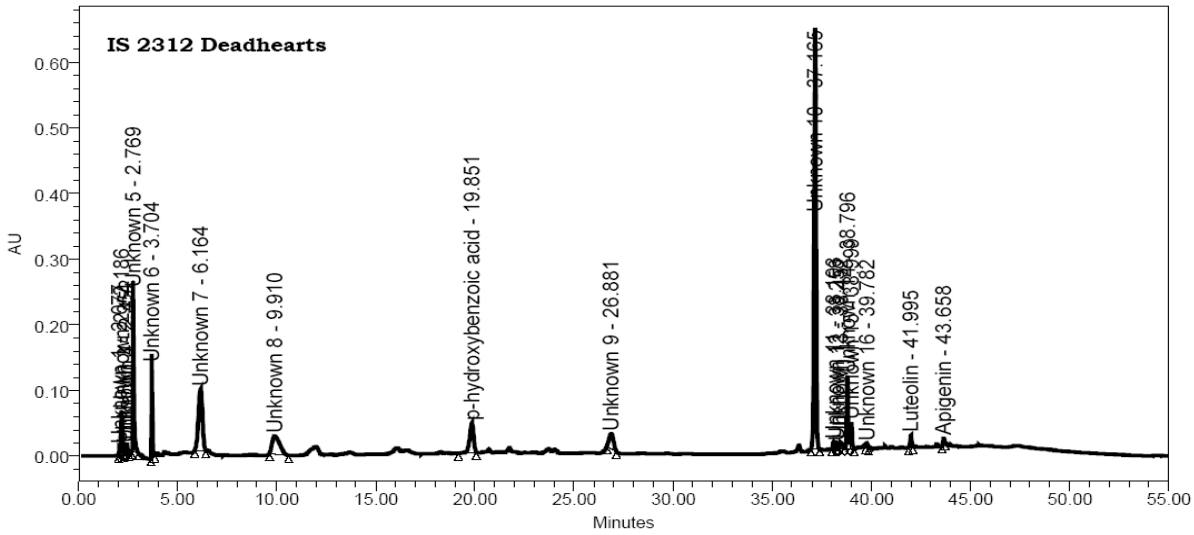

IS 18551 Deadhearts
AU
Minutes
Unknown 5 - 2.741
Unknown 6 - 3.683
Unknown 7 - 5.743
p-hydroxybenzoic acid - 18.660
Luteolin - 41.757

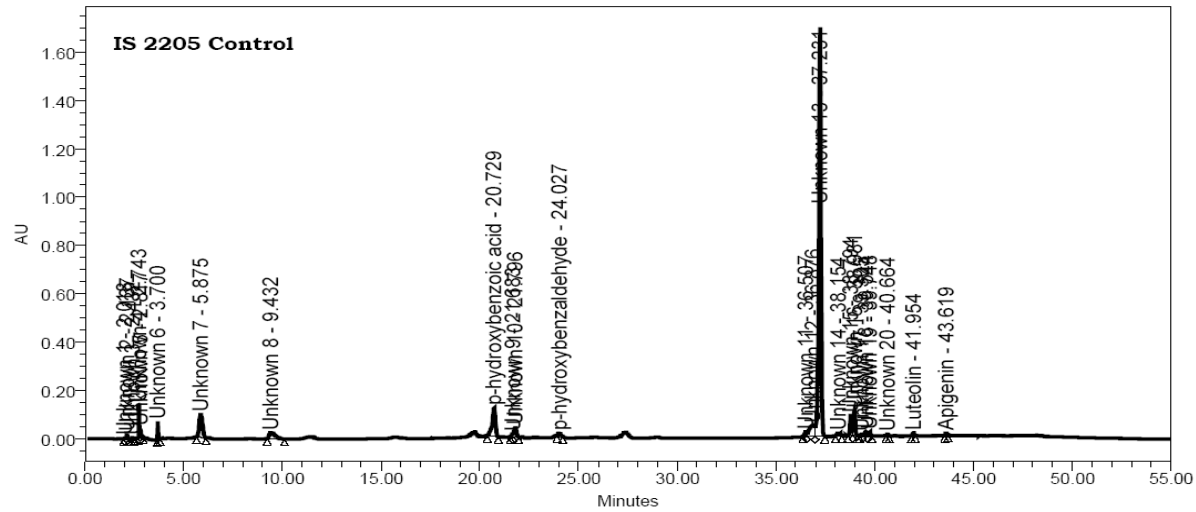

IS 4664 Control
AU
Minutes
Unknown 5 - 3.700
Unknown 6 - 5.977
Unknown 7 - 9.708
Unknown 8 - 19.282
p-hydroxybenzoic acid - 20.449
Unknown 9 - 21.512
Unknown 11 - 26.646
Luteolin - 42.017
Apigenin - 43.681


IS 4664 Deadhearts
AU
Minutes
Unknown 6 - 3.702
Unknown 7 - 4.135
Unknown 11 - 10.186
p-hydroxybenzoic acid - 18.717
Luteolin - 41.831

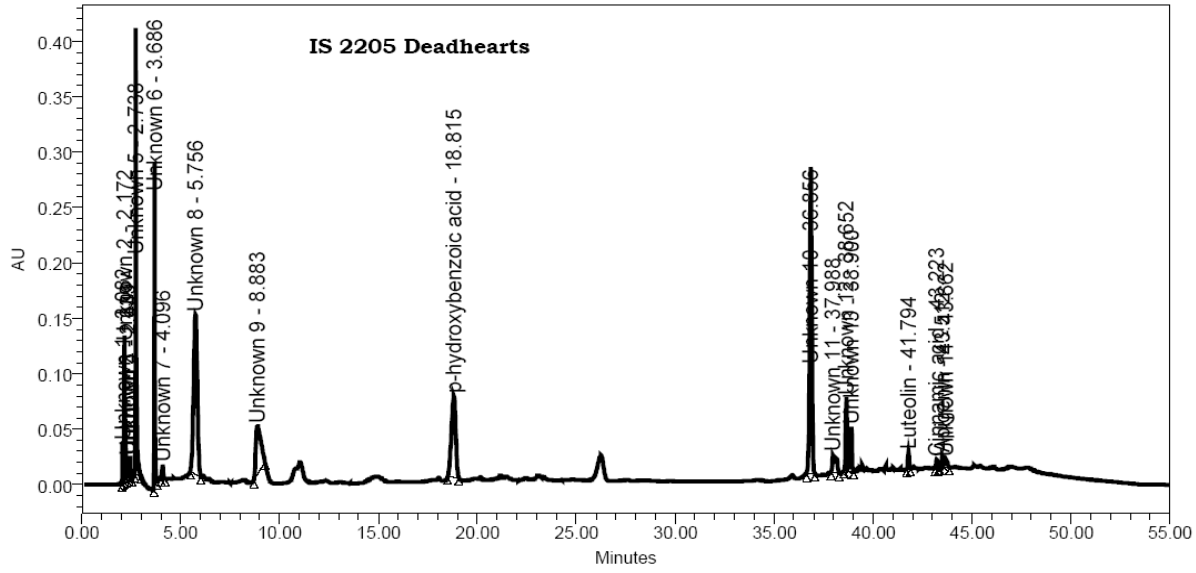
Continued.....

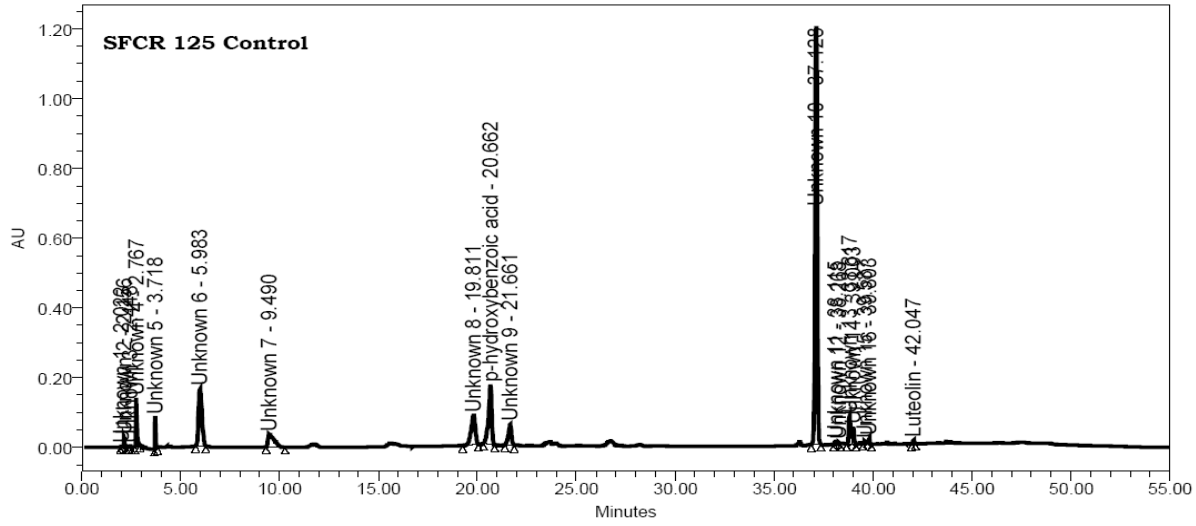

IS 2312 Control
AU
Minutes
Unknown 4 - 3.708
Unknown 5 - 6.124
Unknown 6 - 9.816
p-hydroxybenzoic acid - 20.876
Unknown 7 - 21.929
Unknown 8 - 36.547
Unknown 15 - 40.699
Luteolin - 42.006
Apigenin - 43.650

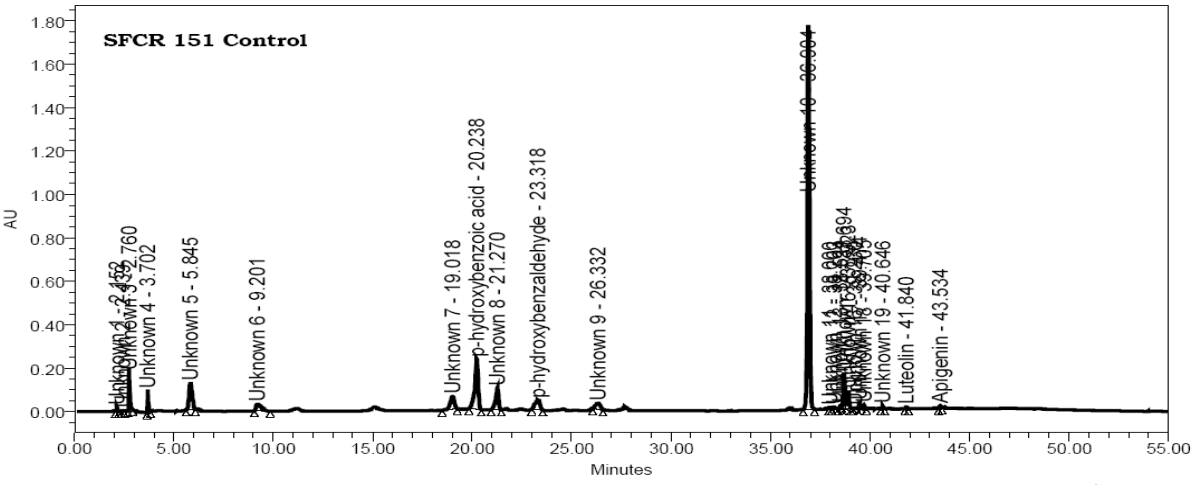

IS 2312 Deadhearts
AU
Minutes
Unknown 5 - 2.769
Unknown 6 - 3.704
Unknown 7 - 6.164
Unknown 8 - 9.910
p-hydroxybenzoic acid - 19.851
Unknown 9 - 26.881
Unknown 16 - 39.782
Luteolin - 41.995
Apigenin - 43.658


IS 2205 Control
AU
Minutes
Unknown 6 - 3.700
Unknown 7 - 5.875
Unknown 8 - 9.432
p-hydroxybenzoic acid - 20.729
p-hydroxybenzaldehyde - 24.027
Unknown 20 - 40.664
Luteolin - 41.954
Apigenin - 43.619

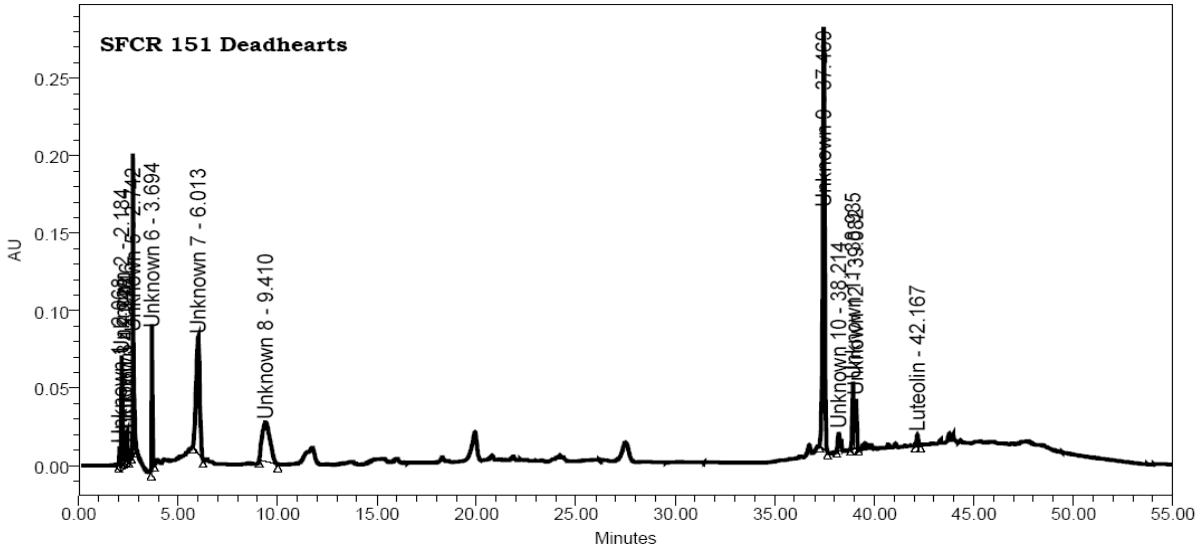
Continued.....

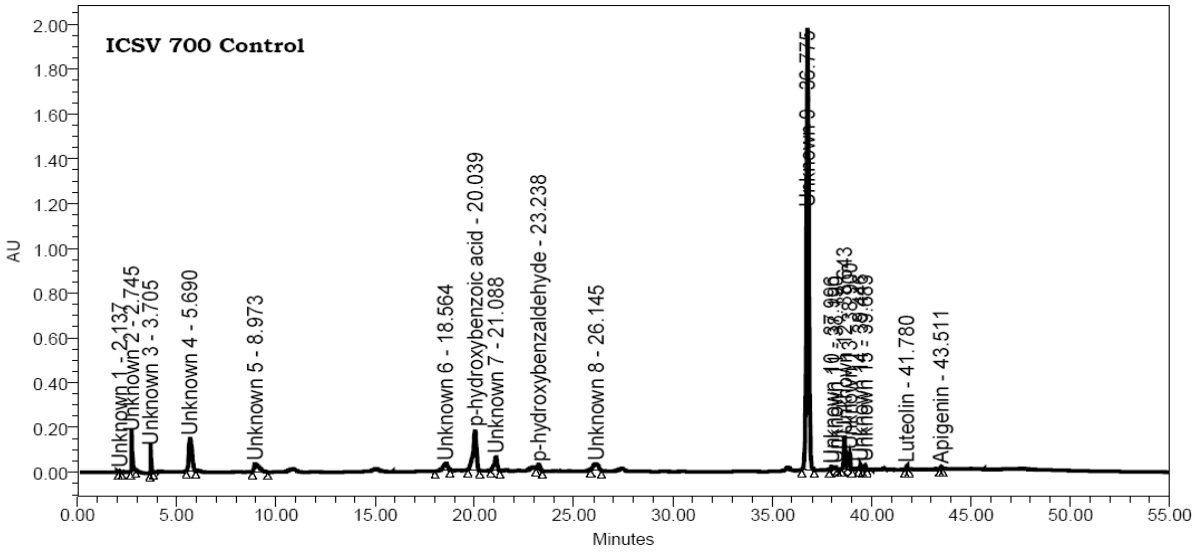

IS 2205 Deadhearts
AU
Minutes
Unknown 6 - 3.686
Unknown 8 - 5.756
Unknown 7 - 4.096
Unknown 9 - 8.883
p-hydroxybenzoic acid - 18.815
Unknown 11 - 37.988
Luteolin - 41.794

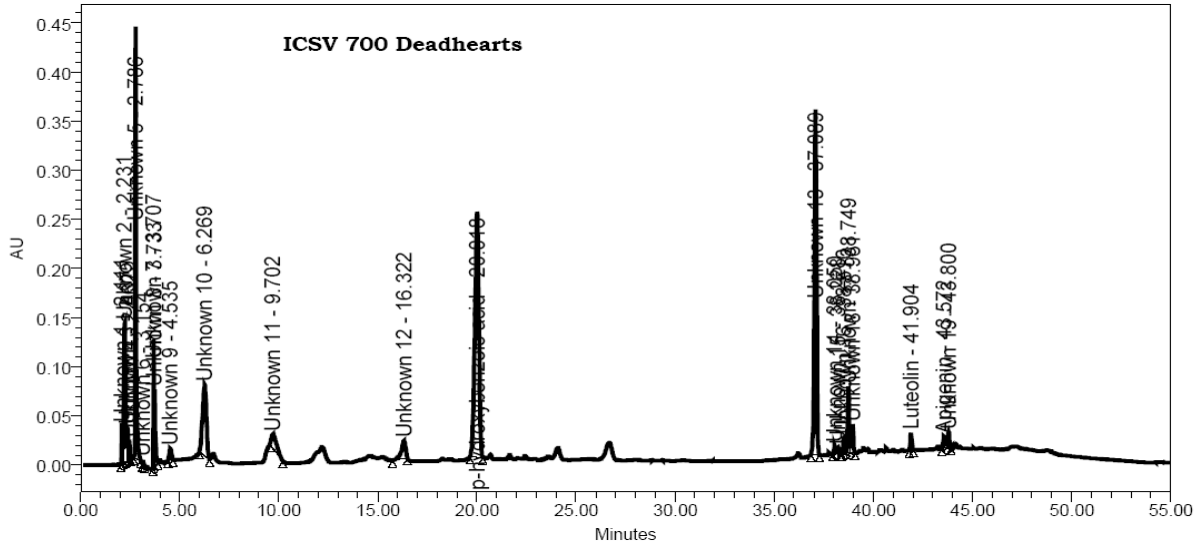

SFCR 125 Control
AU
Minutes
Unknown 5 - 3.718
Unknown 6 - 5.983
Unknown 7 - 9.490
Unknown 8 - 19.811
p-hydroxybenzoic acid - 20.662
Unknown 9 - 21.661
Luteolin - 42.047


SFCR 151 Control
AU
Minutes
Unknown 4 - 3.702
Unknown 5 - 5.845
Unknown 6 - 9.201
Unknown 7 - 19.018
p-hydroxybenzoic acid - 20.238
Unknown 8 - 21.270
p-hydroxybenzaldehyde - 23.318
Unknown 9 - 26.332
Unknown 19 - 40.646
Luteolin - 41.840
Apigenin - 43.534

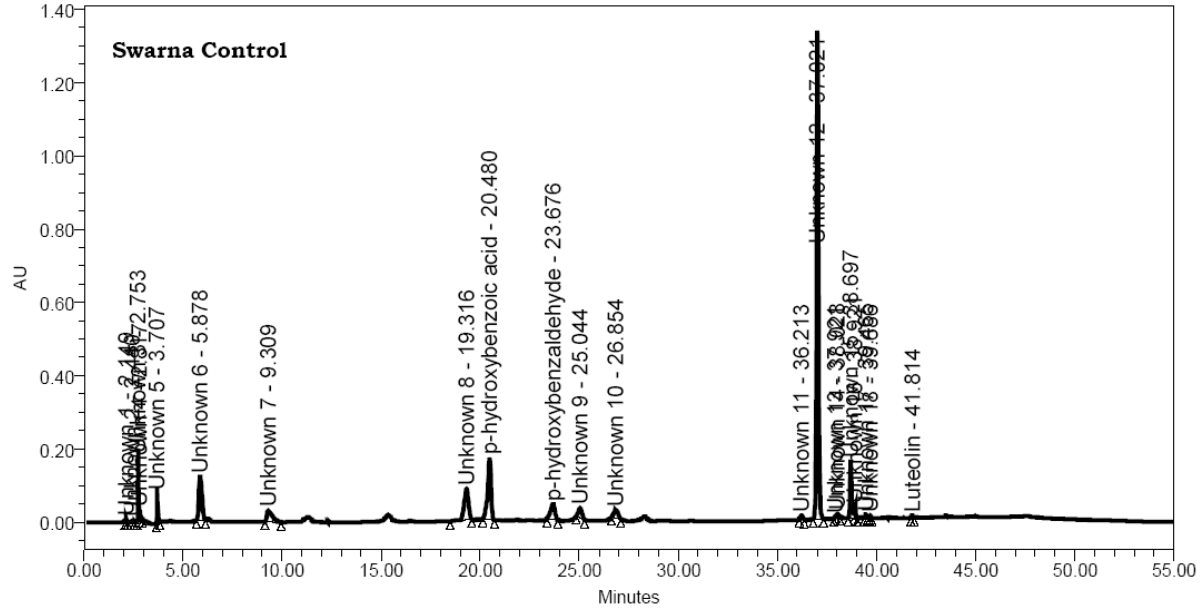
Continued……

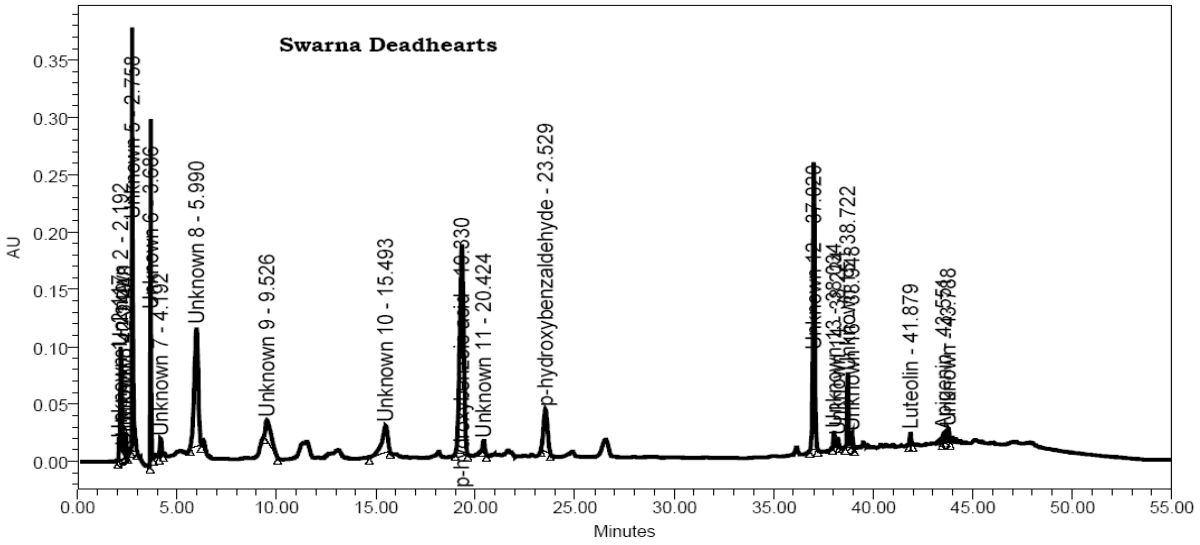

SFCR 151 Deadhearts
AU
Minutes
Unknown 6 - 3.694
Unknown 7 - 6.013
Unknown 8 - 9.410
Unknown 10 - 38.214
Luteolin - 42.167

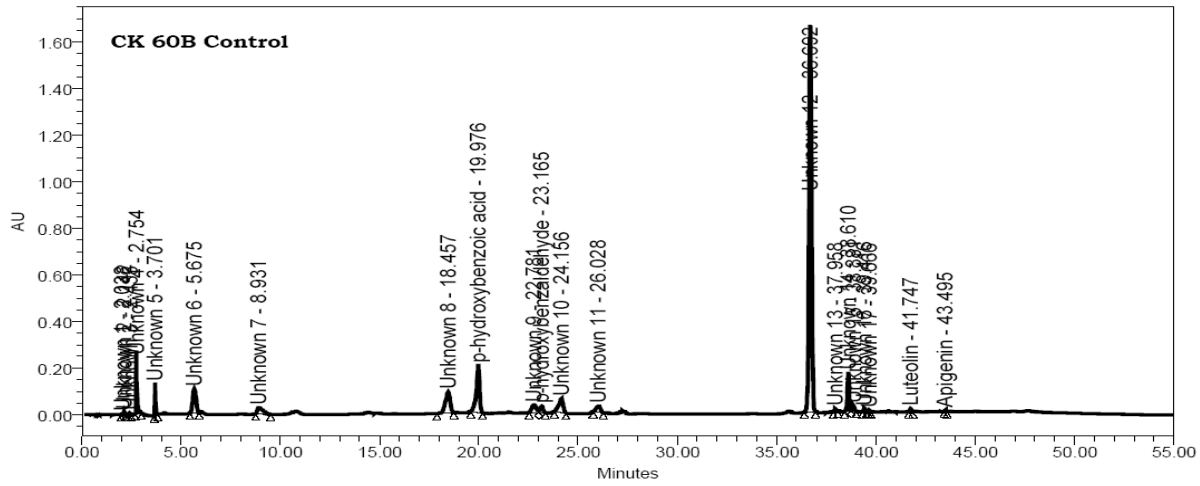

ICSV 700 Control
AU
Minutes
Unknown 3 - 3.705
Unknown 4 - 5.690
Unknown 5 - 8.973
Unknown 6 - 18.564
p-hydroxybenzoic acid - 20.039
Unknown 7 - 21.088
p-hydroxybenzaldehyde - 23.238
Unknown 8 - 26.145
Luteolin - 41.780
Apigenin - 43.511


ICSV 700 Deadhearts
AU
Minutes
Unknown 9 - 4.535
Unknown 10 - 6.269
Unknown 11 - 9.702
Unknown 12 - 16.322
Luteolin - 41.904

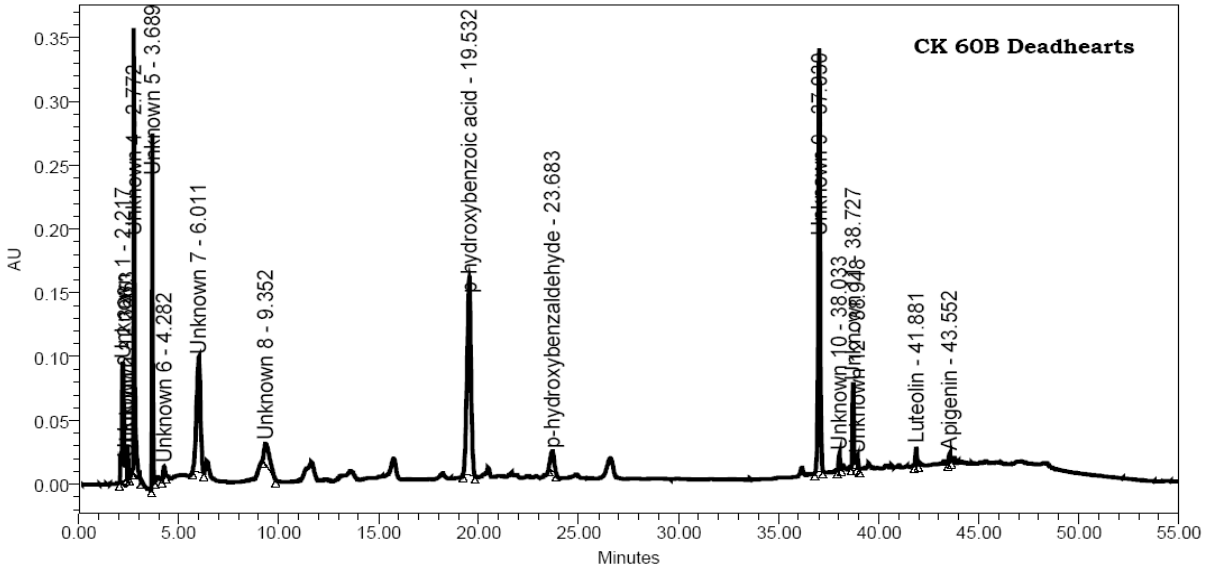
Continued……

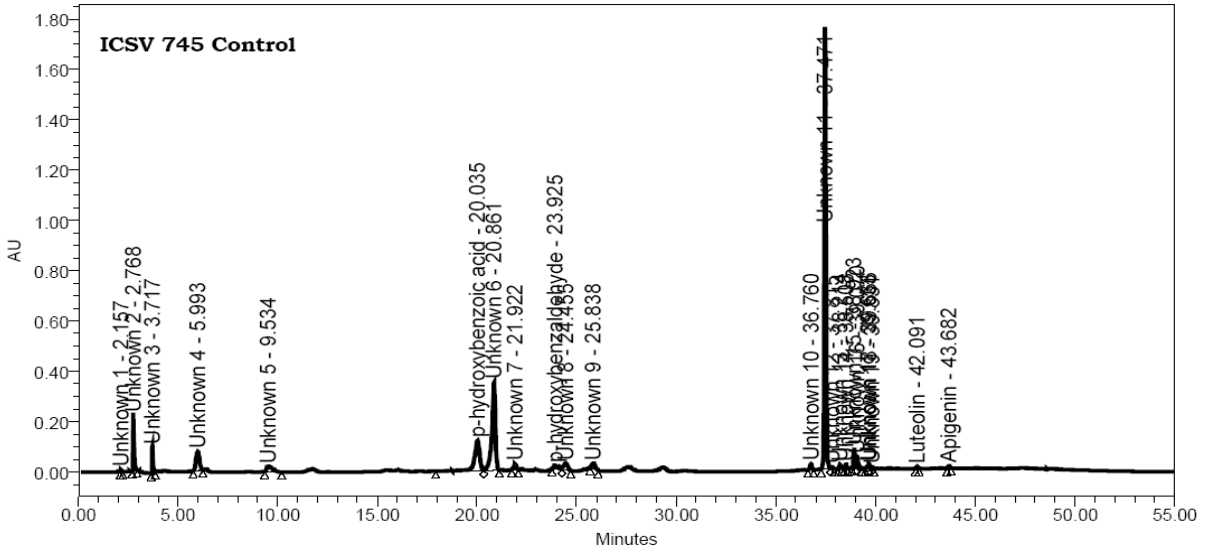

Swarna Control
AU
Minutes
Unknown 5 - 3.707
Unknown 6 - 5.878
Unknown 7 - 9.309
Unknown 8 - 19.316
p-hydroxybenzoic acid - 20.480
p-hydroxybenzaldehyde - 23.676
Unknown 9 - 25.044
Unknown 10 - 26.854
Unknown 11 - 36.213
Luteolin - 41.814

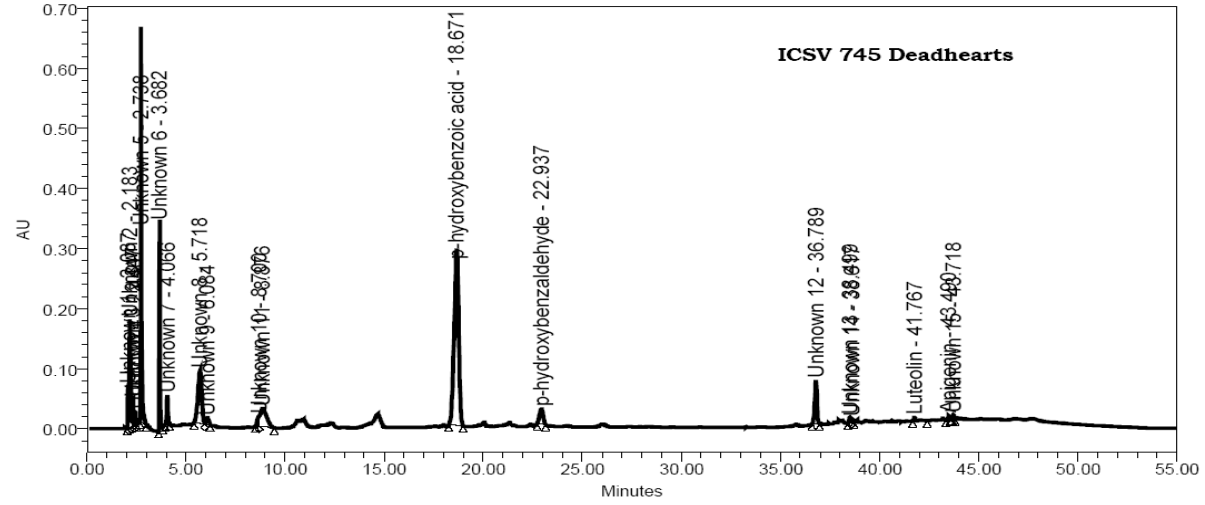

Swarna Deadhearts
AU
Minutes
Unknown 8 - 5.990
Unknown 9 - 9.526
Unknown 10 - 15.493
Unknown 11 - 20.424
p-hydroxybenzaldehyde - 23.529
Luteolin - 41.879


CK 60B Control
AU
Minutes
Unknown 5 - 3.701
Unknown 6 - 5.675
Unknown 7 - 8.931
Unknown 8 - 18.457
p-hydroxybenzoic acid - 19.976
Unknown 10 - 24.156
Unknown 11 - 26.028
Luteolin - 41.747
Apigenin - 43.495

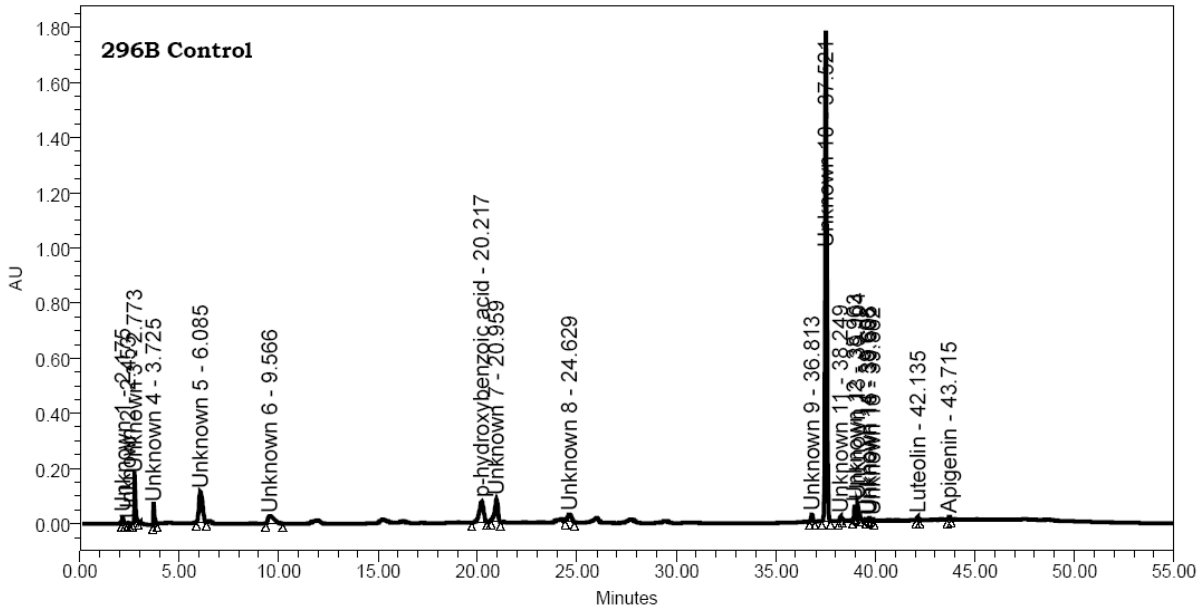
Continued.....

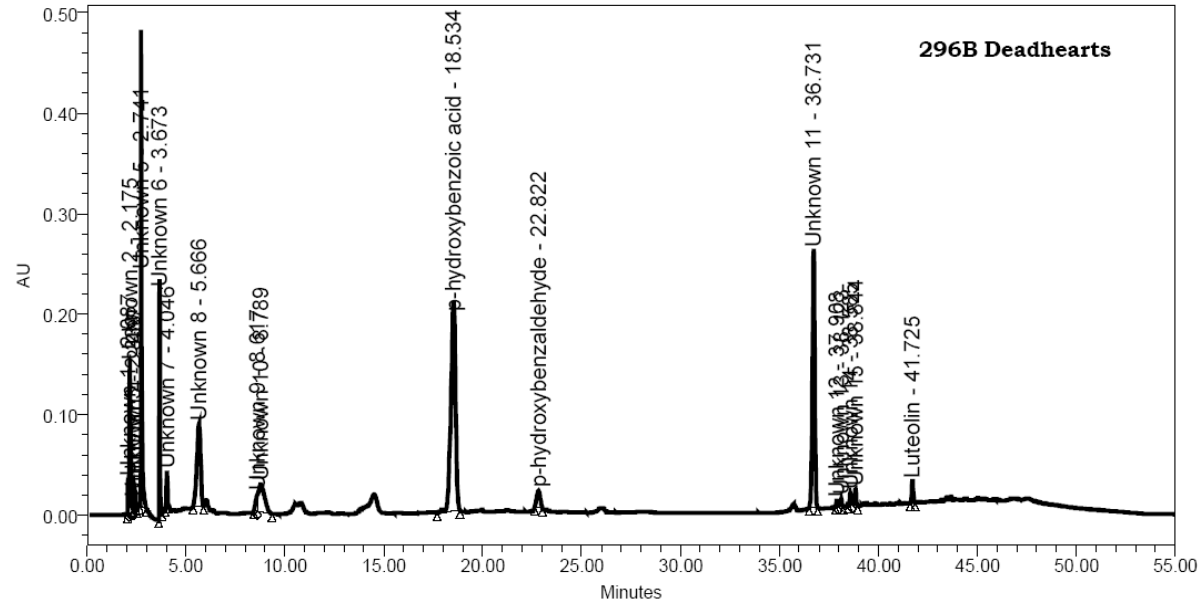

CK 60B Deadhearts
AU
Minutes
Unknown 5 - 3.689
Unknown 6 - 4.282
Unknown 7 - 6.011
Unknown 8 - 9.352
p-hydroxybenzoic acid - 19.532
p-hydroxybenzaldehyde - 23.683
Unknown 10 - 38.033
Luteolin - 41.881
Apigenin - 43.552


ICSV 745 Control
AU
Minutes
Unknown 1 - 2.157
Unknown 2 - 2.768
Unknown 3 - 3.717
Unknown 4 - 5.993
Unknown 5 - 9.534
p-hydroxybenzoic acid - 20.035
Unknown 6 - 20.861
Unknown 7 - 21.922
p-hydroxybenzaldehyde - 23.925
Unknown 9 - 25.838
Unknown 10 - 36.760
Luteolin - 42.091
Apigenin - 43.682

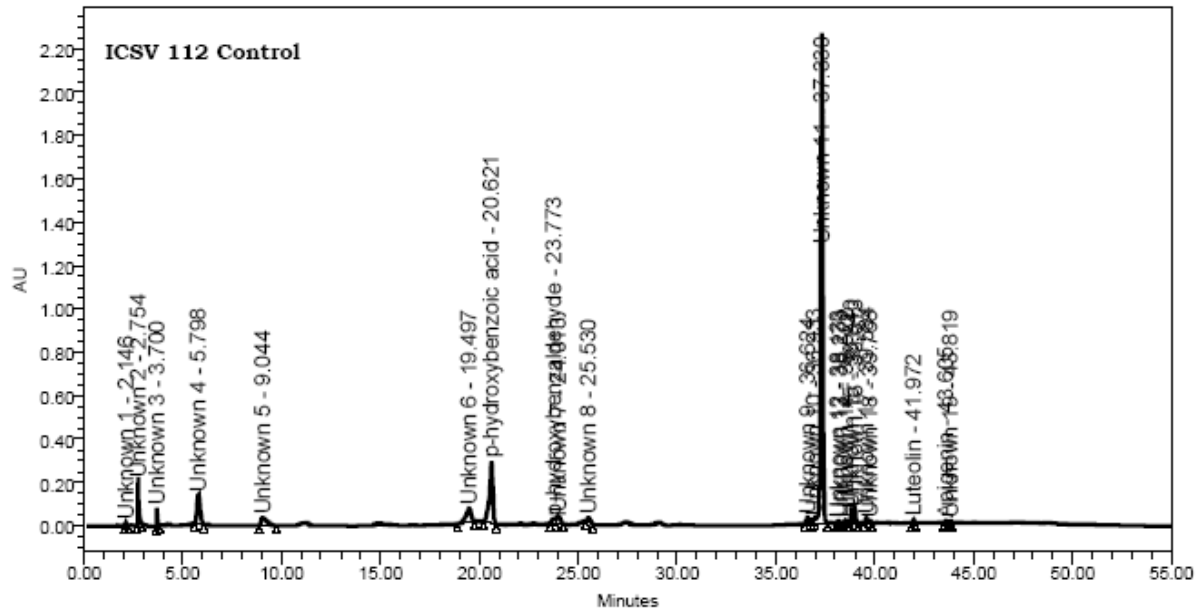

ICSV 745 Deadhearts
AU
Minutes
Unknown 6 - 3.682
Unknown 7 - 4.066
Unknown 9 - 5.718
p-hydroxybenzoic acid - 18.671
p-hydroxybenzaldehyde - 22.937
Unknown 12 - 36.789
Luteolin - 41.767

Continued……


296B Control
AU
Minutes
Unknown 4 - 3.725
Unknown 5 - 6.085
Unknown 6 - 9.566
p-hydroxybenzoic acid - 20.217
Unknown 8 - 24.629
Unknown 9 - 36.813
Luteolin - 42.135
Apigenin - 43.715


296B Deadhearts
AU
Minutes
Unknown 6 - 3.673
Unknown 8 - 5.666
p-hydroxybenzoic acid - 18.534
p-hydroxybenzaldehyde - 22.822
Unknown 11 - 36.731
Luteolin - 41.725

Continued……


ICSV 112 Control
AU
Minutes
Unknown 1 - 2.146
Unknown 2 - 2.754
Unknown 3 - 3.700
Unknown 4 - 5.798
Unknown 5 - 9.044
Unknown 6 - 19.497
p-hydroxybenzoic acid - 20.621
Unknown 8 - 25.530
Luteolin - 41.972

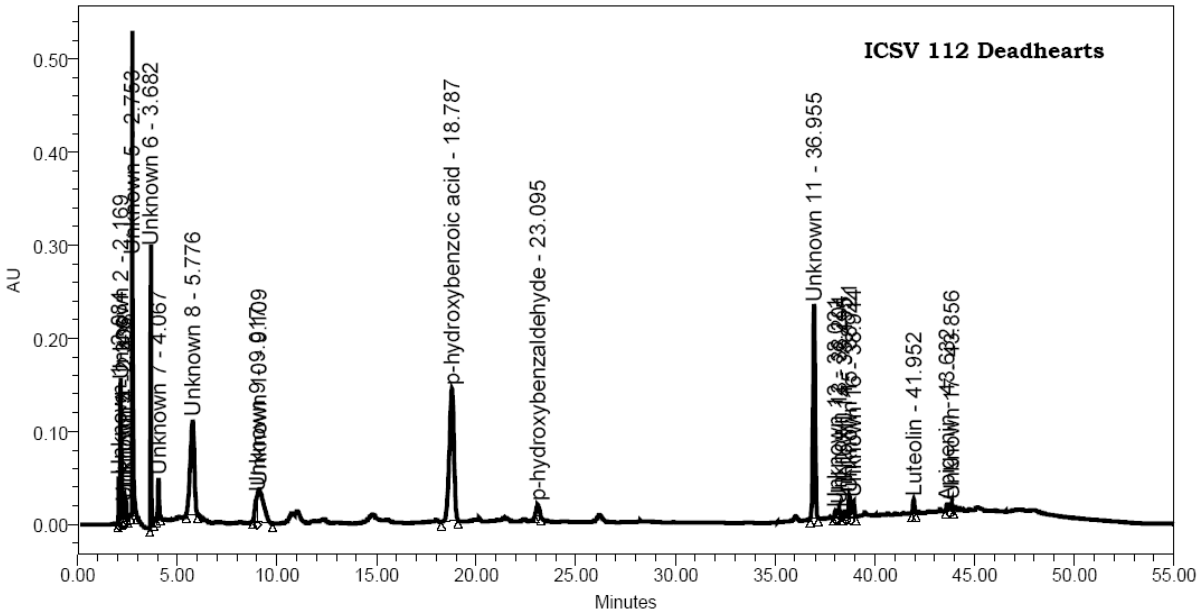
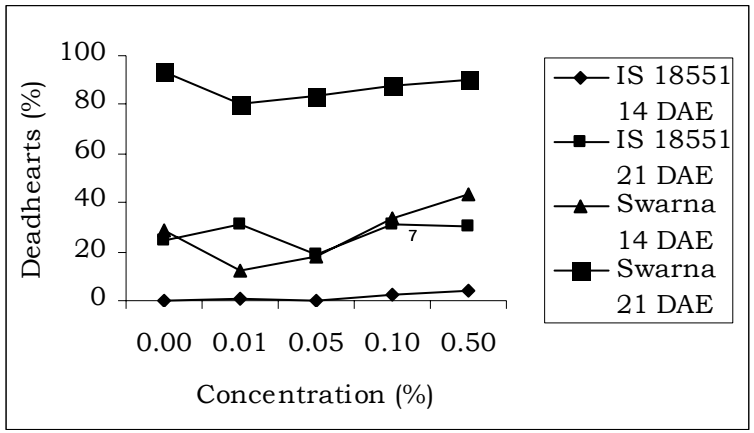
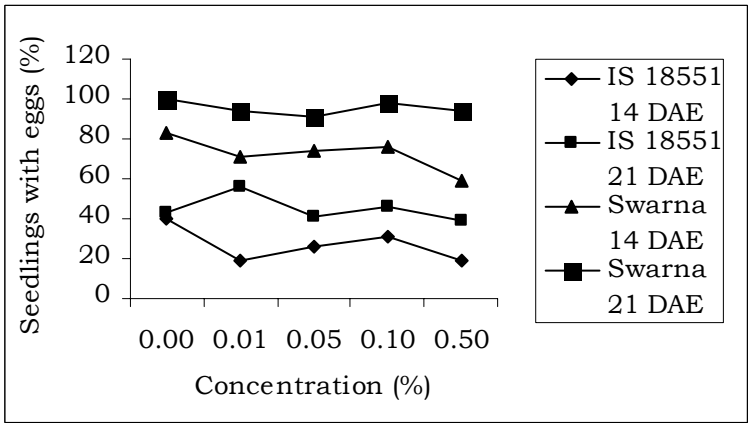
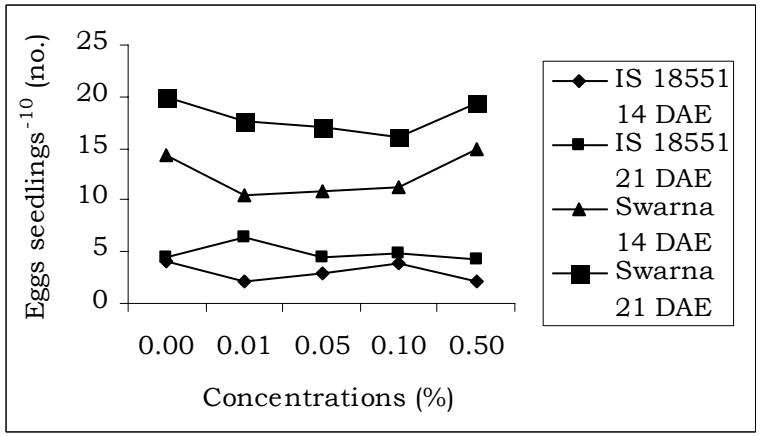

ICSV 112 Deadhearts
AU
Minutes
Unknown 6 - 3.682
Unknown 7 - 4.067
Unknown 8 - 5.776
p-hydroxybenzoic acid - 18.787
p-hydroxybenzaldehyde - 23.095
Unknown 11 - 36.955
Luteolin - 41.952

Fig. 4.12 Effect of p-hydroxy benzaldehyde (PHB) on shoot fly, *A. soccata* damage (ICRISAT, Patancheru, post-rainy season-2006)







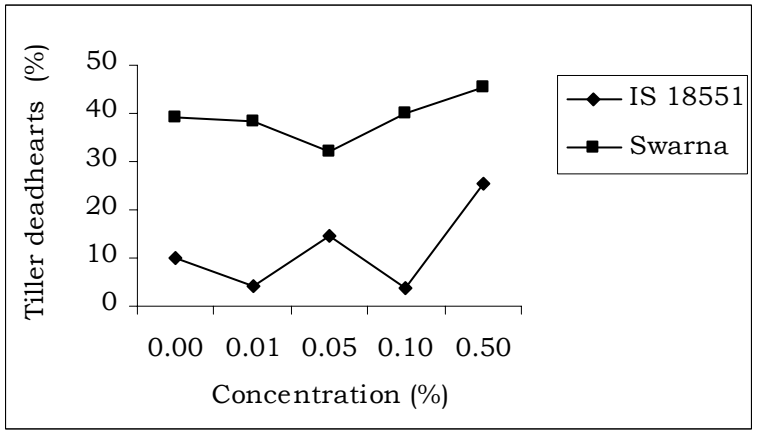
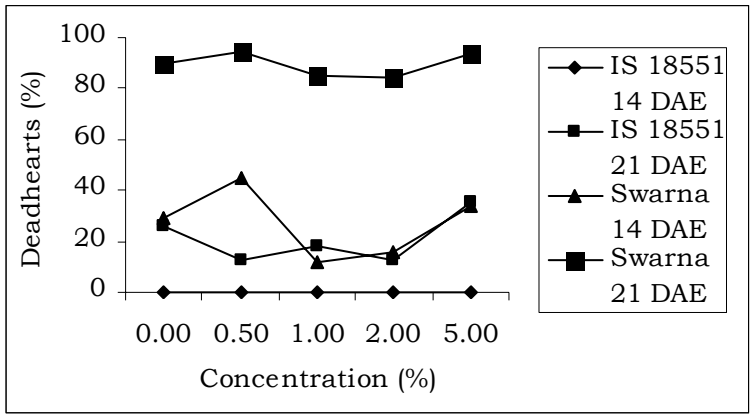
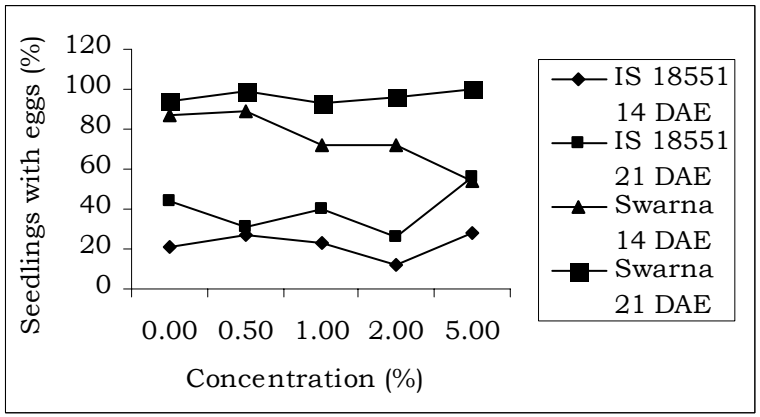
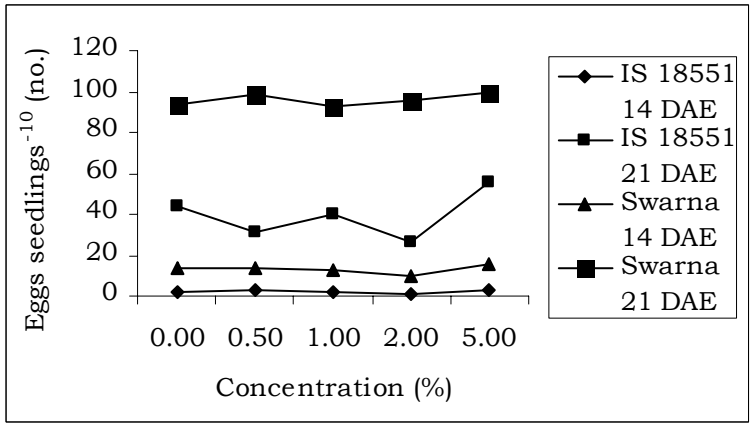
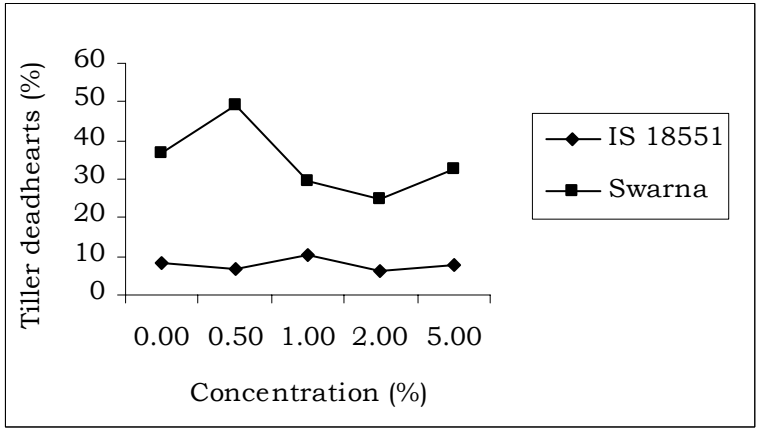


Fig. 4.13 Effect of p-hydroxy benzoic acid (PHBA) on shoot fly, *A. soccata* damage (ICRISAT, Patancheru, post-rainy season- 2006)









4.8 Protein profiles of germinated seeds of sorghum genotypes and expression of resistance to shoot fly, *Atherigona soccata*

RP-HPLC profiling of proteins extracted from geminated seeds of 15 sorghum genotypes showed considerable differences in protein composition among the genotypes. Thirty- seven peaks were recorded in different genotypes, of which 17 peaks were associated with expression of resistance to shoot fly, *A. soccata* (Table 4.27; Fig.4.2).

Peak 1 at RT 2.59 had more peak area in resistant genotypes IS 1057, IS 2146, IS 18551, IS 4664, IS 2205, SFCR 125, and SFCR 151 (except IS 1054, IS 2312 and ICSV 700) as compared to Swarna, CK 60B, ICSV 745, 296B and ICSV 112. Peak 2 at RT 4.08 had more peak area in the susceptible genotypes Swarna, CK 60B, ICSV 745, and ICSV 112 as compared to resistant check, IS 18551. Peak 2 was absent in IS 2312 and IS 2205, which are highly resistant to shoot fly. Peak 3 at RT 5.04 was absent in resistant genotypes (except in IS 1057 and IS 4664), but present in the susceptible genotypes (except CK 60B and ICSV 745). Peak 4 at RT 8.27 had more peak area in the shoot fly susceptible genotypes Swarna, ICSV 745, 296B and ICSV 112 (except CK 60B) as compared to the resistant check, IS 18551.

Peaks 5, 9, and 11 at RTs 11.43, 21.00 and 23.90, respectively, were present in the shoot fly resistant genotypes, but absent in the susceptible genotypes. Peaks 6, 7, and 14 at RTs 14.56, 17.59 and 26.02, respectively, were present in the susceptible genotypes, but

absent in resistant genotypes, although there were a few exceptions. Peak 8 at RT 18.42 had more peak area in the resistant genotypes (IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151 and ICSV 700) than in the susceptible genotypes (Swarna, CK 60B, ICSV 745, 296B and ICSV 112).

Peaks 12 and 13 at RTs 25.64 and 25.78 had more peak area in the susceptible genotypes (Swarna, CK 60B, ICSV 745, 296B, and ICSV 112) than in the resistant genotypes (except peak 12 in IS 2312, and peak 13 in IS 1054). Peak 12 was absent in IS 1054, IS 2205, SFCR 125, SFCR 151, while peak 13 was absent in IS 18551, IS 4664, IS 2312 and SFCR 151. Peak 15 at RT 37.5 had greater peak area in the resistant genotypes IS 1054, IS 2312, IS 2205, SFCR 125, SFCR 151 and ICSV 700 than in the susceptible genotypes ICSV 745, 296B and ICSV 112. This peak was absent in IS 18551, IS 1057, Swarna, and CK 60B, but present in IS 2146 and IS 4664. Peak 16 at RT 49.5 had greater peak area in the susceptible check, Swarna than in the resistant check, IS 18551.

Peak 17 at RT 50.3 had more peak area in the susceptible genotypes Swarna, CK 60B, ICSV 745, 296B and ICSV 112 than in the resistant genotypes IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, IS 2205, SFCR 125, SFCR 151, and ICSV 700 (Table 4.27).

Proteins at peaks 1, 2, 3, 4, 7, 12, 14, 16, and 17 were significantly and positively correlated with deadhearts at 14, 21 and 28 DAE,

seedlings with eggs at 14 and 21 DAE and eggs per 10 seedlings at 14 and 21 DAE, suggesting that these peaks were associated with susceptibility to shoot fly, *A. soccata*. On the other hand, peaks 5, 8, 9, and 11 were significantly and negatively correlated with deadhearts at 14, 21 and 28 DAE, seedlings with eggs at 14 and 21 DAE, and eggs per 10 seedlings at 14 and 21 DAE, although there were few exceptions. These peaks were associated with resistance to shoot fly. Correlation coefficients of the proteins at other retention times with shoot fly damage parameters were nonsignificant (Table 4.28).

Correlations of protein profiles with expression of antibiosis indicated that the peaks 1, 2, 3, 4, 6, 7, 10, 12, 14, 16, and 17 were significantly and negatively correlated with developmental period (except peaks 1, 3 and 10), pupal period (except peaks 2, 3, 6, 7, 10, 12 and 14), and female pupal weight; but significantly and positively correlated with larval survival, adult emergence, and male pupal weight. However, some of the correlation coefficients were nonsignificant. These were associated with susceptibility to shoot fly. On the other hand, peak 8, 9, 11, and 15 were significantly and positively correlated with developmental period, pupal mortality, and female pupal weight; but negatively correlated with larval survival, adult emergence, and male pupal weight, suggesting that these peaks were associated with expression of antibiosis component of resistance to shoot fly, *A. soccata*. Correlation coefficients of the rest of the peaks were nonsignificant (Table 4.29).

Table 4.27 HPLC protein profiles of germinated seeds of sorghum genotypes in relation to expression of resistance to shoot fly, *A. soccata* (ICRISAT, Patancheru, India)

RT (min)	Protein peaks	Areas						
		IS 1054	IS 1057	IS 2146	IS 18551 (R)	IS 4664	IS 2312	IS 2205
2.59	Peak 1	1194303	1399948	1293803	1482006	1305124	704158	784542
4.08	Peak 2	79200	94393	194069	57176	115688	0	0
5.04	Peak 3	0	168347	0	0	271883	0	0
8.27	Peak 4	7041978	7739900	4436295	6628121	7529487	3367532	1597108
11.43	Peak 5	0	0	1084257	584933	835330	0	0
14.56	Peak 6	0	0	158438	0	0	0	0
17.59	Peak 7	0	0	215839	0	0	0	0
18.42	Peak 8	2724868	2400674	3417755	2861381	2693508	2188471	1769159
21.00	Peak 9	662603	647162	792394	615963	349310	472935	349697
21.64	Peak 10	847871	1225387	1204376	1955499	1282071	838647	654256
23.90	Peak 11	717321	520970	0	0	628496	918960	560712
25.64	Peak 12	0	353227	257647	423677	108894	718392	0
25.78	Peak 13	6158321	811267	163709	0	0	0	739329
26.02	Peak 14	0	0	0	0	0	0	0
37.45	Peak 15	1664787	0	714062	0	1061207	2644641	2774302
49.48	Peak 16	17666684	18479623	11236306	12903902	13041405	12703719	12271460
50.28	Peak 17	3726899	2703226	975036	1096549	800437	763437	695873

Continued .....Table 4.27

Continued..... Table 4.27

RT (min)	Protein peaks	Peak areas							
		SFCR 125	SFCR 151	ICSV 700	Swarna (S)	CK 60B	ICSV 745	296 B	ICSV 112
2.59	Peak 1	1207085	964854	948483	1260792	1360100	1549236	1472135	1529352
4.08	Peak 2	95555	58213	208652	247173	155313	265041	69226	188114
5.04	Peak 3	0	0	0	117357	0	0	487126	537187
8.27	Peak 4	4286296	1711967	3073991	7993733	6783683	8730085	8169202	7738666
11.43	Peak 5	527301	297048	351125	0	0	0	0	0
14.56	Peak 6	0	123568	597587	660654	0	0	345289	231254
17.59	Peak 7	205846	0	0	361847	125007	171186	0	173986
18.42	Peak 8	3536801	2295096	3285277	1874179	1738389	1914036	2216116	2033650
21.00	Peak 9	822667	225135	124694	648610	218922	0	0	0
21.64	Peak 10	1290643	882816	631987	1424522	1083016	1258226	1815706	1601508
23.90	Peak 11	729231	332855	398804	0	0	0	0	0
25.64	Peak 12	0	0	504567	528871	1228666	1016799	934905	697354
25.78	Peak 13	236606	0	473924	673363	1187821	1201520	1120632	932980
26.02	Peak 14	0	0	412164	214367	978886	547857	432799	206205
37.45	Peak 15	4353526	2714958	4989217	0	0	1172040	1282597	1263905
49.48	Peak 16	12636748	11224315	11168108	18981628	18927014	18169298	18379777	18426466
50.28	Peak 17	301981	245841	1433945	3485797	5981252	5486946	4766761	4196204

a = Protein unknown peaks.

Table 4.28 Association of protein profiles of 15 sorghum genotypes with expression of resistance to sorghum shoot fly, *A.soccata* (ICRISAT, Patancheru, India)

RT (min)	Protein peaks	Deadhearts (%)			Seedlings with eggs (%)		Eggs seedlings^{-10}		Tiller deadhearts (%)
		14 DAE	21 DAE	28 DAE	14 DAE	21 DAE	14 DAE	21 DAE	
2.59	Peak 1	0.59*	0.65**	0.59*	0.61*	0.64*	0.61*	0.57*	0.61*
4.08	Peak 2	0.67**	0.66**	0.64**	0.62*	0.63*	0.63*	0.61*	0.45
5.04	Peak 3	0.50	0.51*	0.47	0.53*	0.56*	0.55*	0.54*	0.55*
8.27	Peak 4	0.71**	0.72**	0.69**	0.71**	0.74**	0.70**	0.66**	0.60*
11.43	Peak 5	-0.48	-0.46	-0.46	-0.55*	-0.43	-0.53*	-0.45	-0.52*
14.56	Peak 6	0.38	0.34	0.37	0.33	0.38	0.35	0.40	0.15
17.59	Peak 7	0.62*	0.53*	0.53*	0.53*	0.47	0.53*	0.53*	0.40
18.42	Peak 8	-0.58*	-0.52*	-0.53*	-0.58*	-0.50	-0.61*	-0.53*	-0.57*
21.00	Peak 9	-0.48	-0.54*	-0.52*	-0.54*	-0.55*	-0.56*	-0.52*	-0.61*
21.64	Peak 10	0.41	0.41	0.38	0.37	0.43	0.40	0.38	0.51*
23.90	Peak 11	-0.61*	-0.60*	-0.56*	-0.59*	-0.58*	-0.62*	-0.55*	-0.59*
25.64	Peak 12	0.66**	0.67**	0.65**	0.72**	0.65**	0.71**	0.62**	0.73**
25.78	Peak 13	0.01	0.04	0.01	0.12	0.04	0.05	-0.02	-0.11
26.02	Peak 14	0.62*	0.70**	0.70**	0.71**	0.69**	0.68**	0.67**	0.61*
37.45	Peak 15	-0.44	-0.39	-0.36	-0.41	-0.40	-0.43	-0.35	-0.34
49.48	Peak 16	0.83**	0.83**	0.81**	0.89**	0.82**	0.86**	0.79**	0.73**
50.28	Peak 17	0.79**	0.82**	0.79**	0.88**	0.80**	0.84**	0.75**	0.73**

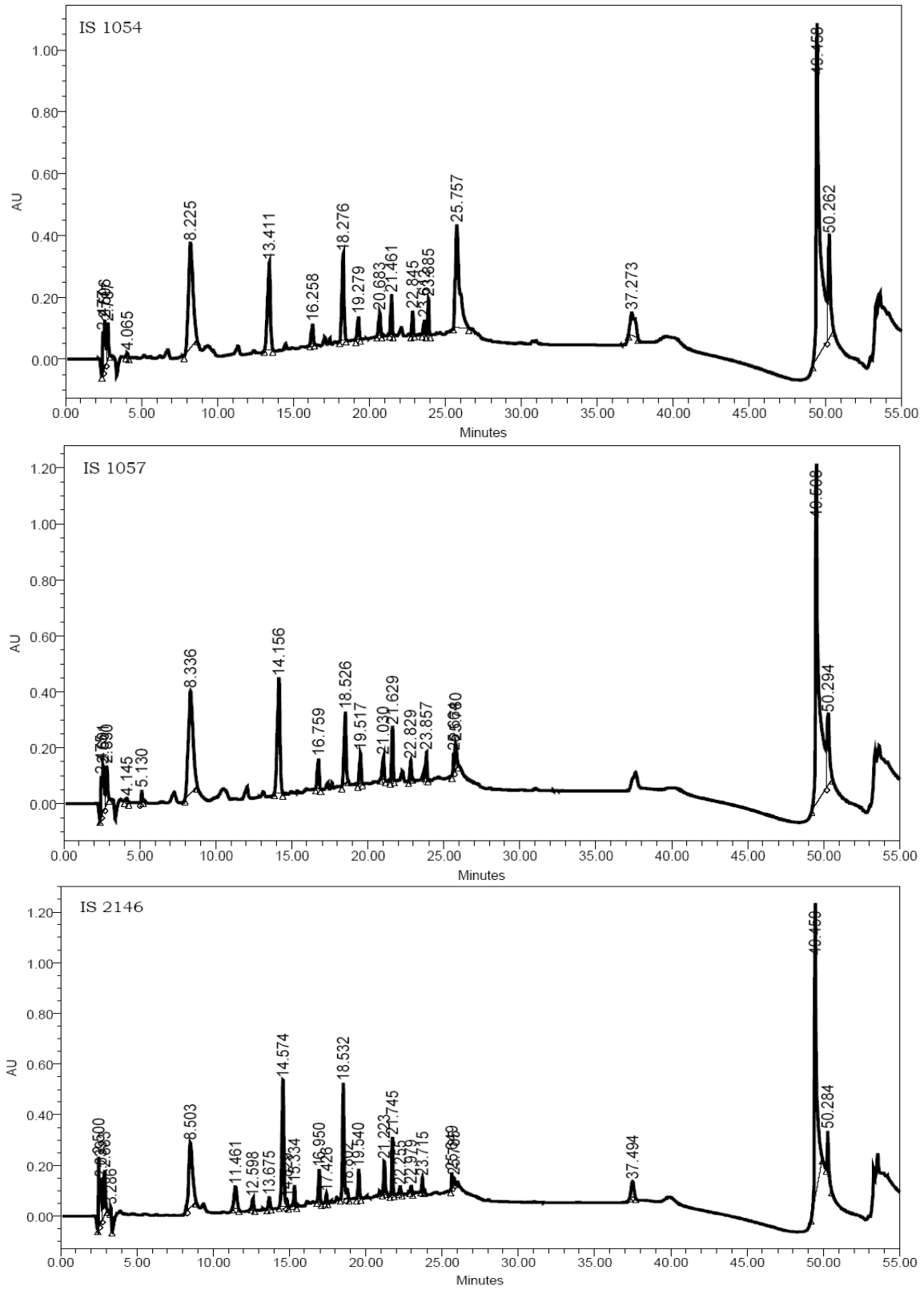
*, ** Correlation coefficients significant at $P = 0.05$ and 0.01, respectively. DAE = Days after seedling emergence.

Table 4.29 Association of protein profiles of 15 sorghum genotypes with expression of antibiosis to sorghum shoot fly, *A. soccata* (ICRISAT, Patancheru, India)

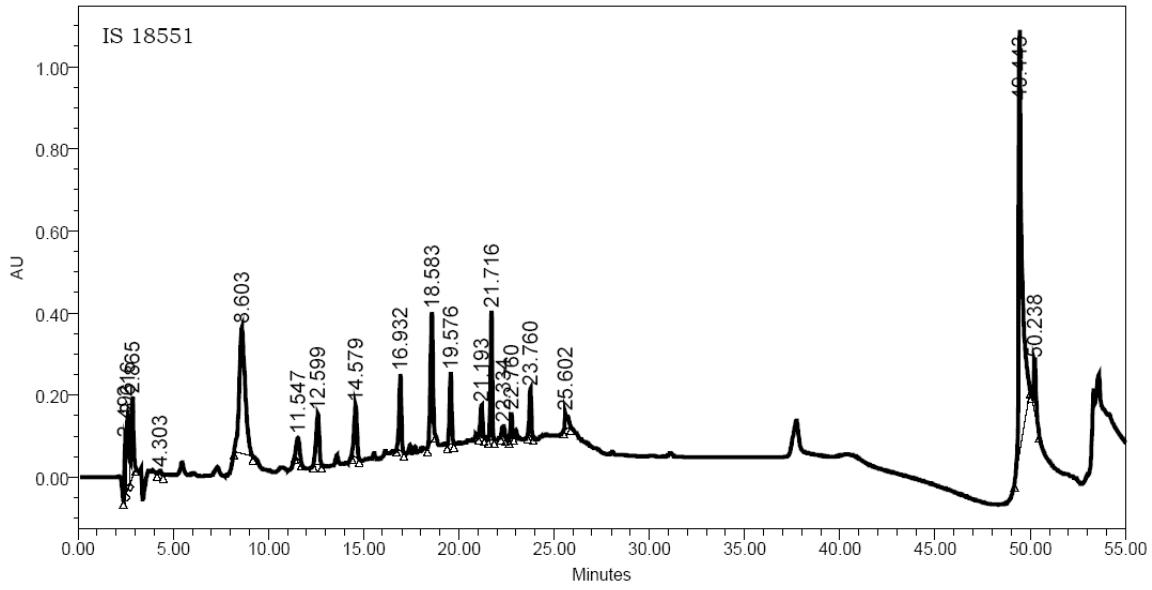
RT (min)	Protein peaks	Antibiosis components in glasshouse						
		Developmental period (days)	Larval survival (%)	Pupal mortality (%)	Adult emergence (%)	Male pupal weight (mg)	Female pupal weight (mg)	Fecundity female^{-1}
2.59	Peak 1	-0.48	0.48	-0.55*	0.72**	0.49	-0.14	-0.02
4.08	Peak 2	-0.55*	0.29	-0.30	0.42	0.45	-0.33	0.18
5.04	Peak 3	-0.41	0.56*	-0.33	0.65**	0.43	-0.12	0.05
8.27	Peak 4	-0.61*	0.45	-0.57*	0.71**	0.49	-0.29	0.10
11.43	Peak 5	0.42	-0.37	0.10	-0.35	-0.03	0.31	-0.18
14.56	Peak 6	-0.32	0.02	-0.05	0.04	0.46	-0.59*	-0.14
17.59	Peak 7	-0.61*	0.21	-0.35	0.39	0.57*	-0.11	-0.07
18.42	Peak 8	0.65**	-0.54*	0.17	-0.53*	-0.01	0.01	-0.35
21.00	Peak 9	0.38	-0.63*	-0.01	-0.48	0.05	0.13	-0.48
21.64	Peak 10	-0.33	0.29	-0.50	0.54*	0.49	-0.01	-0.34
23.90	Peak 11	0.57*	-0.39	0.51*	-0.63*	-0.47	0.15	0.06
25.64	Peak 12	-0.56*	0.32	-0.47	0.55*	0.11	-0.17	0.41
25.78	Peak 13	0.04	0.04	-0.16	0.14	0.04	-0.32	-0.05
26.02	Peak 14	-0.52*	0.33	-0.26	0.43	0.06	-0.42	0.66**
37.45	Peak 15	0.52*	-0.24	0.57*	-0.55*	-0.25	-0.10	0.02
49.48	Peak 16	-0.75**	0.57*	-0.51*	0.77**	0.41	-0.39	0.23
50.28	Peak 17	-0.70**	0.55*	-0.52*	0.76**	0.32	-0.38	0.38

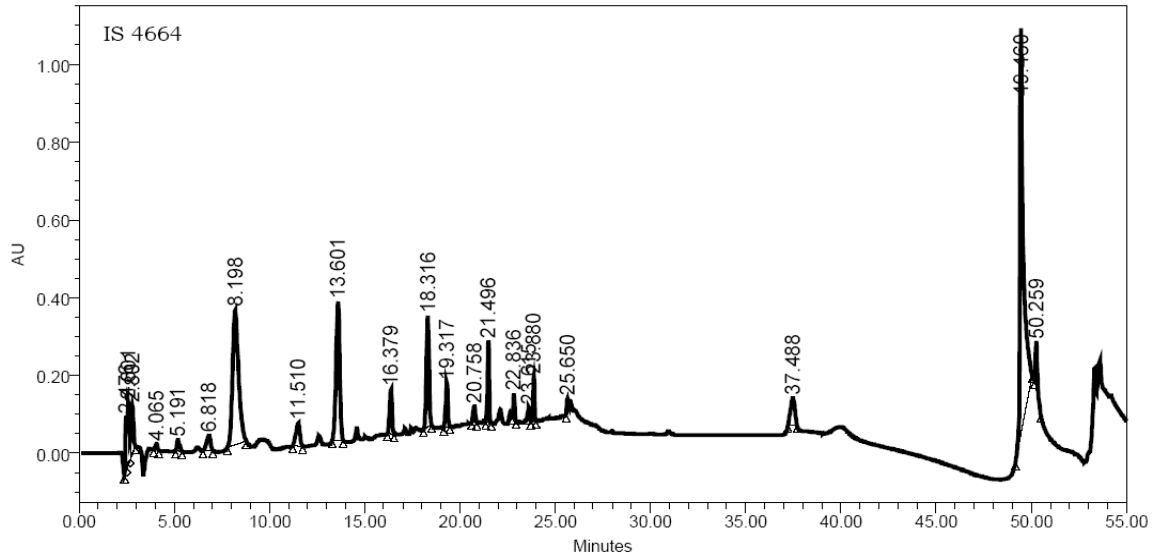
*, ** Correlation coefficients significant at $P = 0.05$ and 0.01, respectively.

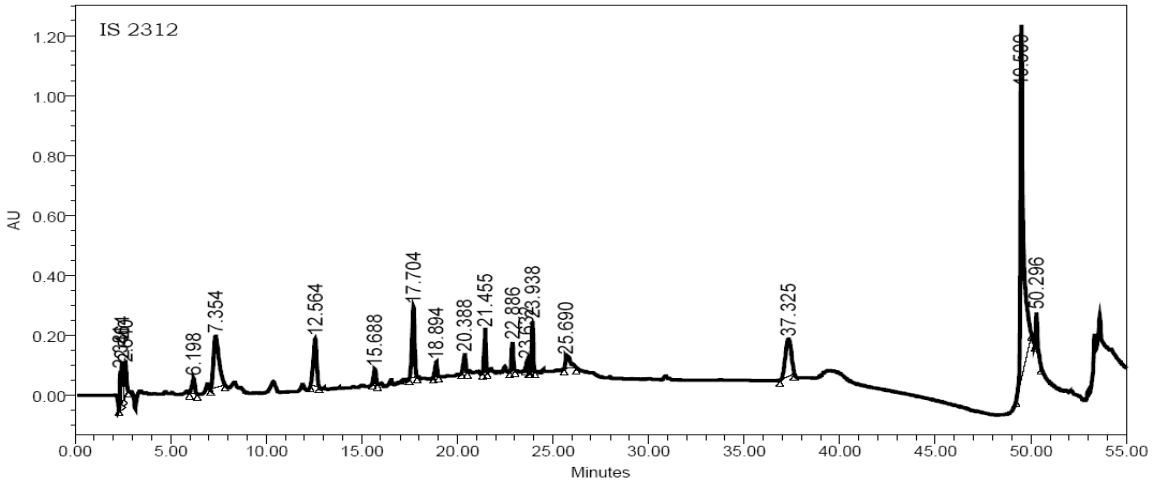
Fig. 4.2 HPLC protein profiles of germinated seeds of 15 sorghum genotypes



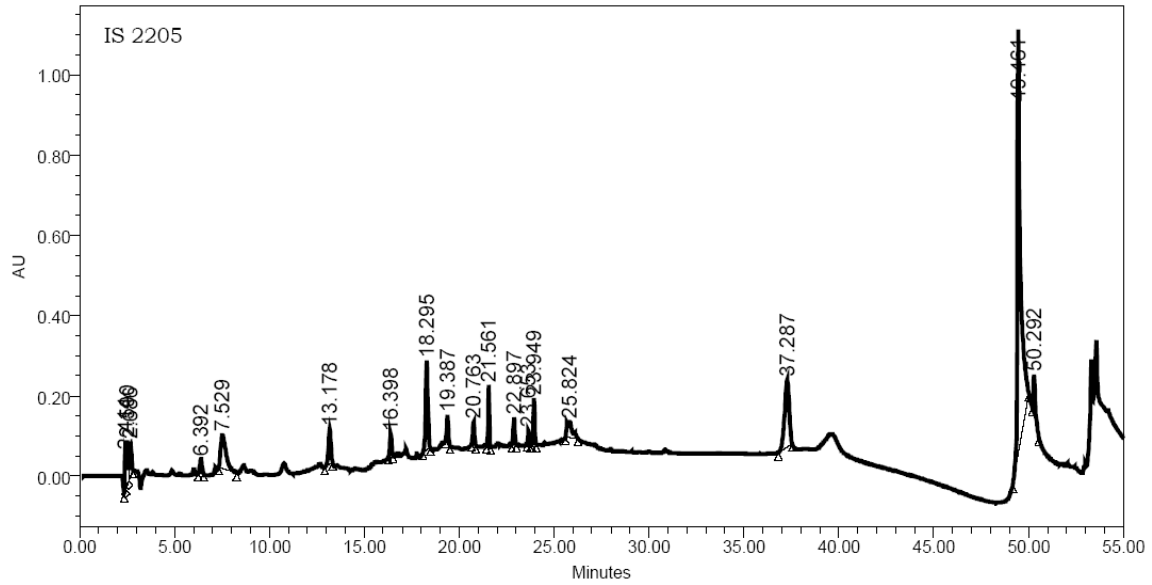
Continued.....

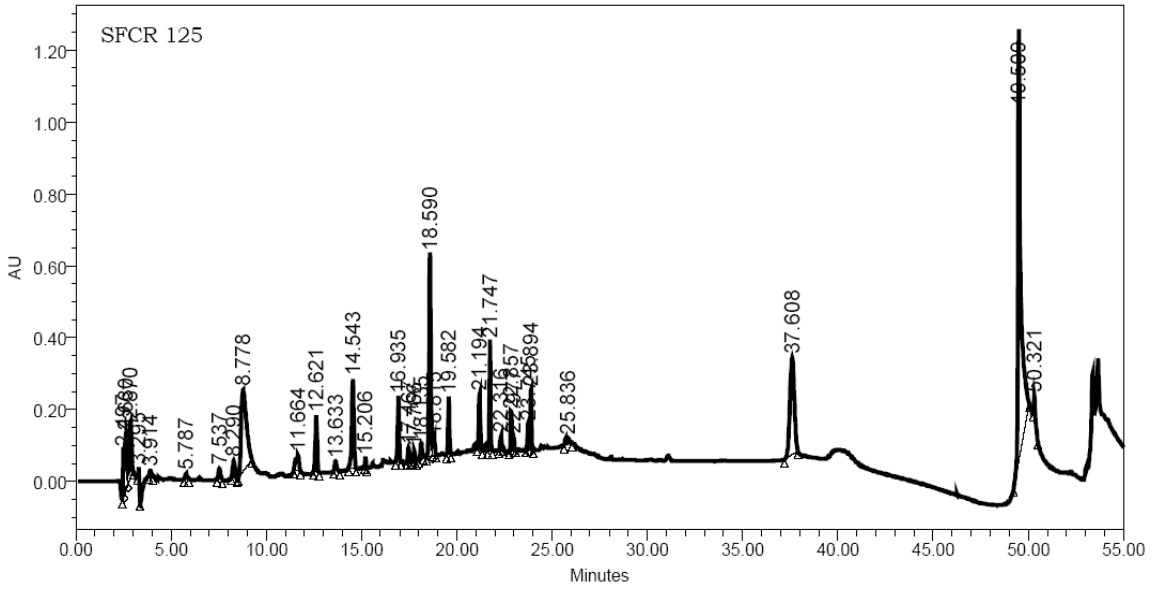

IS 18551
AU
Minutes
8.603
11.547
12.599
14.579
16.932
18.583
19.576
21.716
23.760
25.602
50.238

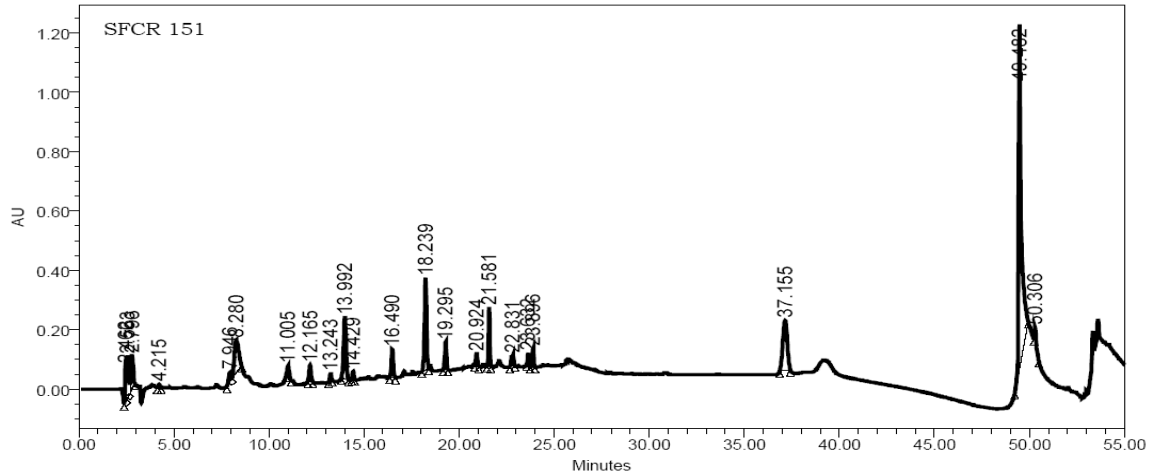

IS 4664
AU
Minutes
8.198
11.510
13.601
16.379
18.316
19.317
21.496
25.650
37.488
50.259


IS 2312
AU
Minutes
6.198
7.354
12.564
15.688
17.704
18.894
20.388
21.455
25.690
37.325
50.296

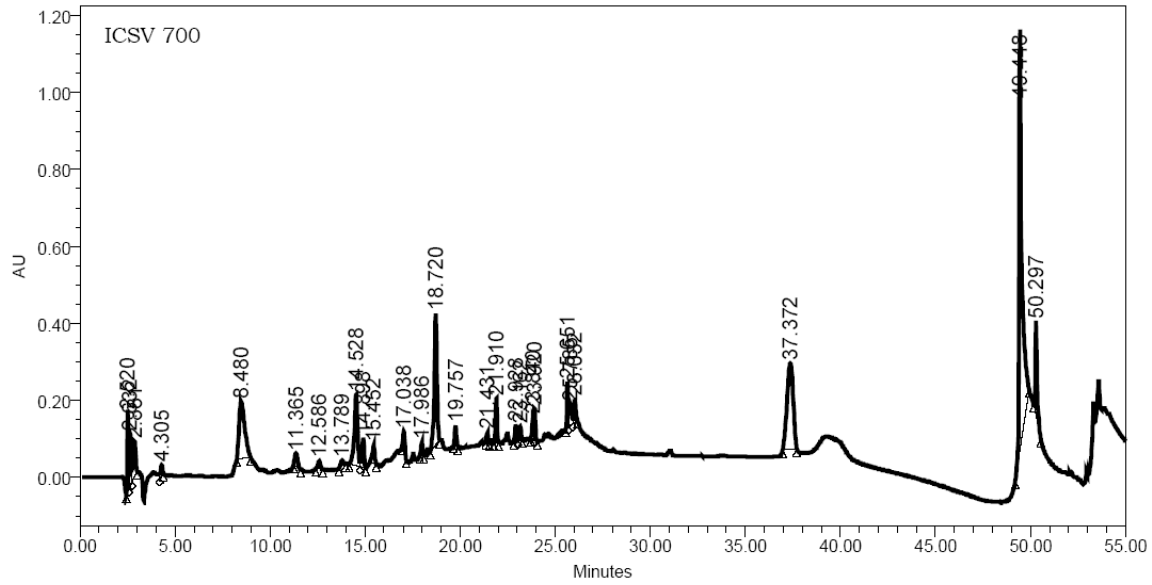
Continued……

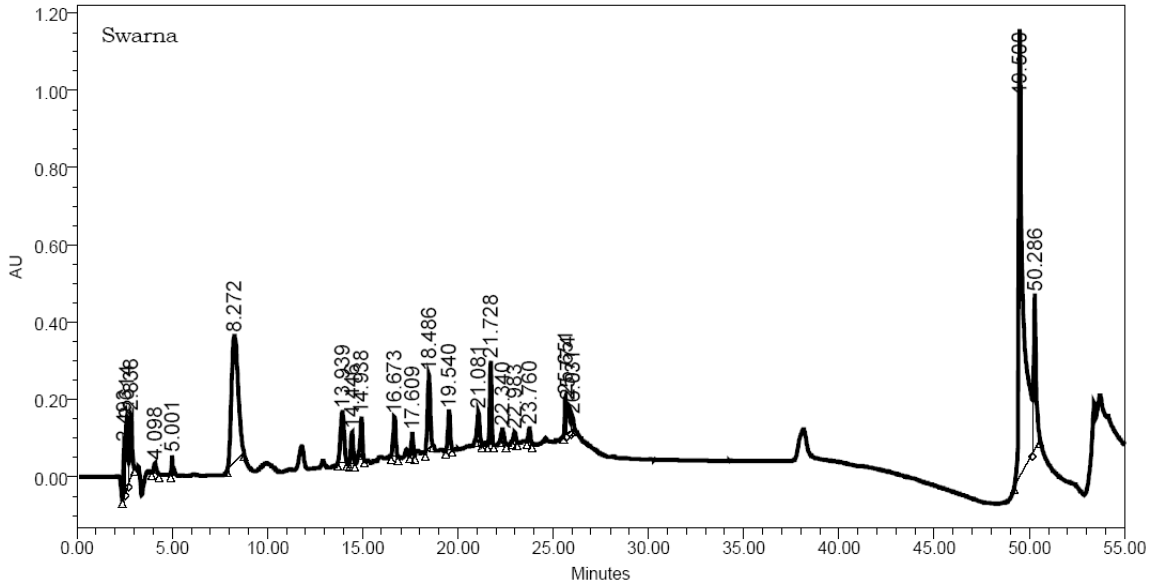

IS 2205
AU
Minutes
6.392
7.529
13.178
16.398
18.295
19.387
20.763
21.561
22.887
25.824
37.287
49.461
50.292

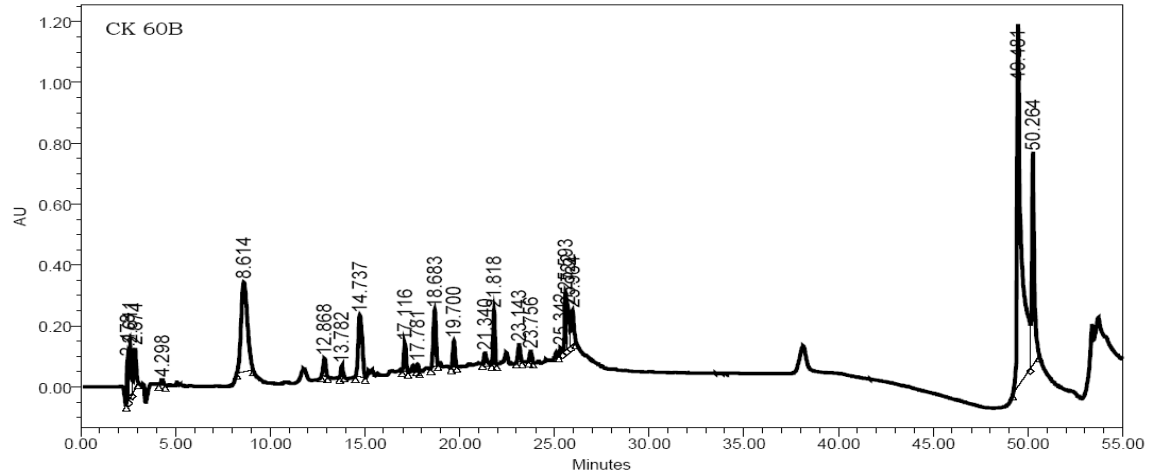

SFCR 125
AU
Minutes
3.914
5.787
7.537
8.778
11.664
12.621
13.633
14.543
15.206
16.935
18.590
19.582
21.194
21.747
25.836
37.608
49.500
50.321


SFCR 151
AU
Minutes
4.215
7.946
8.280
11.005
12.165
13.243
13.992
16.490
18.239
19.295
20.924
21.581
37.155
49.492
50.306

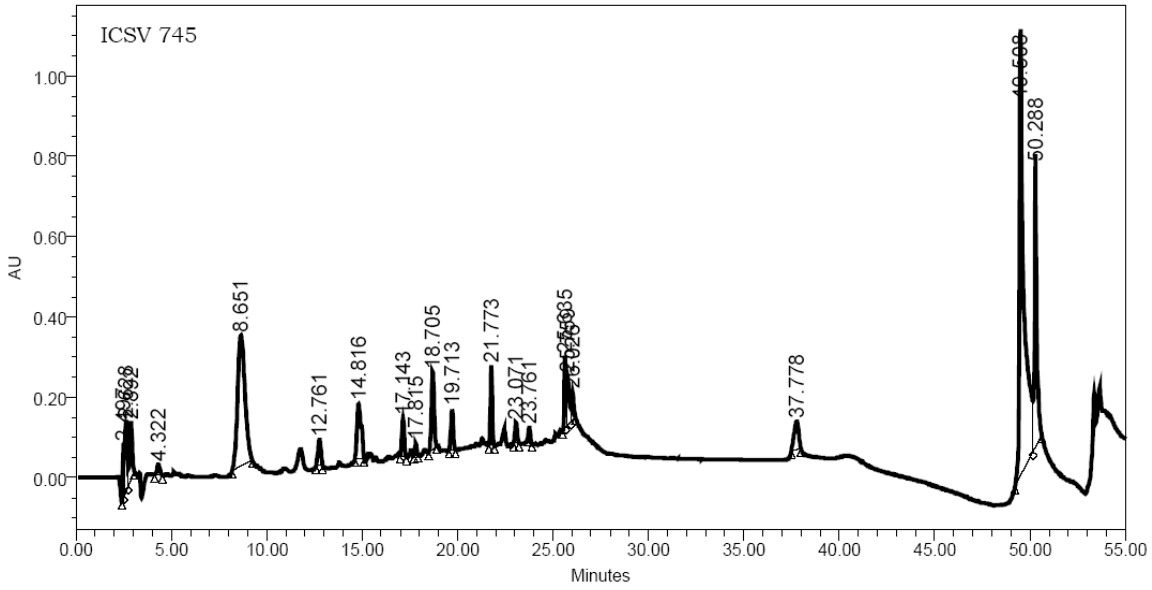
Continued……

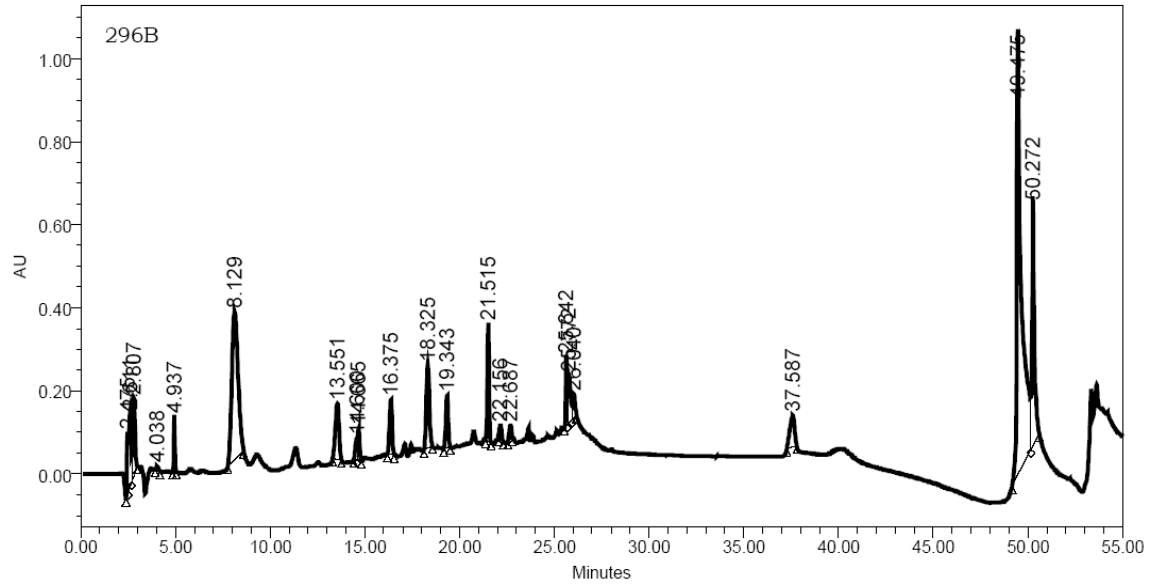

ICSV 700
AU
Minutes
8.480
11.365
12.586
13.789
18.720
37.372
50.297

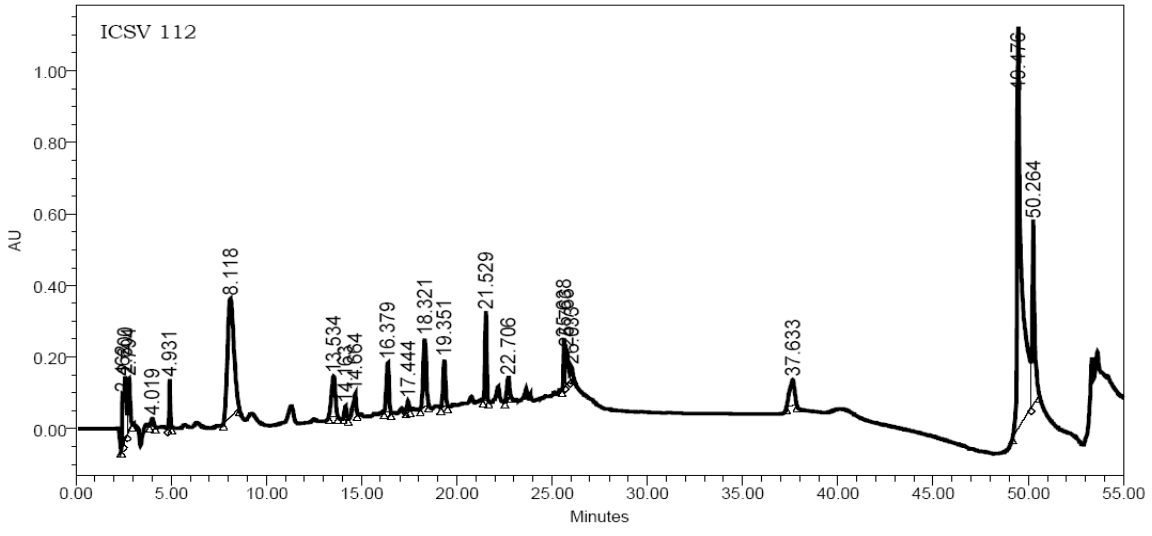

Swarna
AU
Minutes
8.272
18.486
19.540
21.728
50.286


CK 60B
AU
Minutes
8.614
12.868
13.782
14.737
18.683
19.700
50.264

Continued……


ICSV 745
AU
Minutes
8.651
12.761
14.816
18.705
19.713
21.773
37.778
50.288


296B
AU
Minutes
8.129
13.551
16.375
18.325
19.343
21.515
37.587
50.272


ICSV 112
AU
Minutes
8.118
16.379
18.321
19.351
21.529
37.633
50.264

4.9 GC-MS profiles of compounds on the leaf surface of sorghum seedlings and expression of resistance to shoot fly, *Atherigona soccata*

GC-MS profiles of the sorghum leaf surface chemicals revealed considerable differences among the sorghum genotypes tested (Table 4.30) (Fig. 4.3). Of the 150 compounds detected, 10 compounds showed significant association with expression of resistance to *A. soccata*. Of major compounds detected, hexanal at RT 4.15 was present both in IS 18551 and Swarna, but the peak area was greater in the resistant check, IS 18551 as compared to that of the susceptible check, Swarna. Pentadecane, 8 - hexyl at RT 15.34, and lonol 2 at RT 15.8 were present only in the susceptible genotypes, Swarna and CK 60B, but absent in rest of the genotypes.

Dodecane, 2, 6, 11- trimethyl at RT 13.37 was present only in the shoot fly susceptible genotypes CK 60B, ICSV 745, 296B, ICSV 112 and Swarna, but absent in resistant genotypes IS 1054, IS 2146, IS 4664, IS 18551, IS 2312, IS 2205, SFCR 125, SFCR 151, and ICSV 700 (except in genotype IS 1057). Compound 4, 4- dimethyl cyclo octene at RT 7.31 was present in the resistant genotypes IS 2146, IS 2312, and IS 18551, but absent in all other genotypes; while hexane 2, 4-dimethyl at RT 7.31 was absent in IS 2146, IS 2312, IS 18551 and IS 4664, but present in rest of the genotypes. Compound undecane 5-methyl at RT 8.83 was present in all the genotypes, except IS 4664, IS 2205 and Swarna. Its amounts were

greater in SFCR 125, ICSV 745, 296B and ICSV 112. Compound eicosane at RT 14.91 was present in all genotypes, except in the susceptible check, Swarna. More amounts of eicosane were detected in IS 4664. Decane 4-methyl at RT 8.08 was present in all genotypes, but had more peak area in SFCR 125, ICSV 700, CK 60B, ICSV 745, 296B, ICSV 112, and Swarna as compared to the resistant check, IS 18551 (Table 4.30).

Correlations of GC-MS volatile compounds with shoot fly damage indicated that undecane 5- methyl, decane 4- methyl, hexane 2, 4- methyl, pentadecane 8- hexyl, and dodecane 2, 6, 11- trimethyl were significantly and positively correlated with deadhearts and eggs per 10 seedlings, but the correlation of undecane 5- methyl with eggs per 10 seedlings was nonsignificant. These compounds possibly acted as attractants/oviposition stimulants for the sorghum shoot fly, *A. soccata*. The compound 4, 4- dimethyl cyclooctene was significantly and negatively associated with deadhearts and eggs per 10 seedlings, and it might impart resistance to shoot fly. The compounds eicosane, tridecane and hexanal showed a positive, and lonol 2 showed a negative association with shoot fly damage, but the correlation coefficients were nonsignificant (Table 4.31).

Table 4.30 Biochemical constituents on the leaf surface of 15 sorghum genotypes (GC-MS profiles) (ICRISAT, Patancheru, India)

	Area (%)									
Genotype	Hexanal	Hexane 2, 4- dimethyI	4, 4- Dimethyl cyclooctene	Eicosane	Decane4- rnethyI	Undecane 5- rnethyI	Tridecane	Dodecane 2, 6, 11- trimethyl	Pentadecane 8- hexyl	Lonol 2
IS 1054	*	0.56	*	3.04	1.12	0.76	1.65	*	*	*
IS 1057	*	0.54	*	2.74	1.21	0.73	1.29	0.67	*	*
IS 2146	*	*	0.45	3.47	0.94	0.69	1.88	*	*	*
IS 4664	*	*	*	4.88	0.78	*	0.77	*	*	*
IS 2312	*	*	0.49	2.78	1.01	0.66	*	*	*	*
IS 2205	*	0.49	*	2.76	1.1	*	*	*	*	*
SFCR 125	*	0.57	*	3.01	1.33	3.36	1.42	*	*	*
SFCR 151	*	0.49	*	2.9	1.14	0.67	1.38	*	*	*
ICSV 700	*	0.59	*	3.08	1.35	0.93	1.44	*	*	*
CK 60B	*	0.55	*	1.45	1.28	0.89	0.61	0.88	0.67	*
ICSV 745	*	0.66	*	3.18	1.51	3.82	*	0.9	*	*
296B	*	0.56	*	3.32	1.31	3.39	1.54	0.9	*	*
ICSV 112	*	0.54	*	3.21	1.26	3.24	1.48	0.9	*	*
IS 18551 (R)	0.75	*	0.58	3.18	1.15	0.81	1.75	*	*	*
Swarna (S)	0.47	0.56	*	*	1.24	*	*	0.77	0.61	0.39

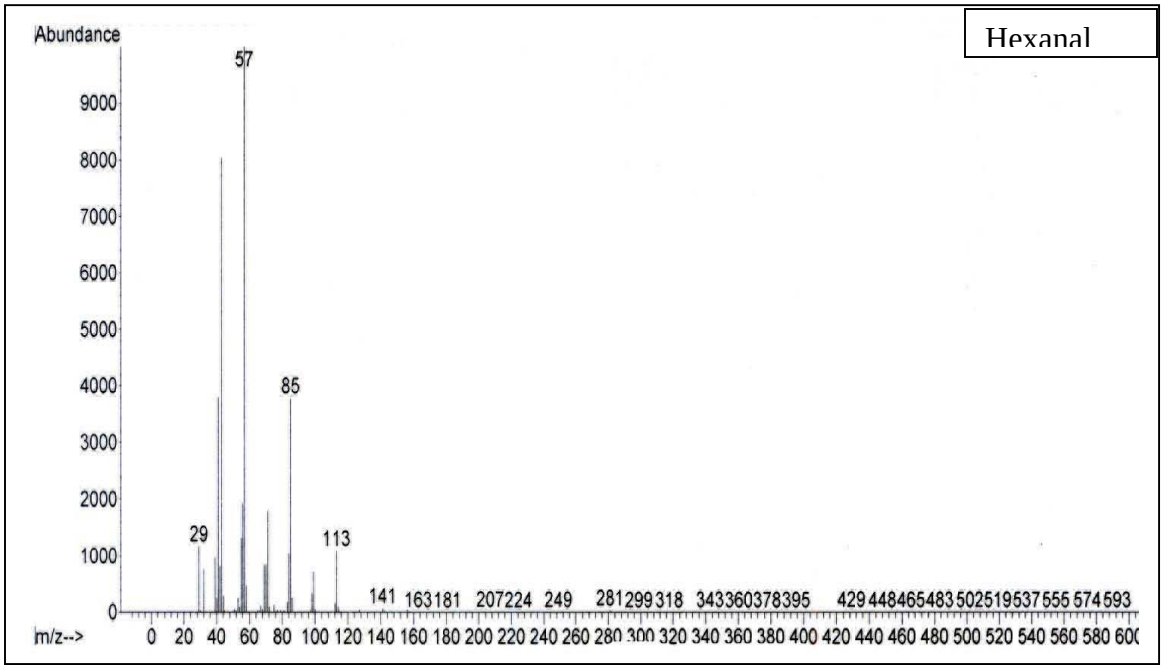
* Undetectable.

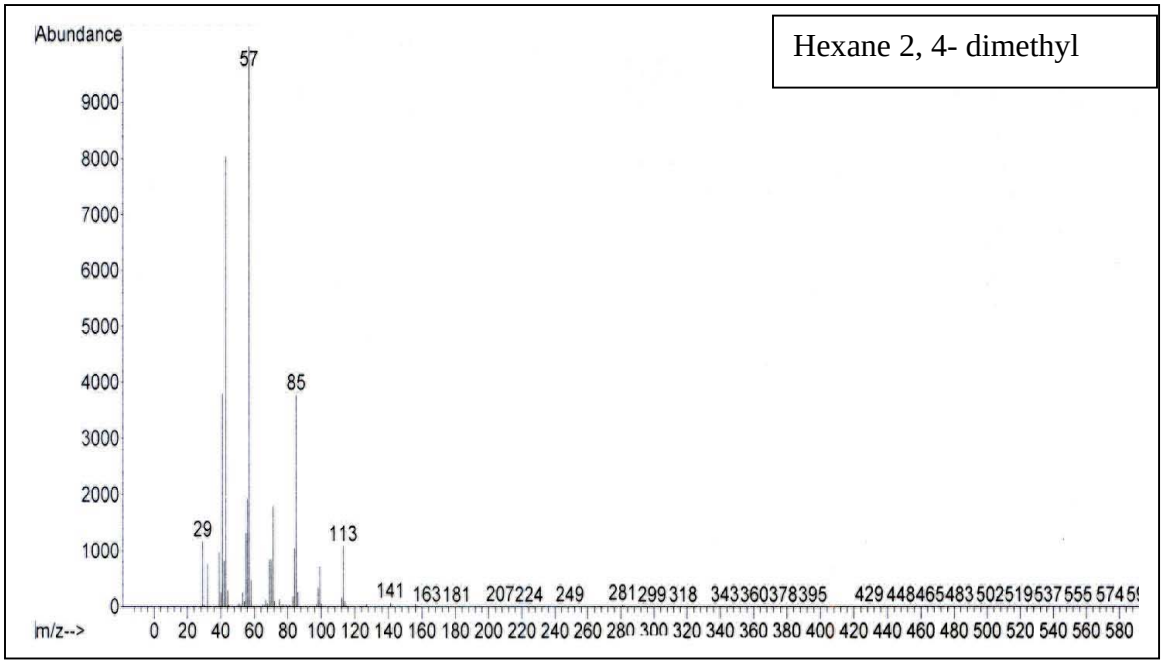
Table 4.31 Association of biochemical constituents on the leaf surface with expression of resistance to sorghum shoot fly, *A. soccata* (ICRISAT, Patancheru, India)

Compound Name	Deadhearts (%) (21 DAE)	Eggs seedlings^{-10} (21 DAE)
Eicosane	-0.36	-0.40
Tridecane	-0.26	-0.28
Hexanal	-0.03	-0.04
Lonol 2	0.38	0.44
Undecane 5- rnethyl	0.54*	0.45
4, 4- Dimethylcyclooctene	-0.53*	-0.59*
Decane 4- rnethyl	0.59*	0.52*
Hexane 2, 4- dimethyl	0.56*	0.56*
Pentadecane 8- hexyl	0.52*	0.57*
Dodecane 2, 6, 11- trimethyl	0.89**	0.88**

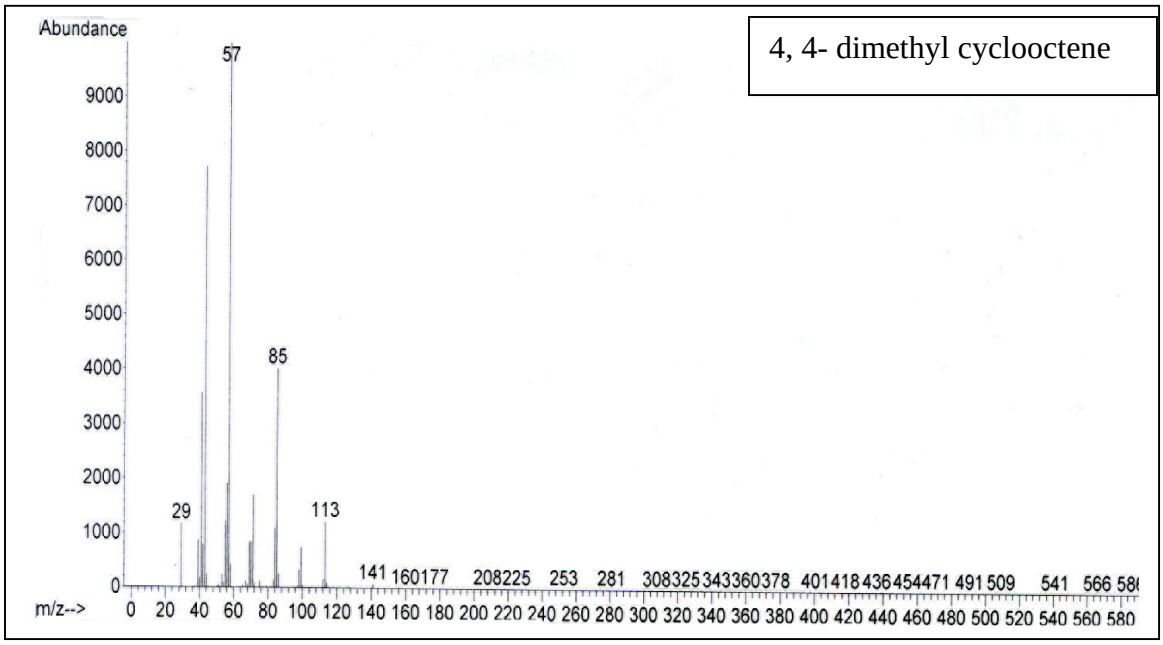
*, ** Correlation coefficients significant at $P = 0.05$ and 0.01, respectively. DAE = Days after seedling emergence.

Fig. 4.3 GC-MS profiles of the chemicals on leaf surface of sorghum genotypes.

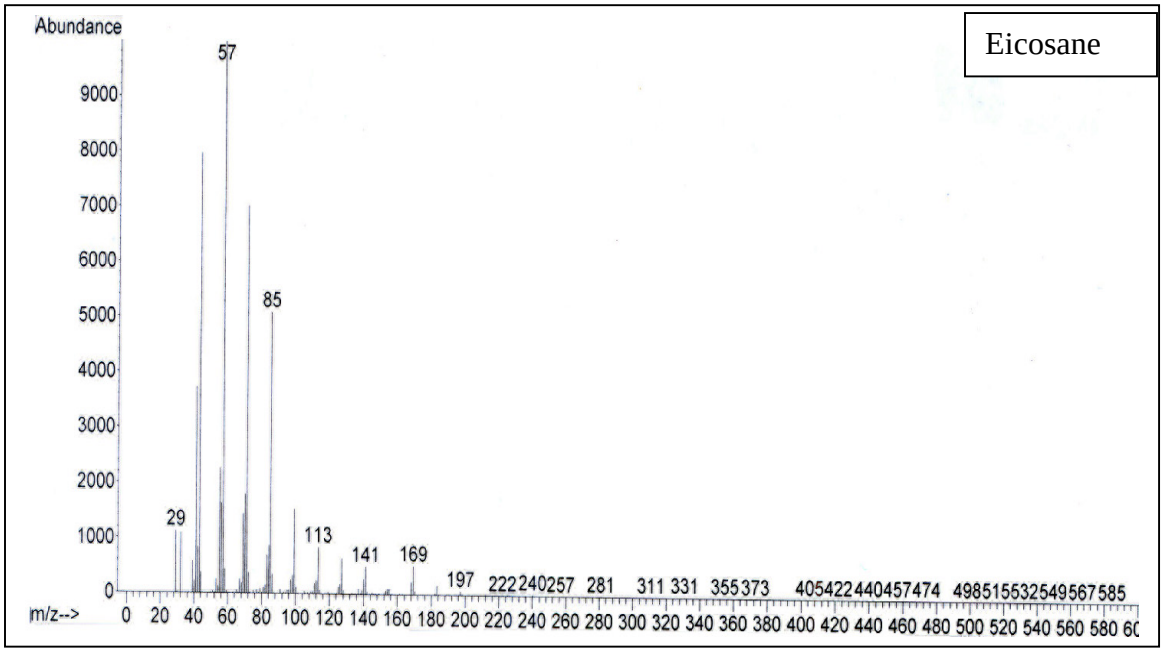




Continued……

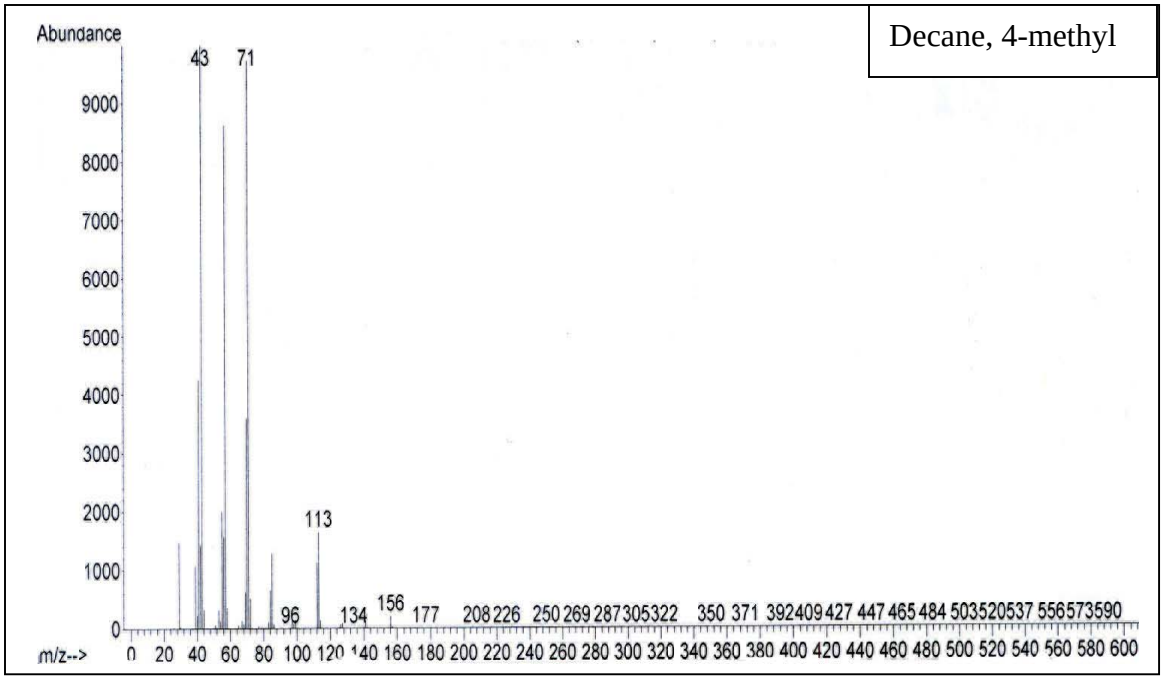


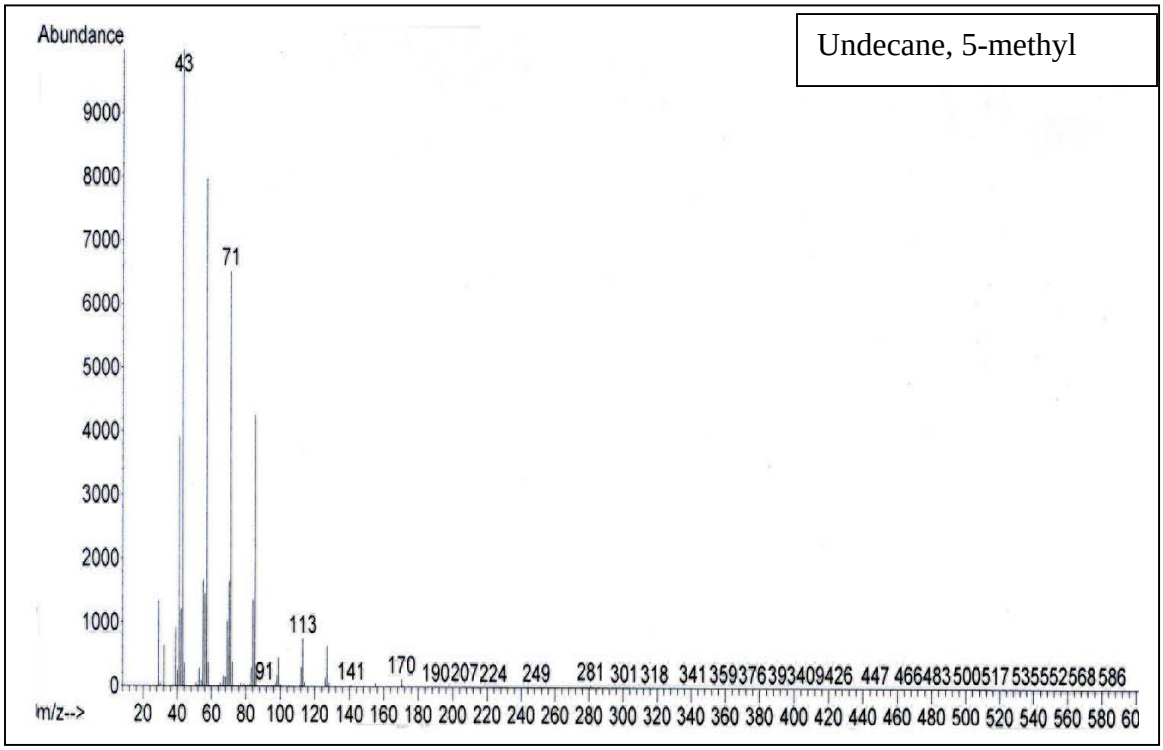
4, 4- dimethyl cyclooctene



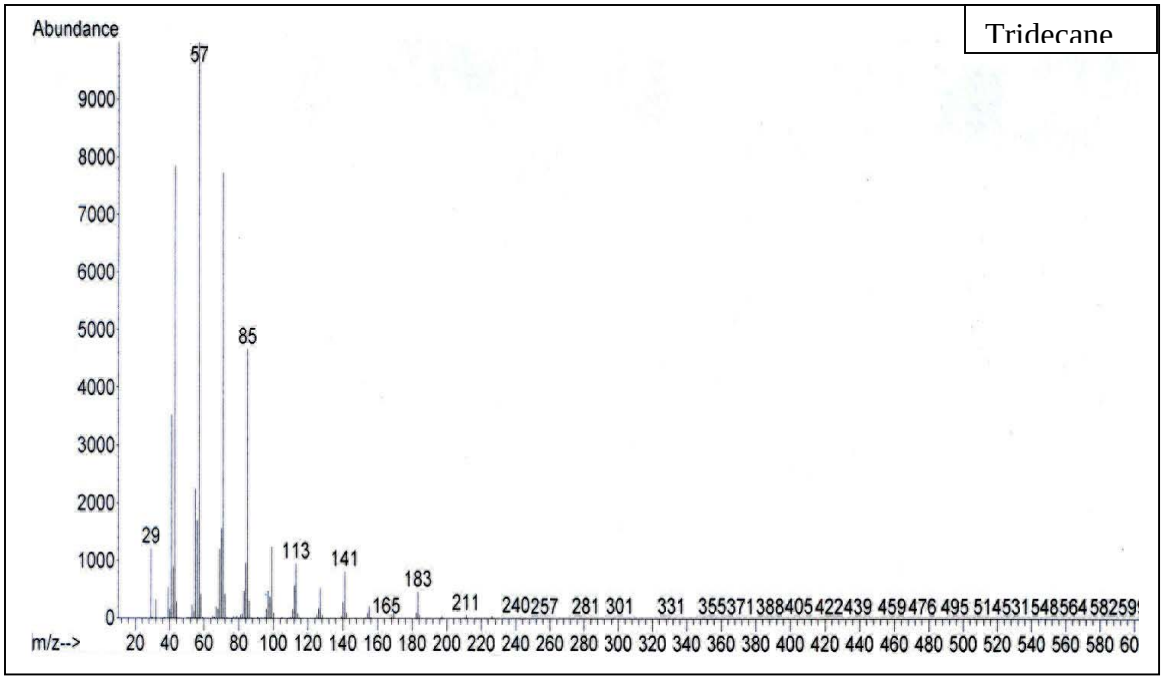
Eicosane

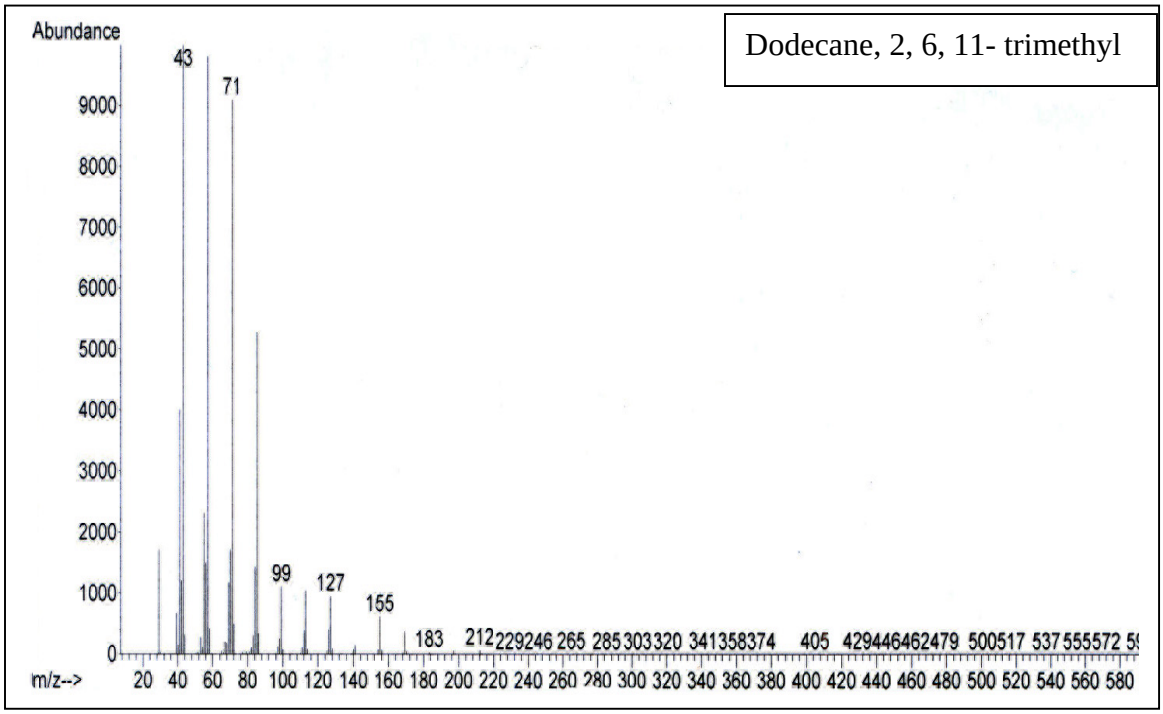
Continued……


Decane, 4-methyl
Abundance
43
71
113
96
134
156
177
m/z-->

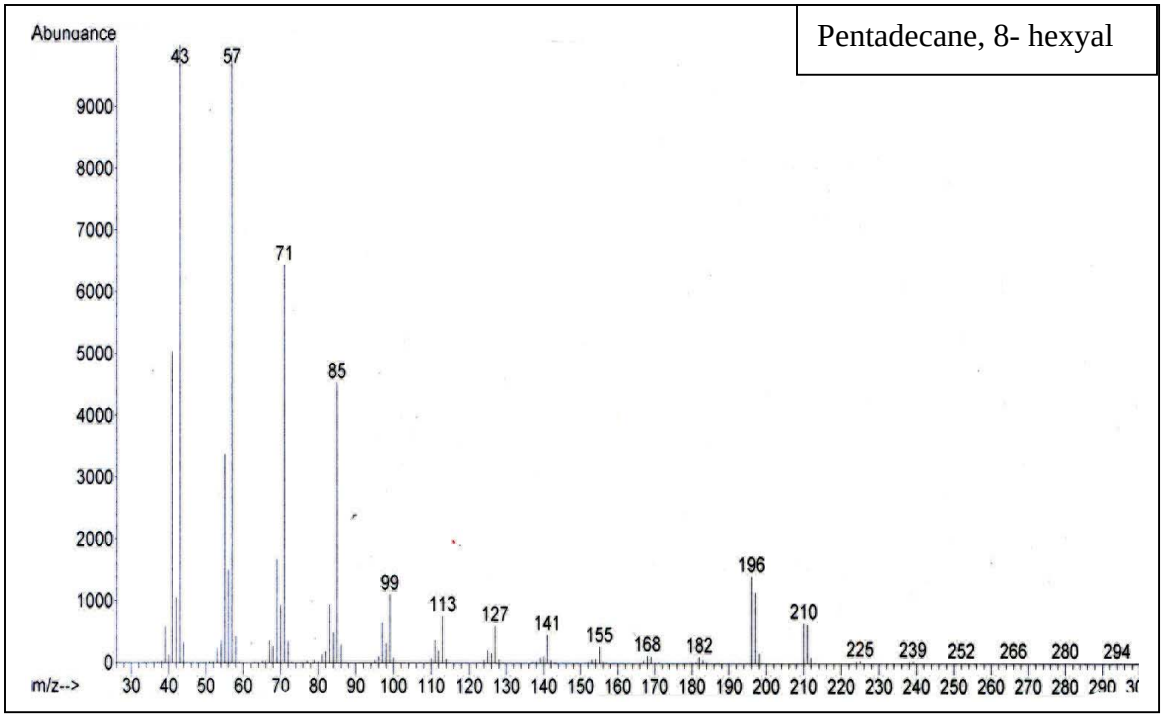

Undecane, 5-methyl
Abundance
43
71
113
91
141
170
m/z-->

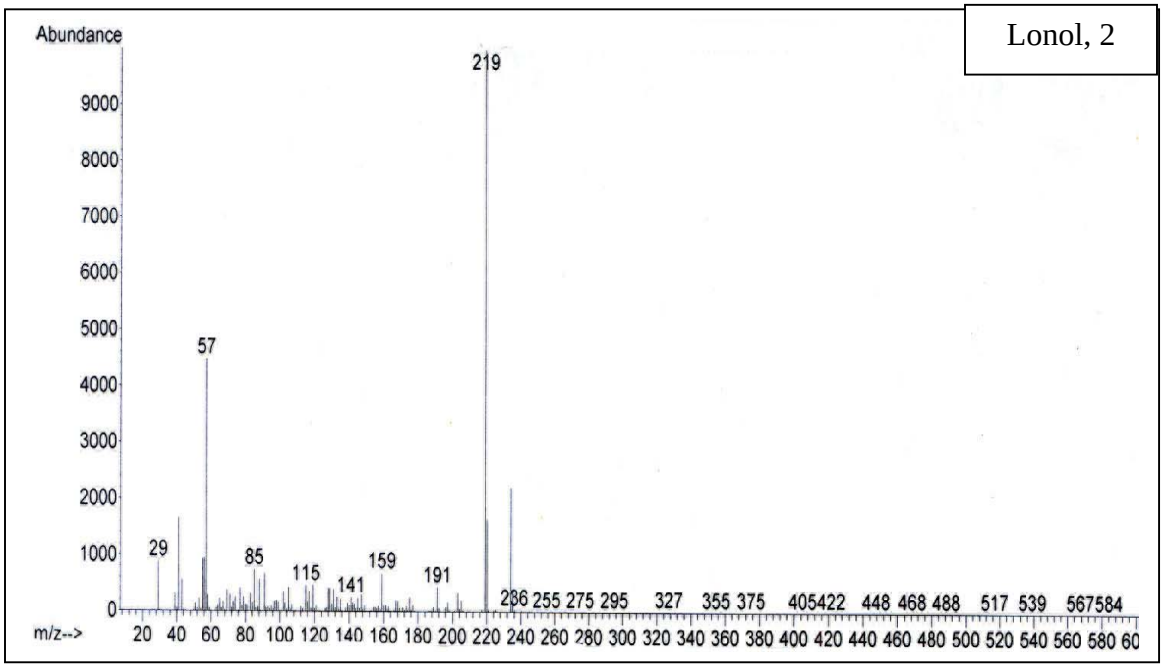
Continued……


Tridecane
Abundance
57
85
29
113
141
183
m/z-->


Dodecane, 2, 6, 11- trimethyl
Abundance
43
71
99
127
155
183
m/z-->

Continued.....


Pentadecane, 8- hexyal
Abundance
m/z-->


Lonol, 2
Abundance
m/z-->

4.10 Diversity of sorghum genotypes and expression of resistance to shoot fly, *Atherigona soccata*

4.10.1 Genotypic diversity based on the biological, morphological, and biochemical parameters

Based on biological parameters (deadhearts at 14, 21, 28 DAE; seedlings with eggs, and egg per 10 seedlings at 14, 21 DAE; tiller deadhearts, and antiobiosis components) of 15 sorghum genotypes, similarity matrix analysis placed the test genotypes into two groups/clusters (Fig. 4.4). First group comprised of resistant genotypes and these were subdivided into three sub-clusters. Cluster I (a) - IS 1054, IS 1057, and SFCR 151; cluster I (b) - IS 4664, SFCR 125, and ICSV 700 and cluster I (c) - IS 2146, IS 2205, IS 18551, and IS 2312. The second group comprised of susceptible genotypes and these were subdivided into two sub-clusters. Cluster II (a) - Swarna and 296B; cluster II (b) - CK 60B and ICSV 745. ICSV 112 formed an out group.

Based on morphological traits (glossy, pigmentation, seedling vigor, adaxial and abaxial trichomes, leaf surface wetness, plant height, days to 50% flowering, and productive tillers) of 15 sorghum genotypes, similarity matrix analysis placed the test genotypes into two groups/clusters (Fig. 4.5). First group comprised of resistant genotypes and these were subdivided into two sub-clusters. Cluster I (a) - IS 1054, SFCR 125, and SFCR 151; cluster I (b) – IS 1057, IS 4664, IS 2146, IS 2312, IS 18551, and IS 2205. ICSV 700 formed an out group. The second

group comprised of susceptible genotypes and these were subdivided into two sub-clusters. Cluster II (a) - Swarna and 296B; cluster II (b) - CK 60B, ICSV 745, and ICSV 112.

Based on biochemical parameters (micronutrients, fats, tannins, polyphenols, proteins, sugars, lignins, and HPLC profiling of undamaged seedlings) of 15 sorghum genotypes, similarity matrix analysis placed the test genotypes into two groups/clusters (Fig.4.6). First group comprised of resistant genotypes and these were subdivided into two sub-clusters. Cluster I (a) - IS 1057, IS 2146, and IS 18551; cluster I (b) – IS 2312, IS 2205, SFCR 125, ICSV 700, and SFCR 151. IS 1054 and IS 4664 formed another group. The second group comprised of shoot fly- susceptible genotypes (Swarna, CK 60B, ICSV 745, 296B, and ICSV 112).

4.10.2 Genetic diversity based on SSR markers

Of the 93 microsatellites primer pairs used in the present study, 79 showed good polymorphism between the sorghum accessions (Fig. 4.7). Of the 93 SSR markers, 7 SSRs (Xgap 01, Xisep 0110, Xisep 0314, Xisep 0128, Xtxp 287, Xtxp 327, Xtxp 343) did not showed any polymorphism, 5 SSRs (gpsb 118, ISEP 0310, Xcup 52, Xtxp 339 and Xtxp 59) were monomorphic, and 2 SSR markers (Xisep 0443 and Xisep 1008) showed high heterozygocity. List of 79 working SSR markers is given in (Table 4.32).

Individual PCR products were pooled based on the product sizes, and separated in capillary electrophoresis using internal size standard. A

total of 332 alleles were detected with an average of 4 alleles per marker, and 2 to 9 alleles per marker with an average of 0.52. Heterozygosity ranged from 0.00 to 0.21, and the mean was 0.03. Marker SbKAFGK 1 showed maximum heterozygosity (0.21) between the genotypes. The polymorphic information content (PIC) values ranged from 0.06 to 0.86. The height level of polymorphism was found with primer pair Xtxp 27 (0.86), followed by Xgap 206 (0.85). Lowest polymorphism was found with the primer pair Xtxp136 (0.06) (Table 4.32).

Factorial analysis of 15 sorghum genotypes with 79 SSR markers placed the sorghum genotypes into 5 divergent groups. First group comprised of IS 18551, IS 2205, IS 2312 and IS 2146; II: IS 1054, IS 1057, IS 4664 and ICSV 700; III: 296B and SFCR 125; IV: Swarna, CK 60B, ICSV 745 and ICSV 112; and V: SFCR 151 (Fig. 4.8). The 15 accessions studied were placed in 2 clusters based on Neighbor-Joining cluster analysis, but ICSV 700 formed an out group (Fig. 4.9). Cluster I was subdivided into two subclusters. Cluster I (a)- CK 60B and Swarna, and Cluster I (b)- ICSV 112 and ICSV 745. SFCR 151, 296B, and SFCR 125 were placed in cluster I. Cluster II was also subclustered into 2 subclusters. Cluster II (a) consisted IS 2205, IS 18551, IS 2312 and IS 2146; while cluster II (b) consisted of IS 1057, IS 1054 and IS 4664. ICSV 700 formed an out group. The results suggested that there is considerable diversity among the sorghum genotypes showing resistance to shoot fly, *A. soccata*. All *durra* races in cluster II (b) and its

intermediate race with *bicolor* II (a) were placed in cluster II. The c*audatum* race along with other intermediate races, and one k*afir* race genotype (CK 60B) were placed in cluster I (Fig. 4.10).

To confirm the association of morphological data with molecular diversity data generated by using 79 SSR markers, we carriedout Darwin analysis to associate morphological traits with molecular diversity. Genetic diversity based on deadheart incidence at 21 DAE placed the genotypes in 3 clusters. Red color clusters included the shoot fly-resistant genotypes IS 18551, IS 2146, IS 2312, IS 2205, and IS 1054; which suffered 25-40% deadhearts at 21 DAE. Green colored cluster included moderately resistant genotypes IS 1057, IS 4664, ICSV 700, SFCR 151, and SFCR 125; which suffered 40-55% deadhearts at 21 DAE. The blue colored cluster included the shoot fly susceptible genotypes Swarna, CK 60B, ICSV 745, 296B, and ICSV 112, which suffered 55-85% at 21 DAE (Fig. 4.11a).

Association of tiller deadhearts with molecular diversity grouped the test genotypes into 2 clusters. Red colored cluster included the shoot fly-resistant genotypes IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2205, IS 2312, SFCR 125, SFCR 151, and ICSV 700, which suffered 20-35% tiller deadhearts; whereas green colored cluster included the susceptible genotypes Swarna, CK 60B, ICSV 745, 296B, and ICSV 112, which showed 35 to 55% tiller deadhearts (Fig. 4.11b)

Association of eggs per 10 seedlings at 14 DAE with molecular diversity placed the test genotypes into 2 clusters. Blue colored cluster included the shoot fly-resistant genotypes: IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2205, IS 2312, SFCR 125, SFCR 151, and ICSV 700; with 1 - 7 eggs per 10 seedlings at 14 DAE. The pink colored cluster included the susceptible genotypes: Swarna, CK 60B, ICSV 745, 296B, and ICSV 112, with 7 - 17 eggs per 10 seedlings at 14 DAE (Fig. 4.11c).

Association of percentage seedlings with eggs at 14 DAE with molecular diversity placed the genotypes into 3 clusters. Blue colored cluster included the shoot fly- resistant genotypes: IS 18551, IS 2146, IS 2205, IS 2312, and SFCR 151; with 25 - 36% plants with eggs. Pink colored cluster included IS 1054, IS 1057, IS 4664, SFCR 125, and ICSV 700; with 36 - 50% plants with eggs. Green colored cluster included the susceptible genotypes: Swarna, CK 60B, ICSV 745, 296B, and ICSV 112 with 50 - 90% seedlings with eggs at 14 DAE (Fig. 4.11d).

Association of leaf surface wetness (LSW) with molecular diversity placed the sorghum genotypes into 2 clusters. Green colored cluster included the shoot fly-resistant genotypes: IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2205, IS 2312, SFCR 125, SFCR 151, and ICSV 700 which had low LSW. Red colored cluster included the susceptible genotypes: Swarna, CK 60B, ICSV 745, 296B, and ICSV 112 which had high LSW (Fig. 4.11e).

Association with leaf glossiness with molecular diversity placed the test genotypes into 3 clusters. Green colored cluster comprised of shoot fly-resistant genotypes; IS 1054, IS 2146, IS 18551, IS 2205, IS 2312, ICSV 700, SFCR 125, and SFCR 151, which were highly glossy. Blue colored cluster comprised of moderately resistant genotypes: IS 1057 and IS 4664 which were moderately glossy. Whereas red colored cluster comprised of susceptible genotypes: Swarna, CK 60B, ICSV 745, 296B, and ICSV 112, which were non-glossy (Fig. 4.11f).

Trichome density and molecular diversity placed the test genotypes into 2 clusters. Red colored cluster comprised of shoot fly- resistant genotypes: IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2205, IS 2312, SFCR 125, SFCR 151, and ICSV 700, which were trichomed. Green colored cluster comprised of the susceptible genotypes; Swarna, CK 60B, ICSV 745, 296B and ICSV 112, which were non-trichomed (Fig. 4. 11g).

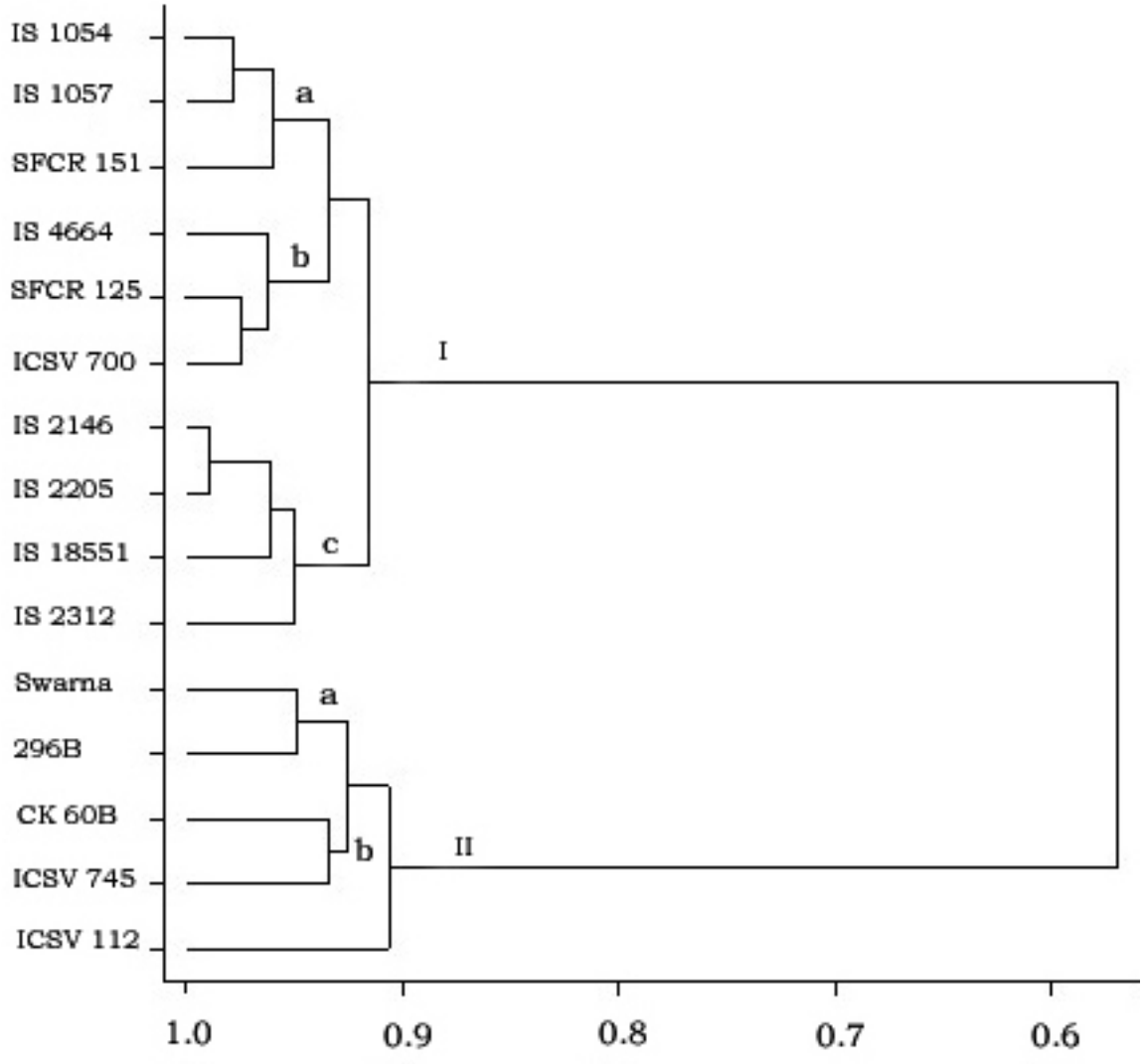


Fig. 4.4 Similarity matrix analysis of 15 sorghum genotypes based on reaction to shoot fly, *A. soccata* under field conditions.

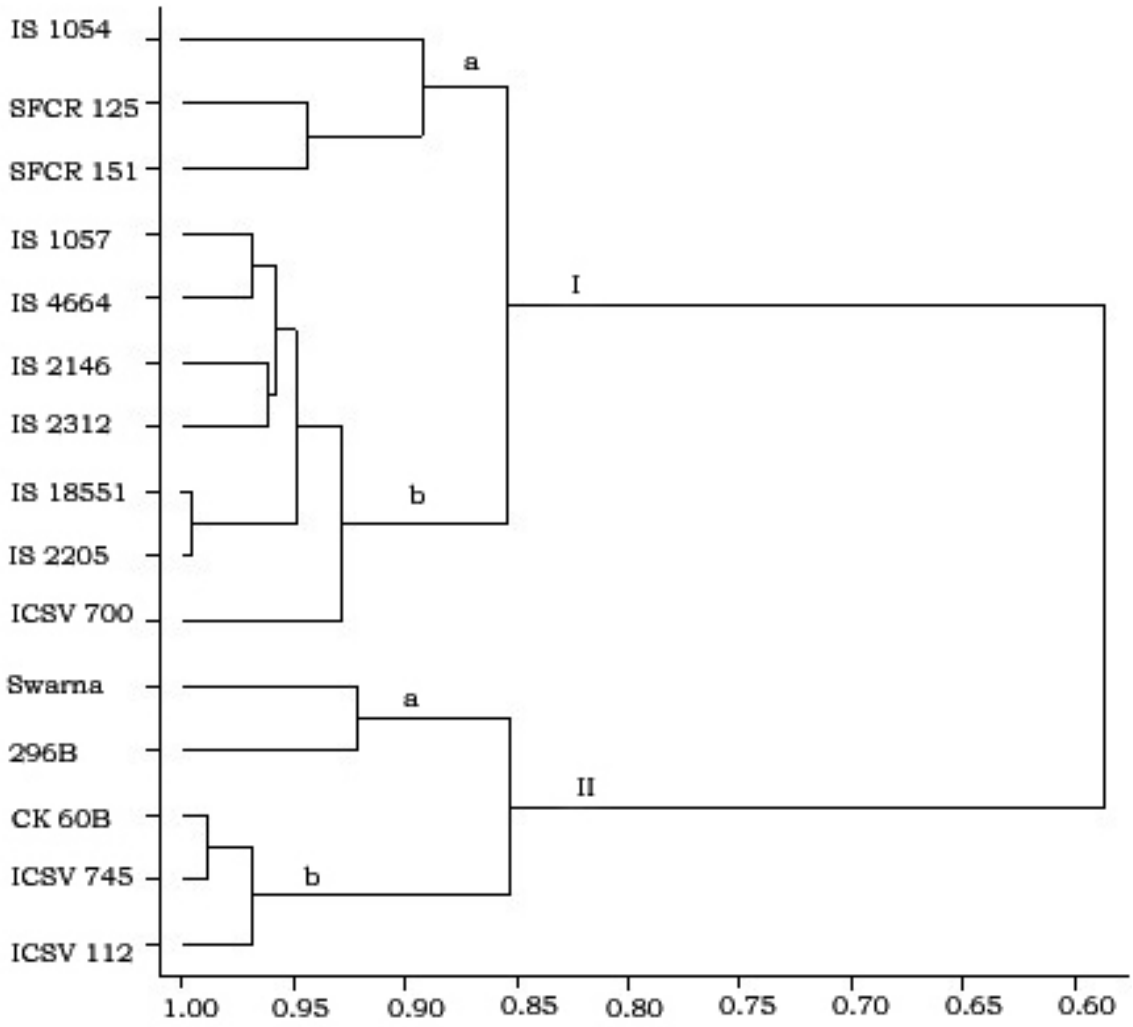


Fig. 4.5 Similarity matrix analysis of 15 sorghum genotypes based on morphological traits.

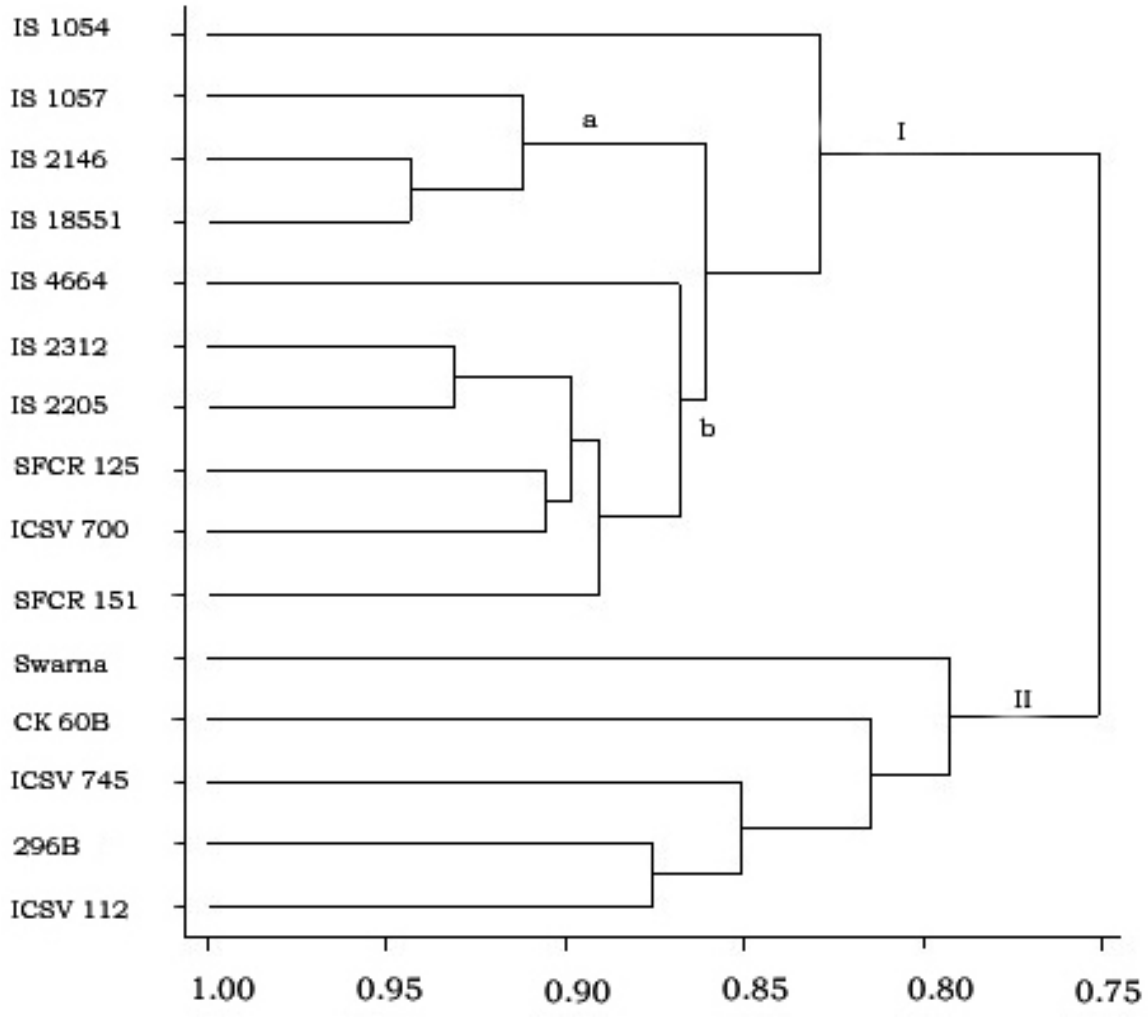


Fig. 4.6 Similarity matrix analysis of 15 sorghum genotypes based on biochemical traits.

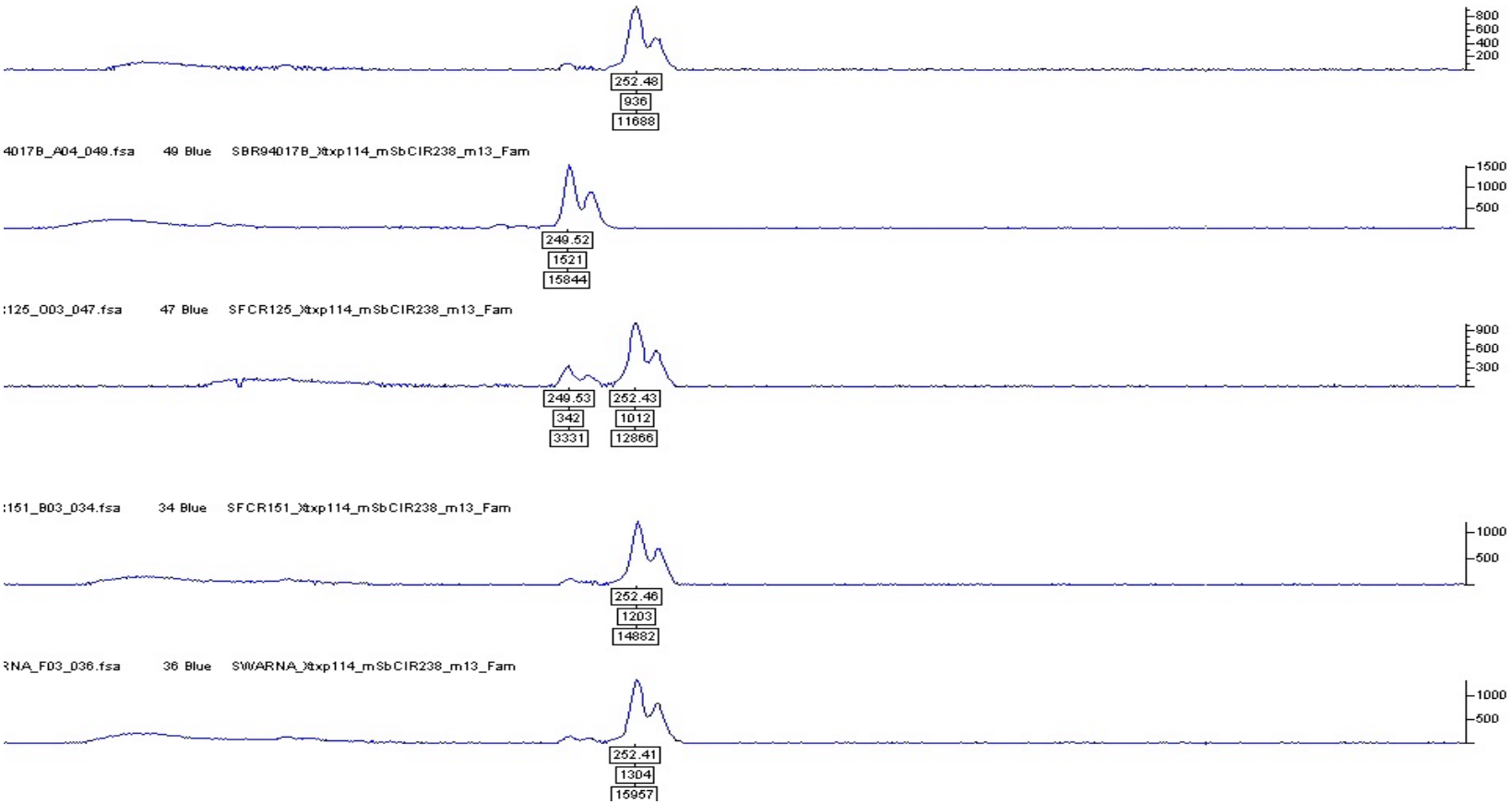


Fig. 4.7 Graphical presentation of sorghum genotypes screened with SSR marker Xtxp 114 using ABI prism 3700 DNA sequencer.

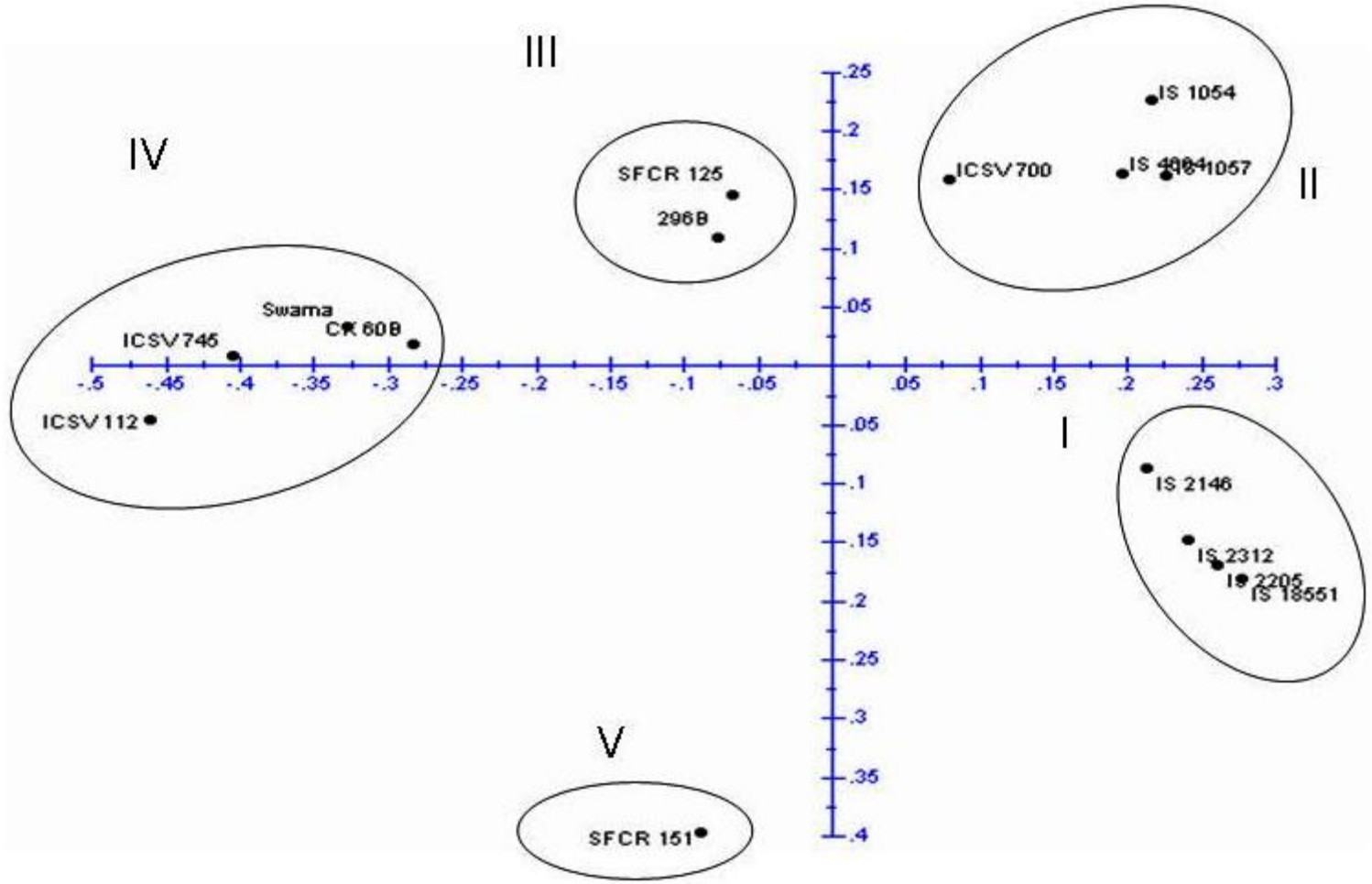
Table 4.32 SSR markers used for assessing genetic diversity of sorghum genotypes (ICRISAT, Patancheru, India)

Marker	AlleleNo	Minimun Allele Size	Maximum Allele Size	GeneDiversity	Heterozygosity	PIC value
gpsp017	3	189	195	0.42	0.00	0.37
gpsb027	6	167	193	0.77	0.00	0.74
gpsb067	4	188	198	0.56	0.00	0.50
gpsb089	4	164	172	0.44	0.00	0.41
gpsb123	3	308	314	0.43	0.00	0.39
gpsb128	2	264	286	0.48	0.00	0.36
gpsb148	3	130	144	0.37	0.07	0.32
mSbCIR223	4	121	133	0.51	0.00	0.46
mSbCIR238	6	90	106	0.77	0.00	0.74
mSbCIR240	4	125	139	0.65	0.07	0.60
mSbCIR246	3	114	120	0.24	0.00	0.23
mSbCIR248	2	110	120	0.23	0.00	0.20
mSbCIR262	2	232	236	0.42	0.07	0.33
mSbCIR276	2	248	252	0.12	0.00	0.12
mSbCIR283	4	132	160	0.54	0.07	0.50
mSbCIR286	5	127	145	0.67	0.07	0.63
mSbCIR300	3	122	128	0.64	0.00	0.57
mSbCIR306	2	139	141	0.50	0.00	0.37
mSbCIR329	4	129	135	0.51	0.00	0.46
SbAGA01	6	86	100	0.78	0.00	0.75
SbAGB02	5	114	140	0.61	0.07	0.57
SbAGE03	2	76	78	0.23	0.00	0.20
SbKAFGK1	4	130	148	0.54	0.21	0.48
Xcup02	4	210	225	0.47	0.07	0.44
Xcup07	3	191	269	0.54	0.00	0.45
Xcup14	3	222	228	0.46	0.07	0.41
Xcup28	3	152	164	0.56	0.00	0.50
Xcup53	3	205	217	0.66	0.07	0.59
Xcup60	2	151	163	0.12	0.00	0.12
Xcup61	2	215	218	0.39	0.00	0.31
Xcup62	2	188	191	0.39	0.00	0.31
Xcup63	2	152	164	0.12	0.00	0.12
Xcup69	2	236	251	0.50	0.00	0.37
Xgap10	4	250	302	0.47	0.07	0.44
Xgap206	9	127	163	0.86	0.00	0.85
Xgap34	2	195	197	0.32	0.00	0.27
Xgap342	6	274	286	0.74	0.07	0.71
Xgap72	4	207	213	0.60	0.00	0.53
Xgap84	6	201	239	0.77	0.07	0.73
Xisep0228	3	215	223	0.59	0.00	0.51

Continued..... Table 32

Xisep0607	3	206	215	0.66	0.13	0.58
Xisep0608	3	228	237	0.64	0.00	0.57
Xisep0632	2	208	212	0.44	0.00	0.35
Xisep0948	2	217	239	0.50	0.00	0.37
Xisep1014	4	214	240	0.44	0.00	0.41
Xtxp010	5	152	168	0.67	0.00	0.61
Xtxp015	6	218	248	0.74	0.00	0.70
Xtxp040	2	154	157	0.44	0.00	0.35
Xtxp057	5	259	271	0.65	0.00	0.61
Xtxp114	3	249	258	0.60	0.00	0.54
Xtxp12	7	192	214	0.78	0.07	0.75
Xtxp136	2	257	260	0.06	0.07	0.06
Xtxp141	5	154	184	0.71	0.07	0.67
Xtxp145	5	231	261	0.73	0.00	0.69
Xtxp20	5	182	222	0.73	0.00	0.69
Xtxp21	3	188	198	0.34	0.00	0.31
Xtxp210	4	185	205	0.75	0.00	0.70
Xtxp215	3	165	169	0.24	0.00	0.23
Xtxp23	7	175	189	0.77	0.07	0.73
Xtxp262	3	166	170	0.56	0.07	0.50
Xtxp265	6	209	227	0.80	0.07	0.77
Xtxp27	9	295	327	0.87	0.00	0.86
Xtxp273	4	235	253	0.69	0.00	0.64
Xtxp278	2	263	269	0.23	0.00	0.20
Xtxp289	5	267	321	0.59	0.00	0.55
Xtxp295	6	166	176	0.74	0.07	0.72
Xtxp304	9	214	313	0.83	0.00	0.81
Xtxp31	8	201	237	0.81	0.07	0.79
Xtxp312	7	138	219	0.77	0.07	0.73
Xtxp320	4	293	305	0.65	0.00	0.59
Xtxp321	7	212	236	0.78	0.13	0.75
Xtxp340	5	181	199	0.65	0.00	0.61
Xtxp354	7	155	167	0.76	0.07	0.73
Xtxp47	3	260	266	0.55	0.00	0.48
Xtxp6	7	81	115	0.80	0.00	0.77
Xtxp65	3	127	133	0.55	0.00	0.46
Xtxp75	5	150	178	0.72	0.00	0.67
Xtxp88	8	105	163	0.78	0.07	0.76
Xtxp95	5	51	99	0.70	0.00	0.66
Min	2	51	78	0.06	0.00	0.06
Max	9	308	327	0.87	0.21	0.86
Mean	4	-	-	0.57	0.03	0.52
Total	332	-	-	-	-	-

Fig. 4.8 Factorial analysis of 15 sorghum genotypes based on 79 SSR markers.



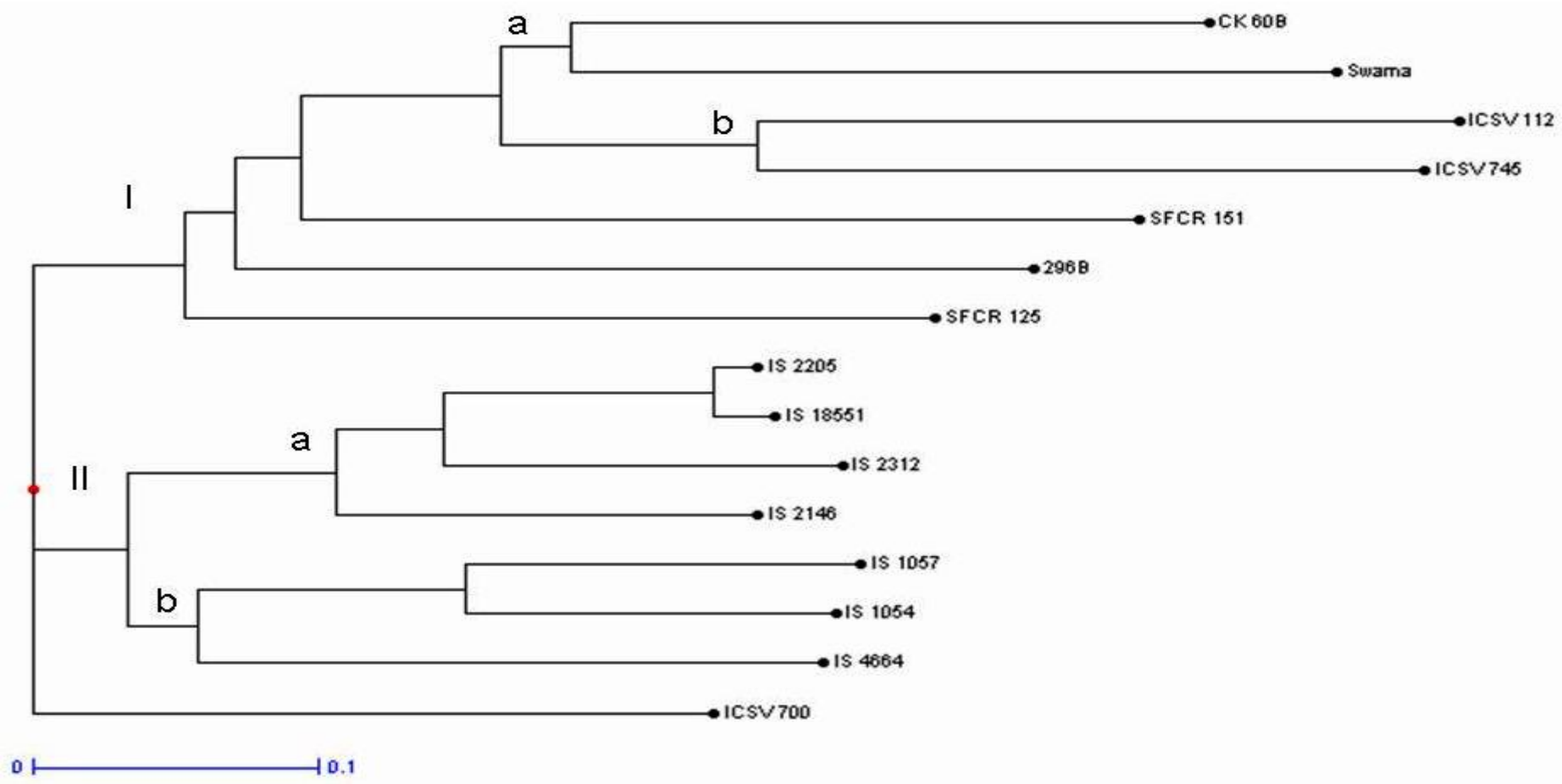
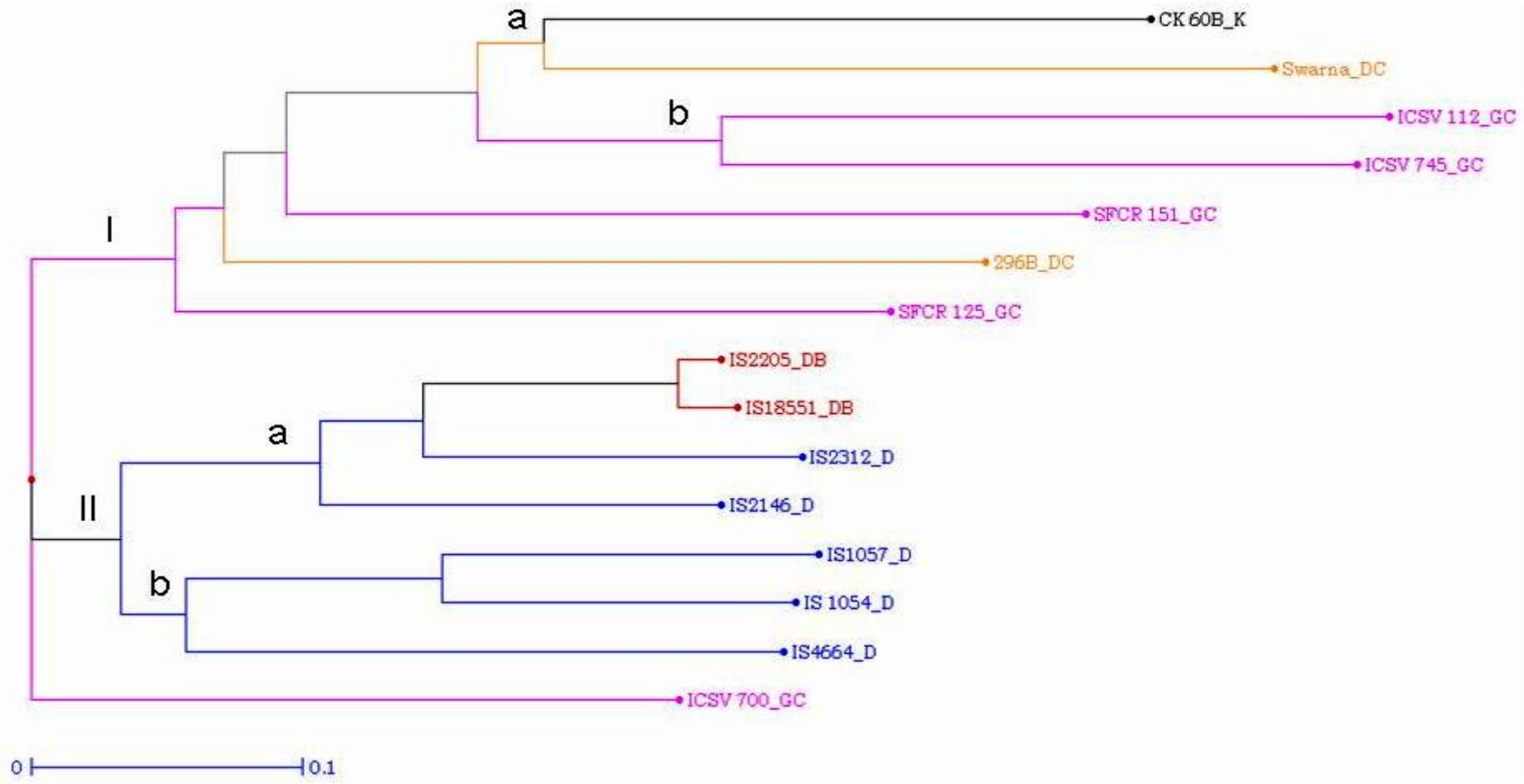


Fig. 4.9 Neighbor-joining cluster analysis of 15 sorghum genotypes based on 79 SSR markers.

Fig. 4.10 Genetic diversity among 15 sorghum genotypes in relation to different races of *sorghum bicolor*



Orange: Drrra caudatum; Black: Kaffir; Pink: Gunea caudatum; Blue: Durra; Red: Durra bicolor

Fig 4.11 Association of morphological traits to 79 SSR markers.

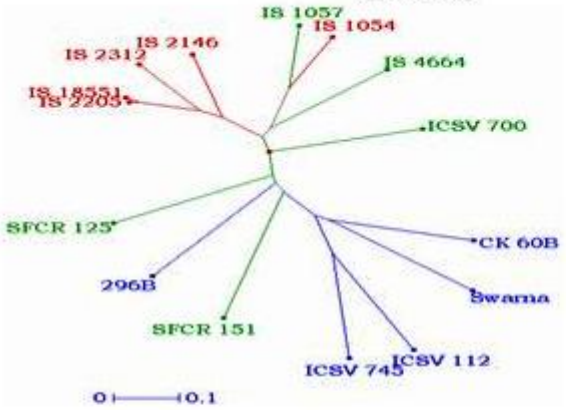


Fig. 4.11a Deadhearts at 21 DAE [red = 25 - 40; green = 40 - 55; and blue = 55 - 85 % plants with deadhearts].

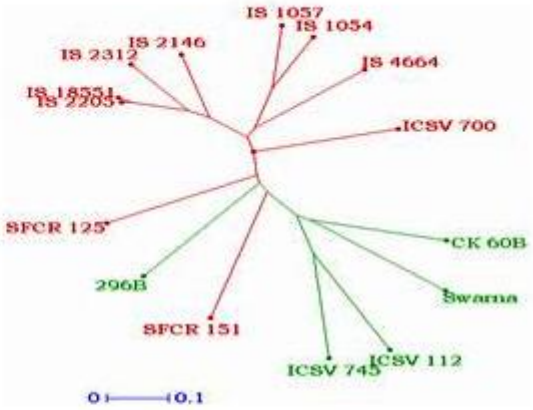


Fig. 4.11b Tiller deadhearts (red = 20 – 35 and green = 35 – 55 % tillers with deadhearts.

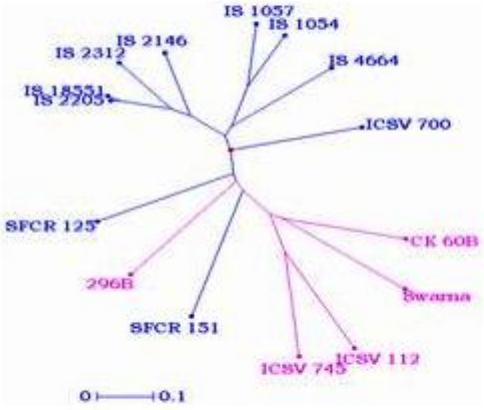


Fig. 4.11c Eggs seedlings^{-10} at 14 DAE [blue = 1 - 7 and pink = 7 - 17 % eggs seedlings^{-10}].

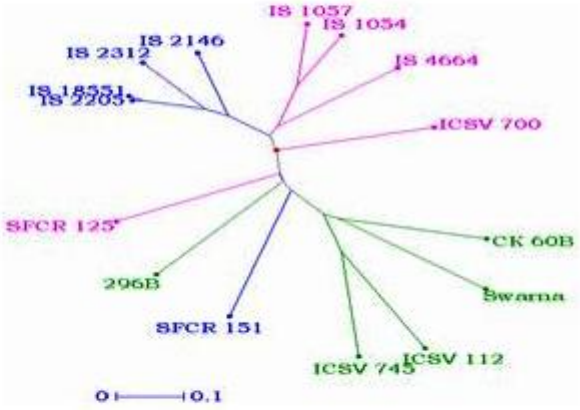


Fig. 4.11d Seedlings with eggs at 14 DAE [blue = 25 - 36; pink = 36 – 50; and green = 50 - 90 % seedlings with eggs].

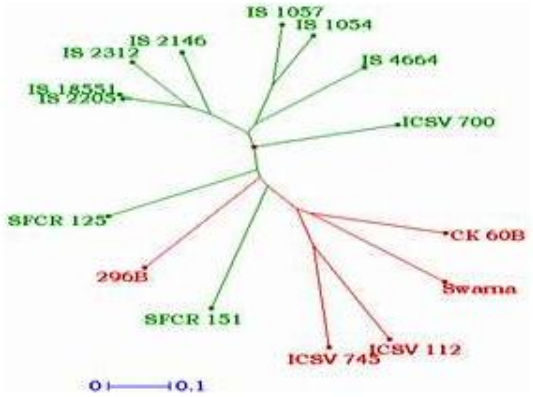


Fig. 4.11e Leaf surface wetness [green = Dry; and red = highly wet].

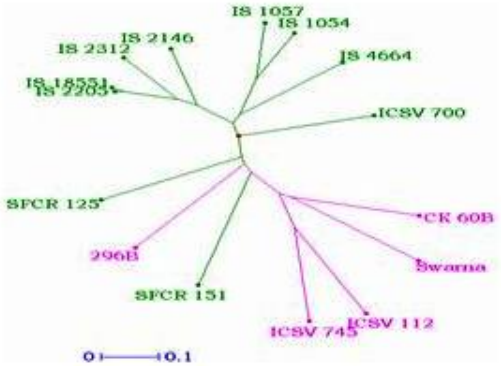


Fig. 4.11f Leaf glossiness [green = glossy; and pink = non-glossy].

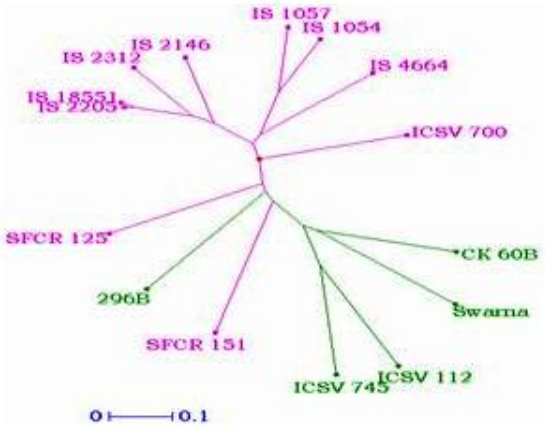


Fig. 4.11g Association of adaxial trichomes with SSR markers [red = trichomed; and pink = non-trichomed].

5. DISCUSSION

Sorghum [*Sorghum bicolor* (L.) Moench] is the fifth most important cereal crop in the world after wheat, rice, corn and barley. The genetic manipulation of sorghum crop since the 1960`s has led to development of several high-yielding varieties and hybrids. However, sorghum yields on the farmer's fields are quite low because of several biotic and abiotic constraints. Among the biotic stresses, the principal limiting factor affecting sorghum production is the sorghum shoot fly, *Atherigona soccata* (Rond.), which causes injury to the growing tip of sorghum seedlings.

To reduce insect damage on crops, farmers often use chemical pesticides that are hazardous to the beneficial organisms and the environment. Shoot fly is not accessible to insecticides sprayed on sorghum crop because the larvae feed inside the leaf whorls. Host plant resistance is one of the important components for minimizing the losses due to this pest. Plant resistance to insects comprises of antixenosis (unattractive for oviposition or feeding), antibiosis (adverse effects on insect biology and population build up), and tolerance (compensating for insect injury, with little or no effect on grain yield). All these mechanisms are under genetic control, suggesting that identification of resistance mechanisms as well as their association with morphological traits, and

biochemical and nutritional factors can be of great help in crop improvement.

A number of genotypes with resistance to shoot fly have been identified, but the levels of resistance are low to moderate (Jotwani, 1978; Taneja and Leuschner, 1985; Sharma *et al.*, 2003). To develop crop cultivars with stable and durable resistance to insects, it is important to study the mechanisms of resistance, identify lines with diverse combination of characters associated with resistance, and combine different components/mechanisms of resistance in the same genetic background. Therefore, it is important to gain an in-depth understanding the factors that regulate these processes. A better understanding of chemical composition of host plants would, therefore, give a better understanding of insect-plant relationships. Finally, integration of molecular technology for genetic enhancement of sorghum will play a pivotal role in increasing the productivity of this crop. Therefore, the present studies were aimed at identifying different physico-chemical traits that influence host plant resistance to shoot fly, *A. soccata*, and identify sorghum genotypes with different combinations of physico-chemical-molecular characteristics conferring resistance to this pest for use in sorghum improvement.

5.1 Expression of resistance to sorghum shoot fly, *Atherigona soccata* under multi, dual, and no-choice conditions

Genotypes preferred for oviposition by the females of sorghum shoot fly, *A. soccata*, also showed high deadheart formation (Rana *et al.*, 1975). Antixenosis for oviposition is the primary component of resistance to shoot fly, *A. soccata* (Blum, 1967; Singh and Narayana, 1978; Maiti and Bidinger, 1979; Singh and Jotwani, 1980a; Taneja and Leuschner, 1985; Dhillon *et al.*, 2005a, b). However, the differences in oviposition preference between resistant and susceptible genotypes tend to narrow down under no-choice conditions (Soto, 1974; Taneja and Leuschner, 1985; Dhillon *et al.*, 2005a, b). The present studies also indicated that though antixenosis for oviposition is the predominant component of resistance to shoot fly under multi-choice conditions in the field, differences in oviposition between the genotypes tested were not significant under no-choice conditions a situation akin to but large-scale planting of a resistant cultivar or very heavy shoot fly pressure under delayed plantings during the rainy season, or early plantings in September during the post rainy season. Antixenosis for oviposition is relative, since there are no known resistant cultivars, which are completely non-preferred for oviposition.

5.1.1 Antibiosis

Antibiosis component of resistant to shoot fly offers exciting possibilities of exerting pressure against insect feeding and development,

resulting in low survival of larvae on the resistant cultivars (Soto, 1974). Retardation of larval development, prolonged larval and pupal development, and reduced larval/pupal survival on the resistant genotypes provides an evidence of antibiosis to shoot fly *A. soccata* in sorghum (Singh and Jotwani, 1980b; Raina *et al.*, 1981; Sharma and Nwanze, 1997; Dhillon *et al.*, 2005a, b). Singh and Jotwani (1980b) observed prolonged larval and pupal periods (8 to 15 days), smaller larvae, and the mortality of neonates on resistant genotypes. However, Dhillon *et al.* (2005 b) reported that larval and pupal periods ranged from 9.1 to 11.0 and 6.5 to 7.4 days, respectively. In the present studies, the larval and pupal periods ranged from 9.1 to 10.7 and 7.2 to 8.1 days, respectively. Larval survival, in general decreases with the age of the plants (Ogwaro and Kokwaro, 1981). There were significant differences in relative growth, development, and adult emergence indices between the shoot fly-resistant and -susceptible genotypes. These results were similar to those reported by Dhillon *et al.* (2005b).

5.1.2 Recovery resistance

Tiller development consequent to deadheart formation in the main shoot and its survival depend on the level of primary resistance as well as shoot fly pressure (Doggett *et al.*, 1970). Tiller survival is related to its faster growth rate with a better chance to escape deadheart formation. Seedling vigor and high rate of recovery are important characteristics of resistant cultivars (Sharma *et al.*, 1977), which may not be related with

seedling height (Dhillon *et al.*, 2005b). The shoot fly-resistant genotypes had significantly less tiller deadhearts than the susceptible ones. Varieties with high recovery resistance compensate for yield loss under shoot fly infestation (Rana *et al.*, 1985).

The genotypes IS 2312, SFCR 125, SFCR 151, ICSV 700, and IS 18551 showing antixenosis, antibiosis, and/or tolerance components of resistance can be used to develop sorghum cultivars for resistance to this pest. Emphasis should be placed on combining different mechanisms of resistance in the same genetic background to increase the levels and diversifying the number of genes contributing to host plant resistance to *A. soccata*.

5.2 Variation in morphological characteristics of different sorghum genotypes

The intensity of leaf glossiness at the seedling stage and trichomes on the adaxial surface of leaves are associated with resistance to shoot fly in sorghum (Maiti and Gibson, 1983; Karanjkar *et al.*, 1992; Sharma and Nwanze, 1997; Dhillon *et al.*, 2005b, 2006; Patil *et al.*, 2006). Leaf surface wetness has been reported to be positively associated with shoot fly susceptibility (Nwanze *et al.*, 1992; Dhillon *et al.*, 2005b). The present results also showed a positive correlation between leaf surface wetness and shoot fly damage. The plumule and leaf sheaths of shoot fly-resistant genotypes have deep pink pigmentation whereas the susceptible genotypes are light green in color (Dhillon *et al.*, 2006). Purple-pigmented

plants with low chlorophyll content are less susceptible to shoot fly damage (Singh *et al.*, 1981; Kamatar *et al.*, 2003; Dhillon, 2004; Dhillon *et al.*, 2005b). This may be due to the reflection of light from the leaf surface and leaf surface chemicals that influence oviposition by the shoot fly females. The present studies also showed that glossy, trichomed, and pigmented genotypes were resistant to shoot fly. Seedling vigor has been reported to be negatively associated with susceptibility of sorghum to shoot fly (Taneja and Leuschner, 1985). However, the results of the present study indicated that when seedling vigor is assessed in plots without shoot fly damage, the genotypes with high seedling vigor were preferred for oviposition, and had high deadheart incidence. Similar results have also been reported earlier by Dhillon *et al.* (2005b).

5.3 Inducible resistance to shoot fly, *Atherigona soccata*

Ingeneral, all the test genotypes had significantly less number of deadhearts, eggs per 10 seedlings, seedlings with eggs, and tiller deadhearts in transplanting seedlings as compared to the normally grown plants. The shoot fly- resistant genotypes had significantly less number of deadhearts, eggs per 10 seedlings, seedlings with eggs, and tiller deadhearts than susceptible genotypes in both the treatments, although there were a few exceptions.

There were no effects of application of 2, 4-D, copper sulphate and potassium iodide on oviposition and deadheart formation by the sorghum shoot fly, *A. soccata* during the 2006 post rainy season. These results are

contrary to those reported earlier in case of soybean (Neupane *et al.*, 1991), cotton (Karban *et al.*, 1986). This may be because of the failure of those compounds to act as inducers of secondary metabolic system in cereals.

5.4 Genetic variability in biochemical composition of sorghum genotypes

Secondary metabolites produced by the plants act as attractants, repellents, or show antibiotic effects on the growth and survival of phytophagous insects (Painter, 1958; Schoonhoven, 1968), and the genotypes lacking these defense mechanisms are vulnerable to insect damage and suffer greater yield loss. Deficiency of nutritional compounds or the presence of antinutritional factors in sorghum genotypes might adversely affect the development and survival of *A. soccata* larvae (Raina, 1985). There is no relationship between moisture content of sorghum seedlings and shoot fly resistance (Singh *et al.*, 2004). Similar results were also obtained in the present studies. However, Rao and Panwar (2002) reported that maize genotypes resistant to stem borer, *Chilo partellus* (Swin.) have low moisture content. Phenolic compounds in sorghum improve resistance to insects (Dreyer *et al.*, 1981), and considerable variation in phenolics among the sorghum cultivars has been observed by Dicko *et al.* (2005). Plant phenolics in wheat provide resistance to *Rhopalosiphum padi* (L.) (Li Sujuan *et al.*, 2001), and stem borer, *C. partellus* in maize (Kabre and Ghorpade, 1998).

However, no significant differences were observed in total phenol content of the test genotypes in the present studies.

The present studies showed that several micronutrients played an important role in the host plant resistance to *A. soccata*. Positive association of N and P with shoot fly oviposition at early seedling stages indicated their role in releasing chemical cues for oviposition (Singh *et al.*, 2004; Bhise *et al.*, 1997). Resistance to fall armyworm, *Spodoptera frugiperda* (J. E. Smith) was positively correlated with higher concentrations of total nitrogen in sorghum leaves (Diawara *et al.*, 1992). Kabre and Ghorpade (1998) indicated that the K was negatively, and P positively correlated with stem borer susceptibility. However, low levels of N, P, and K have earlier been reported to be associated with resistance to shoot fly in sorghum (Singh and Jotwani, 1980; Khurana and Verma, 1983; Chavan *et al.*, 1990; Bhise *et al.*, 1997; Singh *et al.*, 2004). However, no significant association of these nutrients with resistance/susceptibility to shoot fly was observed in the present studies. High amounts of Si and Ca (Chavan *et al.*, 1990) and lignins and phenols (Khurana and Verma, 1983; Kumar and Singh, 1998) were earlier reported to be associated with the shoot fly resistance. However, no significant association of Ca, Cu, lignins, and total polyphenols was observed with resistance/susceptibility to shoot fly in the present studies. Higher amounts of Mg and Zn, and lower amounts of Fe associated with resistance to sorghum shoot fly.

Tannin content in the ovary was associated negatively, while total sugars were associated positively with susceptibility to sorghum midge, *Stenodiplosis sorghicola* (Coq.) (Mohan *et al.*, 1997). Present studies showed a significant and negative correlation between tannin content and shoot fly damage. Similar results have earlier have also been reported by Kamatar *et al.* (2003). Total sugars, reducing sugars, and protein contents have earlier been reported to be positively associated with susceptibility to stem borer (Kabre and Ghorpade, 1999), and shoot fly (Kamatar *et al.*, 2003; Singh *et al.*, 2004).

Genotypes with high amounts of soluble sugars, fats, and greater leaf surface wetness and seedling vigor were susceptible to shoot fly; while leaf glossiness, plumule and leaf sheath pigmentation, trichome density, and high tannin, Mg, and Zn contents were associated with resistance to shoot fly. Leaf glossiness, plumule pigmentation, trichomes, and fat content had direct effects and correlation coefficients in the same direction, and these traits can be used to select for resistance to sorghum shoot fly, *A. soccata*.

5.5 Associations of physico-chemical characteristics of sorghum genotypes with antibiosis to shoot fly

Leaf glossiness, bottom leaf, leaf sheath, and plumule pigmentation were negatively associated with larval survival and adult emergence, but positively associated with developmental period. Plant pigmentation was negatively correlated with pupal mortality, female pupal weight, and

fecundity. Trichomes on the adaxial and abaxial surface of leaves showed a significant and negative association with larval survival, adult emergence, but positive association with developmental period. The results suggested that the genotypes with high leaf glossiness, trichomes and pigmentation contributed to antibiosis to sorghum shoot fly, *A. soccata*.

Total soluble sugars, fats, and leaf surface wetness had significant and positive association with larval survival and adult emergence, but negative association with developmental period. Protein content was significantly and negatively correlated with the pupal mortality, but positively correlated with the adult emergence. Soluble sugars, proteins, fats, and leaf surface wetness contributed to susceptibility to shoot fly. Tannins showed a significant and positive correlation with developmental period. In general, tannins, moisture content, total soluble polyphenols, and lignins contributed to antibiosis to shoot fly.

Nitrogen content showed a significant and negative correlation with pupal mortality, and positive correlation with adult emergence. Potassium content was positively correlated with female pupal weight; while magnesium content was significantly and positively correlated with developmental period, but negatively correlated with larval survival, adult emergence, and male pupal weight. Zinc content showed a significant and negative correlation with larval survival. Phosphorous, calcium, manganese, copper and iron amounts were also associated with

susceptibility to shoot fly, but the correlation coefficients were nonsignificant.

Deadheart formation in the greenhouse was significantly and positively associated with leaf glossiness, leaf sheath pigmentation, total soluble sugars, and leaf surface wetness. However, significant and negative correlations were observed between deadheart incidence and trichomes on abaxial and adaxial surface of leaves, moisture content, tannins, and Mg content. Multiple linear regressions indicated that leaf surface wetness, Mg, total soluble sugars, tannins, fats, leaf glossiness, bottom leaf pigmentation, leaf sheath pigmentation, plumule pigmentation, and trichomes on abaxial and adaxial leaf surfaces explained 76.7% variation in developmental period. Leaf surface wetness, Mg, N, P, total soluble sugars, fats, leaf glossiness, bottom leaf, leaf sheath and plumule pigmentation, and trichomes on abaxial and adaxial leaf surfaces explained 87.6% of the total variability for adult emergence. Stepwise regression indicated that tannins, plumule pigmentation, and trichomes on abaxial and adaxial leaf surfaces explained 92.1% of the total variability for developmental period. Trichomes on abaxial leaf surfaces explained 51% of the total variability for larval survival; while Mg, N, leaf sheath pigmentation, and trichomes on abaxial and adaxial leaf surfaces explained 96.5% of the total variability for adult emergence. These results suggested that biochemical composition of sorghum

seedlings had a significant influence on expression of resistance to shoot fly in sorghum.

5.6 HPLC finger prints of sorghum phenolic compounds

Plant resistance to biotic stresses is often regulated by secondary metabolites. Possible role of seedling chemicals in host selection by the shoot fly has been suggested by Ogwaro (1978), Delobel (1982), and Raina (1982b). Raina (1984) suggested that biochemical deficiencies or the presence of chemical factors in resistant cultivars might adversely affect the development and survival of *A. soccata* larvae.

Present studies on HPLC profiles of phenolic compounds revealed considerable differences between the damaged and un-damaged sorghum seedlings. *p*-hydroxy benzoic acid concentrations were low in the deadhearts of resistant genotypes (IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, IS 2205, SFCR 151 and ICSV 700), and high in the susceptible ones (Swarna, CK 60B, ICSV 745, 296B, and ICSV 112) as compared to the undamaged seedlings. *p*-hydroxy benzaldehyde was present in undamaged seedlings of all the test genotypes, and in the damaged seedlings of the susceptible genotypes. Amounts of *p*-hydroxy benzaldehyde was more in undamaged seedlings of the shoot fly susceptible genotypes: Swarna, CK 60B, ICSV 745 and ICSV 112 as compared with the resistant check, IS 18551. The results indicated that *p*-hydroxy benzoic acid and *p*-hydroxy benzaldehyde conferred

susceptibility to shoot fly. These compounds possibly act as attractants for oviposition by sorghum shoot fly, *A. soccata*.

There was no significant effect of spraying *p*-hydroxy benzaldehyde on oviposition and damage by the sorghum shoot fly, *A. soccata*. Deadheart incidence in the shoot fly resistant genotype, IS 18551 was greater in plots sprayed with 0.01% of *p*-hydroxy benzaldehyde than that in the unsprayed plots at 14 and 21 DAE. During the 2006 post-rainy season, the deadheart incidence in susceptible genotype, Swarna at 14 DAE increased with an increase in concentration of *p*-hydroxy benzoic acid. However, such a trend was not apparent at 21 DAE as the overall shoot fly damage was very high. However, a slight increase in oviposition was observed with an increase in concentration of *p*-hydroxy benzoic acid.

Protocatechuic acid, *p*-coumaric acid, and cinnamic acid were absent in all the genotypes, but protocatechuic acid was present in IS 1054, and *p*-coumaric acid in IS 1054 and ICSV 745 in the undamaged seedlings of sorghum. Small amounts of cinnamic acid were present in damaged seedlings of resistant genotypes IS 2146, IS 4664, and IS 2205, while low quantities of luteolin and apigenin were detected in almost all the sorghum genotypes. Apigenin present in the resistant check, IS 18551, was absent in undamaged seedlings of susceptible check, Swarna and SFCR 125. It was absent in the shoot fly damaged seedlings of IS 18551, SFCR 151 and 296B, but present in the susceptible check, Swarna.

The peaks at RTs 21.44 and 40.66 min were present in the undamaged seedlings of the shoot fly resistant genotypes, while the peak at RT 24.38 min was present only in undamaged seedlings of susceptible genotypes. However, these peaks were absent in damaged seedlings of all the test genotypes. The peaks at RTs 2.34 and 4.15 min were absent in undamaged seedlings, but present in damaged seedlings; while the peaks at RTs 2.13, 20.30, 36.51, 38.88, and 39.56 min were present in undamaged seedlings, and absent in deadheart seedlings. The amount of compounds at RTs 2.76 and 3.70 min (in terms of peak areas) was more in the damaged seedlings than in the undamaged seedlings, and there were significant differences between the resistant and susceptible genotypes. The peak at RT 37.08 min had more peak area in the undamaged seedlings than in damaged seedlings. The compound at RT 37.08 had more peak area in resistant genotypes than in the susceptible check, Swarna. These compounds were possibly linked to primary and induced resistance to shoot fly.

Panday *et al.* (2005) identified six phenolic acids (protoctechuic, *p*-hydroxy benzoic, vanillic, syringic, *p*-coumaric and ferulic acids) from sorghum seedlings by RP-HPLC, and reported considerable variability in phenolic acids contents and their relationship with shoot fly damage. Hahn *et al.* (1983) identified eight phenolic acids (gallic, protoctechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic and cinnamic acids) which were associated with resistance to fungal diseases in sorghum. In

the present studies, we observed, seven phenolic acids (protocatechuic acid, *p*-hydroxy benzoic acid, *p*-hydroxy benzaldehyde, *p*-coumaric acid, cinnamic acid, luteolin and apigenin and unidentified compounds) in sorghum seedlings that were associated with resistance/susceptibility to shoot fly. Woodhead and Bernays (1978) reported that sorghum seedlings contained a mixture of hydroxyl benzoic and cinnamic acids. *p*-hydroxy benzoic acid was in highest concentration, followed by caffeic acid, ferulic acid, *p*-coumaric acid, and *o*-coumaric acid. Gentisic, vanillic, protocatechuic and γ-resorcylic acids were present in lower concentration, *p*-hydroxybenzaldehyde was possibly produced as a result of hydrolysis of dhurrin.

The phenolic compounds are present in the undamaged plant tissues largely in the form of esters, and when the plant cells are ruptured, esterases release the free phenolic acids (Woodhead and Cooper-Driver, 1979). Mixtures of phenolic acids and their esters reduce feeding on artificial media when presented to insects at concentrations similar to those occurring in young plants (Fisk, 1980). The occurrence of *p*-hydroxy benzaldehyde, produced by enzymatic degradation of dhurrin in sorghum seedlings was suspected to act as oviposition stimulant for adults and/or feeding activator for the maggots of shoot fly (Alborn *et al.*, 1992).

David (1997) suggested that amounts of simple phenolic acids in the cell wall did not vary greatly, and ferulic acid and *p*-coumaric acid

levels showed no clear trends over cycles of selection. In the present investigation, absence of protocatechuic, *p*-coumaric, and cinnamic acids in the sorghum seedlings support these observations. Nicholson *et al.* (1987) demonstrated that sorghum mesocotyl accumulates a complex of phenols in response to fungal infection and the two major components identified were 3-deoxyanthocyanidins, apigeninidin, and luteolinidin.

In general, the main feeding deterrent factors are only produced at the time of feeding. This is true of HCN, which is stored as glycoside-dhurrin, and the phenolic acids stored as esters. As a result of damage to the plant tissue, these substrates are brought in contact with enzymes to produce the active compounds. The substrates themselves are not deterrents, but contain phenolic esters and glycosides that deter insect feeding (Woodhead and Bernays, 1978). Pandey *et al.* (2005) observed that protocatechuic, syringic, and *p*-coumaric acids were negatively correlated with shoot fly damage, whereas *p*-hydroxy benzoic acid, vanillic acid, and ferulic acids were positively correlated with shoot fly damage.

5.7 Protein profiling by HPLC

Seeds contain a variety of proteins (Roberts and Selitrennikoff, 1986, 1988, 1990), which appear to play a defensive role against insect pests and pathogens. Several forms of slab gel electrophoresis have been used to separate sorghum proteins (Taylor and Schussler, 1984; Sastry *et al.*, 1986; Hamaker *et al.*, 1995). Reversed-phase high performance

liquid chromatography (RP-HPLC) (Sastry *et al.*, 1986; Smith, 1994), and free-zone capillary electrophoresis (FZCE) (Bean *et al.*, 2000, 2001), have also been used for protein separation. In addition to providing an independent method of resolution based on hydrophobic bonding, RP-HPLC provides a quantitative measure of the separated proteins. This information is useful not only in comparing composition of extracts from different varieties, but is also useful in understanding the differences and similarities between the kafirins and ASGs (alcohol-soluble glutelins) (Sastry *et al.*, 1986). Both isoelectric focusing (IEF) and RP-HPLC are effective in demonstrating genetic variations of kafirins and ASGs extracted from selected inbreds used for development of hybrids. Present studies based on RP-HPLC demonstrated qualitative and quantitative differences in protein composition of shoot fly-resistant and -susceptible genotypes.

RP-HPLC protein profiling of geminated seeds of 15 genotypes revealed considerable differences in their protein composition. Peaks at RT 2.59 and 18.42 had more peak area in resistant genotypes as compared with the susceptible genotypes, while reverse was true for the peaks at RT 4.08 and 8.27. Peaks at RTs 11.43, 21.00 and 23.90, were present in resistant genotypes, but absent in the susceptible genotypes; while the reverse was true for peaks at RTs 5.04, 14.56, 17.59 and 26.02. Peaks at RTs 25.64 and 25.78 had more area in the susceptible genotypes than in the resistant genotypes.

Maiti *et al*. (1994c) suggested that the presence of 54 kDa band in sorghum leaves may be related to shoot fly resistance. Sunitha Kumari *et al*. (1996) indicated that mold- resistant sorghum grains have higher levels of the 18 and 30 kDa antifungal proteins. Levels of the 26 kDa protein increased in the susceptible variety after inoculation of grains with *Fusarium moniliform* (Sheld.), suggesting its inducibility. Present results also showed that there were considerable differences in protein profiles in terms of presence and /or absence among the resistant and susceptible varieties. Differences in protein profiles have earlier been reported in sorghum (Alam and Sandal, 1969; Bhushan, 2006), maize (Asad *et al.*, 2003), and leaf (Hilty and Schmitthemer, 1966) and seeds in soybean (Larsen, 1967). Alam and Sandal (1969) reported 20 bands in male-fertile in contrast to nine in male-sterile anthers. Hilty and Schmitthemer (1966) did not detect differences in protein composition of *Phytophthora megasperma* (var. Sojae.) resistant and susceptible genotypes in soybean. In the present studies seed proteins were estimated by RP-HPLC may not be pure proteins, and it may contain mixture of other compounds as well. Further studies are needed to confirm these proteins by purifying them and study their role in host plant resistance to *A. soccata*.

Peaks 1, 2, 3, 4, 6, 7, 10, 12, 14, 16, and 17 were significantly and negatively correlated with developmental period (except peaks 1, 3 and 10), pupal period (except peaks 2, 3, 6, 7, 10, 12 and 14), and female

pupal weight (except peak 6), but significantly and positively correlated with larval survival, adult emergence, and male pupal weight, however, some of the correlation coefficients were nonsignificant. Peak 8, 9, 11, and 15 were significantly and positively correlated with developmental period, pupal mortality, and female pupal weight, but negatively correlated with larval survival, adult emergence and male pupal weight, where some of the correlation coefficients were non-significant.

5.8 GC-MS profiles of sorghum leaf surface chemicals

Plants are known to produce certain chemical compounds in different quantities and proportions, which affect the host selection behavior of phytophagous insects (Painter, 1958; Beck, 1965; Schoonhoven, 1968). These compounds can be attractants (oviposition and feeding stimulants) or repellents (oviposition and feeding deterrents), and antibiotic (resulting in reduced survival and growth). Reddy (2003) reported that plant volatiles from resistant rice cultivars act as repellents or are toxic to insect pests. Nwanze *et al*. (1998a) reported that females of *A. soccata* are attracted to the volatiles emitted by the susceptible seedlings, and to phototactic (optical) stimuli that may facilitate orientation to its host for oviposition.

Green leaf volatiles are produced from linolenic and linoleic acids through the lipoxygenase pathway (Pare and Tumlinson, 1996). They are liberated from membranes as a result of insect damage, by the action of a lipoxygenase enzyme, that produces hydroperoxides initially. A

hydroperoxide lyase enzyme then converts the hydroperoxides to hexanal (from linoleic acid) and (E)-2-hexenal (from linolenic acid), which undergo further reactions to give other C6 aldehydes, alcohols, and esters (Hatanaka, 1993; Bate and Rothstein, 1998). Present results showed that hexane extracts of 3rd leaf of sorghum seedlings produced hexanal and other compounds, having aldehydes, alcohols, and esters. Robert *et al.* (1992) identified 36 volatile compounds from plum cultivars by continuous vacuum steam distillation/ hexane extraction, and analyzed by capillary GC and GC-MS. Eight major compounds for most cultivars were: hexenal, butyl acetate, (E)-2-hexenal, butyl butyrate, hexyl acetate, linalool, γ-decalactone, and γ-dodecalactone. Minor constituents included eleven esters, two alcohols, four lactones, two terpenes, two saturated hydrocarbons, palmitic acid, three phenyl compounds, and nonanal.

GC-MS profiles of sorghum leaf surface chemicals revealed considerable differences among the sorghum genotypes tested. Of the 150 compounds detected, we selected 10 major compounds, which showed significant association with expression of resistance to *A. soccata.* Of the 10 major compounds detected, hexanal was present both in IS 18551 and Swarna, but the peak area percent was more in the resistant check, IS 18551 as compared to that of the susceptible check, Swarna. Pentadecane, 8-hexyl and lonol 2 were present only in the susceptible genotypes, Swarna and CK 60B, and absent in rest of the genotypes. Dodecane, 2, 6, 11-trimethyl was present only in shoot fly-susceptible genotypes, and absent

in shoot fly-resistant genotypes, except IS 1057. Compound 4, 4-dimethyl cyclooctene was present in resistant genotypes IS 2146, IS 2312, and IS 18551, and absent in all other genotypes, while hexane 2, 4-dimethyl was absent in IS 2146, IS 2312, IS 18551, and IS 4664, and present in rest of the genotypes. Eicosane was present in all genotypes, except in the susceptible check, Swarna. More amount of eicosane were detected in IS 4664. Decane 4-methyl was present in all genotypes, but had more peak area percent in SFCR 125, ICSV 700, CK 60B, ICSV 745, 296B, ICSV 112, and Swarna compared with the resistant check, IS 18551. Undecane 5- methyl, decane 4- methyl, hexane 2, 4- methyl, pentadecane 8- hexyl, and dodecane 2, 6, 11- trimethyl were significantly and positively correlated with deadhearts and eggs per 10 seedlings, suggesting that these compounds act as attractants/ oviposition stimulants for the sorghum shoot fly, *A. soccata*. 4, 4- dimethyl cyclooctene was negatively correlated with deadhearts and eggs per 10 seedlings, and might impart resistance to shoot fly. The compounds eicosane, tridecane and hexanal showed a positive, and lonol 2 negative association with shoot fly damage, but the correlation coefficients were nonsignificant.

Further studies are needed to determine the role of leaf surface chemicals in host plant resistance to shoot fly in sorghum.

5.9 Molecular diversity of sources of resistance to sorghum shoot fly, *Atherigona soccata*

Molecular markers are an excellent tool for the assessment of genetic relationships. Restriction Fragment Length Polymorphisms (RFLPs) (Ahnert *et al.*, 1996; Deu *et al.*, 1994; Tao *et al.*, 1993), Randomly Amplified Polymorphic DNA (RAPDs) (Ayana *et al.*, 2000; Tao *et al.*, 1993; Uptmoor *et al.*, 2003), microsatellites (SSRs) (Uptmoor *et al.*, 2003; Menz *et al.*, 2004; Anas and Yoshida, 2004; Folkertsma *et al.*, 2005; Dhillon *et al.*, 2006), and Amplified Fragment Length Polymorphisms (AFLPs) (Menz *et al.*, 2004; Uptmoor *et al.*, 2003) have been used to quantify levels of genetic diversity in sorghum. SSR markers have been found to give good discrimination between closely related individuals of sorghum even when only a few loci were used (Djè *et al.*, 1999; Smith *et al.*, 2000; Ghebru *et al.*, 2002; Deu *et al.*, 2008). Agrama and Tuinstra (2003) compared SSRs with RAPDs, and suggested that SSRs were more polymorphic compared to RAPDs. Ghebru *et al.* (2002) also studied genetic diversity of Eritrean sorghum landraces using SSRs.

Most studies have indicated that geographic origin and/or racial classification are associated with the organization of genetic diversity. However, RAPD markers alone are not enough to separate accessions into discrete racial or geographic groups in a large collection of sorghum (Menkir *et al.*, 1997). This was probably due to nature of evolutionary mechanisms underlying the variation measured by different markers,

repetitive sequence revealed by some RAPD markers, lack of allelism of RAPD bands of similar sizes, and unequal distribution in the genome (Powell *et al.*, 1996b; Noli *et al.*, 1997). Differentiation among races or geographic origins of sorghums was not observed by Djé *et al.* (2000) using a limited number of SSR markers. Dhillon *et al.* (2006) differentiated shoot fly-resistant and susceptible parents and their hybrids using SSR loci mapped QTLs for shoot fly resistance in sorghum. They have also suggested that the resistance gene frequencies in the hybrids are dependent on the gene frequencies in the CMS lines.

In the present studies, assessment of genetic diversity based on SSR markers was based on 15 sorghum lines with different levels of resistance/susceptibility to sorghum shoot fly, *A. soccata*. Of the 93 microsatellites primer pairs used in the present study, 79 showed clear polymorphism between sorghum accessions studied. A total of 332 alleles were detected with an average of 4 alleles per marker. Heterozygosity ranged from 0.00 to 0.21, with a mean of 0.03. Marker SbKAFGK 1 showed maximum heterozygosity (0.21) between the genotypes. The polymorphic information content (PIC) values ranged from 0.06 to 0.86. The polymorphism was maximum with the primer pair Xtxp 27 (0.86), followed by Xgap 206 (0.85). Lowest polymorphism was observed with Xtxp136 (0.06).

The average number of alleles per locus identified in this study were similar to those reported for maize (7.8) (Romero Severson *et al.*,

2001; Matsuoka *et al.*, 2002), elite sorghum lines (5.9) (Smith *et al.*, 2000), sorghum landraces (8.7) from Southern Africa (Uptmoor *et al.*, 2003), and sorghum parents and their hybrids (5.8) (Dhillon *et al.*, 2006). Barnaud *et al.* (2007) detected relatively low genetic diversity at the scale of single village average gene diversity (GD) per landrace 0.32, and average gene diversity per locus 0.51. Djè *et al.* (1999) reported GD 0.83 for a sample throughout five regions in Morocco, while Uptmoor *et al.* (2003) reported GD 0.59 for 23 landraces from southern Africa. Smith *et al.* (2000) reported mean PIC values of 0.58 for SSRs. Caniato *et al.* (2007) used 15 SSR loci, which produced 130 alleles for the 47 sorghum lines. The number of alleles per SSR locus ranged from 2 to 12, with an average of 8.7 alleles per locus. Abu Assar *et al.* (2005) studied the genetic diversity among 96 sorghum accessions from Sudan, ICRISAT and Nebraska, using 16 SSRs, total of 117 polymorphic bands was detected, with a mean of 7.3 alleles per SSR locus. The PIC ranged from 0.46 to 0.87. In the present studies, similar results were obtained with respect to number of alleles and PIC values. Generally, PIC values increase with an increase in heterozygosity. However, this trend was not consistent in the present studies for SSR markers Xisep 0607 and Xtxp 321, both had 0.13 heterozygocity, but different PIC values (0.58 and 0.75, respectively). These result were also supported by Hokanson *et al.* (1998). Casa *et al.* (2005) used SSR markers to quantify and characterize diversity in a selection of cultivated and wild sorghums. His studies

revealed that wild and cultivated sorghums formed different groups, which contained mixtures of racial types.

Based on factorial analysis 79 SSR markers, the 15 sorghum genotypes were grouped into 5 divergent groups. First group comprised of IS 18551, IS 2205, IS 2312 and IS 2146; II: IS 1054, IS 1057, IS 4664, and ICSV 700; III: 296B and SFCR 125; IV: Swarna, CK 60B, ICSV 745, and ICSV 112; and V: SFCR 151. All 15 accessions were grouped into 2 clusters based on Neighbor-Joining cluster analysis, but ICSV 700 formed a separate group. Cluster I was subdivided into two subclusters, cluster I (a) CK 60B and Swarna, and Cluster I (b) ICSV 112 and ICSV 745. SFCR 151, 296B and SFCR 125 were placed in cluster I. Cluster II was also divided into two subclusters. Cluster II (a) consisted of IS 2205, IS 18551, IS 2312 and IS 2146, while cluster II (b) consisted of IS 1057, IS 1054, and IS 4664. ICSV 700 was placed independently. In general, sorghum genotypes are grouped into five races and 10 intermediate races, based on panicle and spikelet morphology (Harlan and De Wet, 1972). In the present study, all the genotypes clustered according to races. All durra races (IIb) and its intermediate race with *bicolor* (IIa) were placed in cluster II. The race *caudatum* along with other intermediate races, and one *kafir* genotype (CK 60B) were clustered into cluster I. The test genotypes were also placed in different groups according to morphological and biochemical characteristics (Fig. 11a to 11g). Diversity analysis of sorghum genotypes based on biochemical and molecular

markers indicated that sorghum genotypes with resistance to shoot fly are quite diverse. Genotypes placed in different groups can be used to diversify the basis of resistance to shoot fly, *A. soccata*.

6. SUMMARY AND CONCLUSIONS

Sorghum is the fifth most important cereal crop after wheat, rice, corn, and barley, and an important cereal crop in the semi-arid tropics. Nearly 150 insect species have been reported as pests on sorghum, of which shoot fly, *Atherigona soccata* (Rond.) is one of the most important constraints in sorghum production. A number of genotypes with resistance to shoot fly have been identified, but the levels of resistance are low to moderate. To develop crop cultivars with durable resistance to insect pests, it is important to identify lines with diverse combinations of factors associated with resistance, and combine different components/mechanisms of resistance in the same genetic background.

Several physico-chemical characteristics of the plant affect orientation, oviposition, development, and fecundity of insects. Finally, integration of molecular technology with conventional crop improvement approaches is important to gain an understanding of the genetics of important traits associated with resistance to insect pests. Therefore, the present studies were undertaken on constitutive and inducible resistance to shoot fly in sorghum, the physico-chemical traits that influence host plant resistance to shoot fly, and identify sorghum genotypes with different combinations of physico-chemical and molecular characteristics conferring resistance to this pest for use in sorghum improvement.

The present investigations entitled “Biochemical mechanisms of resistance to shoot fly, *Atherigona soccata* (Rondani) in sorghum, *Sorghum bicolor* (L.) Moench” were taken up to study the mechanisms of resistance to shoot fly. The experimental material consisted of a diverse array of 15 sorghum genotypes comprising of seven germplasm lines (IS 1054, IS 1057, IS 2146, IS 18551, IS 4664, IS 2312, and IS 2205) and three breeding lines (SFCR 125, SFCR 151, and ICSV 700) with low to moderate levels of resistance to sorghum shoot fly, and five commercial cultivars (Swarna, CK 60B, ICSV 745, 296B, and ICSV 112) susceptible to shoot fly. The experiments were conducted under field and greenhouse conditions. Biochemical, and molecular diversity was studied under laboratory conditions at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India, during the 2004-07 rainy and post rainy seasons.

Genotypes IS 2312, SFCR 125, SFCR 151, ICSV 700, and IS 18551 exhibited antixenosis, antibiosis, and tolerance components of resistance to shoot fly, and may be used in sorghum improvement to develop sorghum cultivars with resistance to this pest. Sorghum genotypes with high amounts of soluble sugars, more leaf surface wetness and fats, and better seedling vigor were susceptible to shoot fly; while those with glossy leaf trait, pigmented plumule and leaf sheath, tall with high trichome density; and high tannin, Mg, and Zn contents showed resistance to shoot fly. Leaf surface wetness, Mg, Zn, soluble sugars, tannins, fats, leaf

glossiness, leaf sheath and plumule pigmentation, and trichome density explained 99.8% of the variation for deadhearts. Path coefficient analysis revealed that correlation coefficients and direct effects of leaf glossiness, plumule pigmentation, trichomes on adaxial leaf surface, Mg and fat contents were in the same direction, and these traits can be used to select for shoot fly resistance.

Leaf glossiness, leaf sheath and plumule pigmentation, high trichome density, tannins, moisture, total soluble polyphenols, lignins, and Mg were associated with antibiosis to shoot fly. However, soluble sugars, proteins, fats, leaf surface wetness, N, and P contents were associated with susceptibility to shoot fly. Stepwise regression analysis indicated that tannins, plumule pigmentation, trichomes on abaxial and adaxial leaf surfaces explained 92.1% of the variability for developmental period. Trichome on abaxial leaf surface explained 51% of the variability for larval survival; while Mg, N, leaf sheath pigmentation, and trichomes on abaxial and adaxial leaf surfaces explained 96.5% of the variability for adult emergence.

Phenolic compounds: *p*-hydroxy benzaldehyde, *p*-hydroxy benzoic acid, luteolin and unknown peaks at RTs 24.38 and 3.70 were associated with susceptibility to shoot fly, whereas protocatechuic acid, *p*-coumaric acid, cinnamic acid, and apigenin were associated with resistance to shoot fly, *A. soccata*.

Protein peaks 1, 2, 3, 4, 7, 12, 14, 16, and 17 were positively associated with susceptibility to shoot fly, the peaks 5, 8, 9, 11, and 15 were associated with resistance to shoot fly, *A. soccata*. Peaks 1, 2, 3, 4, 6, 7, 10, 12, 14, 16, and 17 were negatively correlated with developmental period, pupal period, and female pupal weight, but positively correlated with larval survival, adult emergence, and male pupal weight, indicating that those were associated with susceptibility to shoot fly. On the other hand, peaks 8, 9, 11 and 15 were associated with antibiosis to shoot fly, although some of the correlation coefficients were nonsignificant.

Compounds undecane 5- methyl, decane 4- methyl, hexane 2, 4- methyl, pentadecane 8- hexyl and dodecane 2, 6, 11- trimethyl, present on the leaf surface of sorghum seedlings, were associated with susceptibility to shoot fly. While 4, 4- dimethyl cyclooctene was associated with shoot fly resistance.

Similarity matrix analysis indicated considerable diversity among the sorghum genotypes based on morphological, biochemical, and molecular markers. Factorial analysis and Neighbor-Joining cluster analysis based on 79 SSRs markers placed the shoot fly-resistant and-susceptible genotypes separately. There was considerable diversity between the races *guinea*, *durra*, and their intermediate races. Genotypes placed in different groups, and with diverse combination of characteristics associated with resistance to sorghum shoot fly can be

used to broaden the genetic base, and increase the levels of resistance to sorghum shoot fly, *A. soccata*.

6.2 Future thrust of work

- Gene pyramiding for resistance to shoot fly.
- Alteration of metabolic pathways to increase the effectiveness of secondary metabolites in sorghum for resistance to insect pests.
- Isolation and identification of proteins associated with resistance/ susceptibility to shoot fly.
- Identification of highly polymorphic molecular markers associated with resistance to shoot fly for use in marker- assisted selection.
- Develop cultivars with multiple resistances to insect pests.

7. BIBLIOGRAPHY

1. Abu Assar A. H., Uptmoor R., Abdelmula A. A., Salih M., Ordon F. and Friedt W. 2005. Genetic variation in sorghum germplasm from Sudan, ICRISAT, and USA assessed by simple sequence repeats (SSRs). Crop Science 45: 1636-1644.
2. Adewusi S. R. A. 1990. Turnover of dhurrin in green sorghum seedlings. *Plant Physiology* 94: 1219-1224.
3. Agrama H. A. and Tuinstra M. R. 2003. Phylogenetic diversity and relationships among sorghum accessions using SSRs and RAPDs. *African Journal of Biotechnology* 10: 334-340.
4. Agrawal B. L. and Abraham C. V. 1985. Breeding sorghum for resistance to shoot fly and midge. In: *Proceedings, 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 502324, Andhra Pradesh, India. Pp. 371-383.
5. Agrawal B. L. and House L. R. 1982. Breeding sorghum for resistance in sorghum. In: *Sorghum in Eighties, Proceeding, International Symposium on Sorghum*, 2-7 November 1981 (House, L. R., Mughogho, L. K. and Peacock, J. M. eds.), International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India. Pp. 435-446.

6. Ahnert D., Lee M., Austin D. F., Livini C., Woodman W. L., Openshaw S. J., Smith J. S. C., Porter K. and Dalton G. 1996. Genetic diversity among elite sorghum inbred lines assessed with DNA markers and pedigree information. *Crop Science* 36: 1385-1392.
7. Akazawa T., Miljanich P. and Conn E. E. 1960. Studies on cyanogenic glycosides of *Sorghum vulgare*. *Plant Physiology* 35: 535-538.
8. Alam S. and Sandal P. C. 1969. Electrophoretic analysis of anther proteins from male-fertile and male-sterile sudangrass, *sorghum vulgare* var. *sudanense* (Piper). *Crop Science* 9: 157-159.
9. Alborn H., Stenhagen G. and Leuschner K. 1992. Biochemical selection of sorghum genotypes resistant to sorghum shoot fly (*Atherigona soccata*) and stem borer (*Chilo partellus*): role of allelochemicals. In: *Allelopathy: Basic and Applied Aspects* (Rizvi, S.H. and Rizvi, V. eds.). Chapman & Hall, London. Pp. 101-117.
10. Anas and Yoshida T. 2004. Genetic diversity among Japanese cultivated sorghum assessed with simple sequence repeats markers. *Plant Production Sciences* 7: 217-223.
11. AOAC (Association of Official Analytical Chemists). 1984. 14th Edition. Associaion of Official Analytical Chemists, INC 1111, North Nineteenth Street, Suite 210, Arlington, VA 22209, USA, pp.187-188.

12. AOCS (American Oil Chemist`s Society). 1981. Official and Tentative Methods of the American Oil Chemist`s Society. Third Edition. (Ab 3-49). 508 South, Sixth Street, Champaign, Illinois 61820, USA.

13. Arabjafari K. H. and Jalali S. K. 2007. Identification and analysis of host plant resistance in leading maize genotypes against spotted stem borer, *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae). *Pakistan Journal of Biologocal Sciences* 10: 1885-1895.

14. Asad H. S., Muhammad F. K. and Abdul K. 2003. Genetic characterization of some maize (*Zea mays* L.) varieties using SDS-PAGE. *Asian Journal of Plant Sciences* 2: 1188-1191.

15. Awika J. M. and Rooney L. W. 2004. Sorghum phytochemicals and their potential impact on human health. *Phytochemistry* 65: 1199-1221.

16. Ayana A., Bryngelsson T. and Bekele E. 2000. Genetic variation of Ethiopian and Eritrean sorghum (*Sorghum bicolor* (L.) Moench) germplasm assessed by random amplified polymorphic DNA (RAPD). *Genetic Resources and Crop Evoluation* 47: 471-482.

17. Barnaud A., Deu M., Garine E., McKey D. and Joly H. I. 2007. Local genetic diversity of sorghum in a village in northern Cameroon: structure and dynamics of landraces. *Theoretical and Applied Genetics* 114: 237-248

18. Bate N. J. and Rothstein S. J. 1998. C_6 volatiles derived from the lipoxygenase pathway induce a subset of defence-related genes. *Plant Journal* 16: 561-569

19. Bate Smith E. C. 1969. Luteoforol (3', 4, 4', 5, 7-pentahydroxyflavan) in *Sorghum vulgare* L. *Photochemistry* 8: 1803-1810.

20. Bean S. R., Hicks C., Tuinstra M. and Lookhart G. L. 2001. The use of SDS to extract sorghum and maize proteins for free zone capillary electrophoresis (FZCE) analysis. *Cereal Chemistry* 78: 84-87.

21. Bean S. R., Lookhart G. L. and Bietz J. A. 2000. Acetonitrile as a buffer additive for the separation of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) storage proteins by HPCE. *Journal of Agriculture and Food Chemistry* 48: 318-327.

22. Beck S. D. 1965. Resistance of plants to insects. *Annual Review of Entomology* 10: 207-232.

23. Bell E. A. and Charwood B. V. 1980. Secondary plant products. Springer Verlag, Berlin, Germany.

24. Bene G. del. 1986. Note sulla biologia di *Atherigona soccata* Rondani (Diptera: Muscidae) in Toscanae Lazio. *Redia* 69: 47-63.

25. Beyermann B., Nurnberg P., Weihe A., Meixner M., Epplen J. T. and Borner T. 1992. Fingerprinting plant genomes with oligonucleotides probes specific for simple repetitive DNA sequences. *Theoretical and Applied Genetics* 83: 691-649.

26. Bhattramakki D., Dong J., Chhabra A. K. and Hart G. E. 2000. An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. *Genome* 43: 988-1002.

27. Bhise H. T., Desai B. B. and Chavan H. D. 1996. Effects of chemical constituents on resistance of shoot fly in sorghum. *Journal of Research, Maharashtra Agricultural Universities* 21: 293-294.

28. Bhise H. T., Desai B. B. and Chavan U. D. 1997. Assessments of some biochemical parameters responsible for shoot fly resistance in sorghum. *Journal of Maharashtra Agricultural Universities* 21: 127-129.

29. Bhushan R. K. 2006. Studies on biochemical and molecular parameters for identification of varieties and hybrids of sorghum. *Ph.D. Thesis*, 114, Department of Biochemistry, Sant Gadge Baba Amravati University, Amravati, India.

30. Biradar S. G., Borikar S. T. and Chandurwar R. D. 1986. Trichome density in some progeny of sorghum. *Sorghum Newsletter* 29: 107.

31. Blum A. 1963. The penetration and development of the sorghum shoot fly in susceptible sorghum plants. *Hassadesh* 44: 23-25.

32. Blum A. 1967. Varietal resistances of sorghum to the sorghum shoot fly (*Atherigona varia soccata*). *Crop Science* 7: 461-462.

33. Blum A. 1972. Sorghum breeding for shoot fly resistance in Israel. In: *Control of Sorghum Shoot fly* (Jotwani M. G. and Young W. R. eds.) Oxford and IBH Publishing Co., New Delhi. Pp. 180-191.

34. Board P. K. and Mittal V. P. 1983. Assessment of losses caused by pest complex to sorghum hybrid, CSH 5. In: *Crop Losses due to Insect Pests, Special Issue of Indian Journal of Entomology* (Krishnamurthy Rao B. H. and Murthy K. S. R. K. eds.). Entomological Society of India, Rajendranagar, Hyderabad, Andhra Pradesh, India. Pp. 271-278.

35. Botstein D., White R. L., Skolnick M. and Davis R. W. 1980. Construction of genetic linkage map in man using restriction fragment length polymorphisms: *American Journal of Human Genetics* 32: 314-331.

36. Bough W. A. and Gander J. E. 1971. Exogenous l-tyrosine metabolism and dhurrin turnover in sorghum seedlings. *Phytochemistry* 10: 67-77.

37. Brandon M. J., Foo L. Y., Porter L. and Meredith P. 1982. Proanthocyanidins of barley and sorghum compositon as a funation of maturity of barley ears. *Phytochemistry* 12: 2953-2957.

38. Bredemeijer G. M. M., Arens P., Wouters D., Visser D. and Vosman B. 1998. The use of semi-automated fluorescent microsatellite analysis for tomato cultivar identification. *Theoretical and Applied Genetics* 97: 584-590

39. Brown S. M., Hopkins S. M., Mitchell S. E., Senior M. L, Wang T. Y., Duncan R. R., Gonzalez-candelas F. and Kesovichsel S. 1996. Multiple methods for true identification of polymorphic simple sequence repeats (SSR) in sorghum (*Sorghum bicolor* (L.) Moench). *Theoretical and Applied Genetics* 93: 190-198.

40. Caetano Anolles G., Bassam B. J. and Grasshof P. M. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Bio Technology* 9: 553-557.

41. Caniato F. F., Guimaraes C. T., Schaffert R. E., Alves V. M. C., Kochian L. V., Borem A., Klein P. E. and Magalhaes J. V. 2007. Genetic diversity for aluminum tolerance in sorghum. *Theoritical and Applied Genetics* 114: 863-876

42. Carroll M. J., Schmelz E. A., Meagher R. L. and Teal P. E. A. 2005. Attraction of *Spodoptera frugiperda* larvae to volatiles from herbivore-damaged maize seedlings. *Journal of Chemical Ecology* 32: 1911-1924.

43. Casa A. M., Mitchell S. E., Hamblin M. T., Sun H., Bowers J. E., Paterson A. H., Aquadro C. F. and Kresovich S. 2005. Diversity and selection in sorghum: simultaneous analyses using simple sequence repeats. *Theoretical and Applied Genetics* 111: 23-30

44. Chavan M. H., Phadnawis B. N., Hudge V. S. and Salunkhe M. R. 1990. Biochemical basis of shoot fly tolerant sorghum genotypes. *Annals of Plant Physiology* 4: 215-220.

45. David J. B., John T. A. and Robert I. H. 1997. Phytochemical changes during recurrent selection for resistance to the European corn borer. *Crop science* 37: 1567-1572

46. Davies J. C. and Reddy K. V. S. 1981a. Observations on oviposition of sorghum shoot fly, *Atherigona soccata* Rond. (Diptera: Muscidae). *Sorghum Entomology, Progress Report 4*. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India.

47. Davies J. C., Reddy K. V. S. and Skinner J. D. 1976. *Cereal entomology Part II, Annual Report* (March 1975 - May 1976), International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India.

48. Dean R. E., Dahlberg J. A., Hopkins M. S., Mitchell S. E. and Kresovich S. 1999. Genetic redundancy and diversity among 'Orange' accessions in the US national sorghum collection as assessed with simple sequence repeat (SSR) markers. *Crop Science* 39: 1215-1221.

49. Delobel A. G. L. 1982. Effect of sorghum density on oviposition and survival of the sorghum shoot fly, *Atherigona soccata*. *Entomologia Experimentalis et Applicata* 31: 170-174.

50. Delobel A. G. L. and Unnithan G. C. 1981. The status of *Sorghum arundinaceum* as a host of *Atherigona soccata* Rondani (Diptera: Muscidae) in Kenya. *Insect Science and its Applicata* 2: 67-71.

51. Deu M., Gonzalez de leon D., Glaszmann J. C., Degremont J., Chantereau I., Lanaud C. and Hamon P. 1994. RFLP diversity in cultivated sorghum in relation to racial differentiation. *Theoritical and Applied Genetics* 88: 838-844.

52. Deu M., Sagnard F., Chantereau J., Calatayud C., Herault D., Mariac C., Pham J. L., Vigouroux Y., Kapran I., Traore P. S., Mamadou A., Gerard B., Ndjeunga J. and Bezancon G. 2008. Niger-wide assessment of in situ sorghum genetic diversity with microsatellite markers. *Theoritical and Applied Genetics* (in press).

53. Dhawan P. K., Singh S. P., Verma A. N. and Arya D. R. 1993. Antibiosis mechanism of resistance to shoot fly, *Atherigona soccata* (Rondani) in sorghum. *Crop Research* 6: 306-310.

54. Dhillon M. K. 2004. Effects of cytoplasmic male-sterility on expression of resistance to sorghum shoot fly, *Atherigona soccata* (Rondani). Ph.D. Thesis, 382 pp. Department of Entomology, Chaudhary Charan Singh Haryana Agricultural University, Hisar 125004, Haryana, India.

55. Dhillon M. K., Sharma H. C., Folkertsma R. T. and Chandra S. 2006. Genetic divergence and molecular characterization of sorghum

hybrids and their parents for reaction to *Atherigona soccata* (Rondani). *Euphytica* 149: 199-210.

56. Dhillon M. K., Sharma H. C., Ram Singh and Naresh J. S. 2005b. Mechanisms of resistance to shoot fly, *Atherigona soccata* in sorghum. *Euphytica* 144: 301-312.

57. Dhillon M. K., Sharma H. C., Reddy B. V. S., Ram Singh. and Naresh J. S. 2006b. Inheritances of resistance to sorghum shoot fly, *Atherigona soccata*. *Crop science* 46: 1377-1383.

58. Dhillon M. K., Sharma H. C., Reddy B. V. S., Ram Singh., Naresh J. S. and Kai Z. 2005a. Relative susceptibility of different male-sterile cytoplasms in sorghum to shoot fly, *Atherigona soccata. Euphytica* 144: 275-283.

59. Dhillon M. K., Sharma H. C., Ram Singh and Naresh J. S. 2006a. Influence of cytoplasmic male-sterility on expression of physico-chemical traits associated with resistance to sorghum shoot fly, *Atherigona soccata* (Rondani). *SABRAO Journal of Breeding and Genetics* 38: 105-122.

60. Diawara M. M., Wiseman B. R., Isenhour D. J. and Hill N. S. 1992. Sorghum resistance to whorl feeding by larvae of the fall armyworm (Lepidoptera: Noctuidae). *Journal of Agricultural Entomology* 9: 41-53.

61. Dicko M. H., Gruppen H., Traore A. S., Van Berkel W. J. H and Voragen A. G. J. 2005. Evaluation of the effects of germination, the

content of phenolic compounds and antioxidant activities in sorghum varieties. *Journal of Agricultural and Food Chemistry* 53: 2581-2588.

62. Dje Y., Forcioli D., Ater M., Lefebvre C. and Vekemans X. 1999. Assessing population genetic structure of sorghum landraces from North-western Morocco using allozyme and microsatellite markers. *Theoritical and Applied Genetics* 99: 157-163

63. Dje Y., Heuertz M., Lefebvre C. and Vekemans X. 2000. Assessment of genetic diversity within and among germplasm accessions in cultivated sorghum using microsatellite markers. *Theoritical and Applied Genetics* 100: 918-925.

64. Doggett H. and Mjisu B. N. 1965. Sorghum breeding research. *Annual Report, East African Agriculture and Forestry Research Organization,* pp: 70-79.

65. Doggett H., Starks K. J. and Eberhart S. A. 1970. Breeding for resistance to the sorghum shoot fly. *Crop Science* 10: 528-531.

66. Dreyer D. L., Reese J. C. and Jones K. C. 1981. Aphid feeding deterrents in sorghum: bioassay isolation and characterization. *Journal of Chemical Ecology* 7: 273-284.

67. Dubois M., Gilles K. A., Hamilton J. K., Rebers P. A. and Smith F. 1956 Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* 28: 350-356.

68. Ebel J. 1986. Phytoalexin synthesis: The biochemical analysis of the induction process. *Annual Review of Phytopathology* 24: 235-264.

69. Einhellig F. A., Muth M. S. and Schon M. K. 1985. Effects of allelochemicals on plant-water relationship. *In* A.C. Thompson (ed.), The Chemistry of Allelopathy, American Chemical Society, Washington, D.C., pp. 170-195.

70. Ejeta G., Goldsbrough M. R., Tunistra E. M., Grote A., Menkir Y., Ibrahim Y., Cisse N., Weerasuriya Y., Melake Berhan A. and Shaner C. A. 2000. Molecular marker application in sorghum. In: *Application of molecular markers in plant breeding, Training manual for a seminar held at IITA, Ibadan, Nigeria, 16-17 August 1999* (Haussmann B. I. G., Geiger H. H., Hess D. E., Hash C. T. and Bramel Cox P. eds.). International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India. Pp. 81-89.

71. Emma Mace S., Ling X., David R. J., Kirsten H., Dipal K. P., Eric H., Peter W. and Andrzej K. 2008. DArT markers: diversity analyses and mapping in Sorghum bicolor**.** *BMC Genomics* 9: 26

72. F. A. O. 2002. Food and Agriculture Organization, New York. Year book, 56.

73. F. A. S. 2005. http://fas.usda.gov/ FAS Online. United States Department of Agriculture, Foreign Agricultural service.

74. Fisk J. 1980. Effects of HCN, phenolic acids and related compounds in *sorghum bicolor* on the feeding behaviour of the planthopper *peregrinus maidis*. *Entomologia Experimentalis et Applicata* 27: 211-222.

75. Folkertsma R. T., Rattunde H. F., Chandra S., Raju G. S. and Hash C. T. 2005. The pattern of genetic diversity of guinea race *sorghum bicolor* (L.) Moench land races revealed with SSR markers. *Theoretical and Applied Genetics* 111: 399-409

76. Fukuoka S., Inoue T., Miyao A., Manna L., Zhang H. S., Sasaki T. and Minobe Y. 1994. Mapping of sequence tagged sites in rice by single strand conformation polymorphism. *DNA Research* 1: 271-277.

77. Ghareyazie B. N., Huang G., Second J., Bennett and Khush G. S. 1995. Classification of rice germplasm I. Analysis using ALP and PCR-based RFLP. *Theoretical and Applied Genetics* 91: 218-227.

78. Ghebru B., Schmidt R. J. and Bennetzen J. L. 2002. Genetic diversity of Eritrean sorghum landraces assessed with simple sequence repeat (SSR) markers. *Theoretical and Applied Genetics* 105: 229-236.

79. Gibson P. T. and Maiti R. K. 1983. Trichomes in segregating generations of sorghum matings. I. Inheritance of presence and density. *Crop Protection* 23: 73-75.

80. Gillingham J. T., Shirer M. M., Starnes J. J., Page N. R. and McClain E. F. 1969. Relative occurrence of toxic concentrations of cyanide and nitrate in varieties of sudangrass and sorghum-sudangrass hybrids. *Agronomy Journal* 61: 727-730.

81. Gorz H. J., Haskins F. A., Morris R. and Johnson B. E. 1987. Identification of chromosomes that condition dhurrin content in sorghum seedlings. *Crop Science* 27: 201-203.

82. Gous F. 1989. Tannins and phenols in black sorghum. Ph.D. Dissertation, Texas A & M University, College Station, TX, USA.

83. Grayer R. J., Kimmins F. M., Padgham D. E., Harborne J. B. and Rangarao D. V. 1992. Condensed tannin levels and resistance of groundnuts (*Arachis hypogea*) against *Aphis craccivora*. *Phytochemistry* 31: 3795-3800.

84. Grenier C., Deu M., Kresovich S., Bramel Cox P. J. and Hamon P. 2000. Assessment of genetic diversity in three subsets constituted from the ICRISAT sorghum collection using randon vs non-random sampling procedures. B. Using molecular markers. *Theoretical and Applied Genetics* 101: 197-202.

85. Gujer R., Magnolato D. and Self R. 1986. Lucosylated flavonoids and other phenolic compounds from sorghum. *Phytochemistry* 25: 1431-1436.

86. Gupta R. K. and Haslam E. 1980. Vegetative tannins: structure and biosynthesis. ln J.H. Hulse, ed. *Polyphenols in cereals and legumes*: 15-24.

87. Hahn D. H., Faubion J. M. and Rooney L. W. 1983. Sorghum phenolic acids, their performance liquid chromatography separation and their relation to fungal resistance. *Cereal Chemistry* 60: 255-259.

88. Hahn D. H., Rooney L. W. and Earp C. F. 1984. Tannins and phenols of sorghum. *Cereal Foods World* 29: 776-779.

89. Haley C. S., Knott S. A. and Elsen J. M. 1994. Mapping quantitative trait loci in crosses between outbred lines using least squares. *Genetics* 136: 1195-1207.

90. Halkier B. A. and Moller B. L. 1989. Biosynthesis of the cyonogenic glucoside dhurrin in seedlings of *sorghum bicolour* (L.) Moench and partial purification of the enzyme system involved. *Plant physiology 90*: 1552-1559.

91. Hamaker B. R., Mohamed A. A., Habben J. E., Huang C. P. and Larkins B. A. 1995. Efficient procedure for extracting maize and sorghum kernel proteins reveals higher prolamin contents than the conventional method. *Cereal Chemistry* 72: 583-588.

92. Harlan J. R. and Dewet J. M. J. 1972. A simplified classification of cultivated sorghum. *Crop Science*. 12: 172-176

93. Hatanaka A. 1993. The biogeneration of green odour by green leaves. *Phytochemistry* 34: 1201-1218

94. Hearne C. M., Ghosh S. and Todd J. 1992. Micro satellites for linkage analysis of genetic traits. *Trends in Genetics* 8: 288-294.

95. Hedin P. A. and Waage S. K. 1986. Role of flavonoids in plants resistance to insects. In: Cody V, Middleton E, Harborne J. B. (eds) plant flavonoids in biology and medicine Vol. 1. Biochemical, pharmacological and structure activity relationships. Alan R. liss, New York. 87-100.

96. Heller W. and Forkman G. 1993. Biosynthesis of flavonoids. In: The flavonoids, advances in research since 1986 (Harborne J. B. ed.). Chapman and Hall, London, United Kingdom.

97. Hilty J. W. and Schmitthenner A. F. 1966. Electrophoretic comparisons of soybean leaf proteins from varieties resistant and susceptible to *Phytophthora megasperma* var. Sojae. *Phytopathology* 56: 287-291.

98. Hokanson S. C., Szewc McFadden A. K., Lamboy W. F. and McFerson J. R. 1998. Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a Malus × domesticaborkh. core subset collection. *Theoretical and Applied Genetics* 97: 671- 683.

99. Hulbert S. H., Rechter T. E., Axxell J. D. and Bennetzen J. L. 1990. Genetic mapping and characterization of sorghum and related crops

by means of maize DNA probes. *Proceedings of the National Acadamy of Sciences,* USA 87: 4251-4255.

100. Ingham J. L., Keen N. T., Mulheirn L. J. and Lyne R. L. 1981. Inducibly formed isoflavonoids from leaves of soybean. *Phytochemistry* 20: 795-798.

101. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). 1992. The medium term plan, Vol. 1, ICRISAT, Patancheru, Andhra Pradesh, India.

102. Jadhav S. S. and Mote U. N. 1986. Nature of injury and survival of shoot fly on resistant and susceptible sorghum varieties. *Sorghum Newsletter* 29: 75.

103. Jadhav S. S., Mote U. N. and Bapat D. R. 1986. Biophysical plant characters contributing to shoot fly resistance. *Sorghum Newsletter* 29: 70.

104. Jain K. K. and Bhatnagar M. P. 1962. Studies on varietal resistance to the jowar shoot fly. *Indian Journal of Genetics* 22: 224-229.

105. Jarman A. P. and Wells R. A. 1989. Hypervariable minisatellites: recombinators or innocent bystanders? *Trends in Genetics* 5: 367-371.

106. Jayanthi P. D. K., Reddy B. V. S., Gour T. B. and Reddy D. D. R. 2002. Early seedling vigour in sorghum and its relationship with

resistance to shoot fly, *Atherigona soccata* Rondani. *Journal of Entomological Research* 26: 93-100.

107. Jayanthi P. D. K., Reddy B. V. S., Gour T. B. and Reddy D. D. R. 1999. Genetics of glossy and trichome characters in sorghum hubrids of cytoplasmic male sterile lines. *Journal of Maharashtra Agricultural University* 24: 251-256.

108. Jones J. B. Jr., Wolf B. and Mills H. A. 1991. Plant Analysis Handbook: A practical sampling, preparation, and interpretation guide; Micro-Macro publishing, Inc.: Athens, GA.

109. Jotwani M. G. 1978. Investigations on insect pests of sorghum and millets with special reference to host plant resistance. *Final Technical Report (1972-1977)*. Research bulletin of the division of entomology, Indian Agricultural Research Institute, New Delhi, India. 114 pp.

110. Jotwani M. G. and Srivastava K. P. 1970a. Studies on sorghum lines resistant against shoot fly, *Atherigona varia soccata* Rond. *Indian Journal of Entomology* 32: 1-3.

111. Jotwani M. G., Marwah K. K., Srivastava K. P. and Young W. R. 1970b. Seasonal incidences of shoot fly (*Atherigona varia soccata* Rond.) in *Jowar* hybrids at Delhi. *Indian Journal of Entomology* 32: 7-15.

112. Jotwani M. G., Young W. R. and Teetes G. L. 1980. Elements of integrated control of sorghum pests. *FAO Plant Production and Protection Paper,* FAO, Rome, Italy: 159 pp.

113. Kabre G. B. and Ghorpade S. A. 1998. Studies on association of some chemical constituents of maize genotypes with susceptibility of stem borer. *Journal of Maharashtra Agricultural University* 22: 301-304.

114. Kabre G. B. and Ghorpade S. A. 1999. Susceptibility to maize stem borer, *Chilo partellus* (Swinhoe) in relation to sugars, proteins and free amino acids content of maize germplasm and F_1 hybrids. *Journal of Insect Science* 12: *37-40.*

115. Kadire G., Jagadish P. S., Gowda K. N. M., Ramesh S. and Gowda K. 1996. Biochemical basis of resistance to shoot fly (*Atherigona pulla Wiede)* in little millet. *Journal of Agricultural Sciences* 9: 56-62.

116. Kamatar M. Y. and Salimath P. M. 2003. Morphological traits of sorghum associated with resistance to shoot fly, *Atherigona soccata* Rondani. *Indian Journal of Plant Protection* 31: 73-77.

117. Kamatar M. Y., Salimath P. M., Nayakar N. Y. and Deshpande V. P. 2002. Biochemical parameters responsible for resistance to sorghum shoot fly (*Atherigona soccata* (R.). In: *Proceedings, National Seminar on Resource Management in Plant Protection in 21st Century*, 14-15 November 2002, National Plant Protection Association of India, Hyderabad, India. Pp. 49.

118. Kamatar M. Y., Salimath P. M., Ravi Kumar R. L. and Swamy Rao T. 2003. Heterosis for biochemical traits governing resistance to shoot

fly in sorghum [*Sorghum bicolor* (L.) Moench]. *Indian Journal of Genetics* 63: 124-127.

119. Kambal A. E. and Bate Smith E. C. 1976. Genetic and biochemical study on pericarp pigments in a cross between two cultivars of grain sorghum, *Sorghum bicolor*. *Heredity* 37: 413-416.

120. Karanjkar R. R., Chundurwar R. D. and Borikar S. T. 1992. Correlations and path analysis of shoot fly resistance in sorghum. *Journal of Maharashtra Agricultural Universities* 17: 389-391.

121. Karban R. 1986. Induced resistance against spider mites in cotton filed verification. *Entomologia Experimentb alis et Applicata* 42: 239.

122. Khan Z. R., Norris D. M., Chiang H. S. and oosterwyk A. S. 1986. Light induced susceptibility in soybean to cabbage looper, *Trichoplusia ni* (Lepidoptera: Noctuidae). *Environmental Entomology* 15: 803-808.

123. Khurana A. D. and Verma A. N. 1983. Some biochemical plant characters in relation to susceptibility of sorghum to stem borer and shoot fly. *Indian Journal of Entomology* 45: 29-37.

124. Kong L., Dong J. and Hart G. E. 2000. Characteristics, linkage-map positions, and allelic differentiation of *Sorghum bicolor* (L.) Moench DNA simple sequence repeats (SSRs). *Theoretical and Applied Genetics* 101: 438-448.

125. Krueger C. G., Vestling M. M. and Reed J. D. 2003. Matrix-assisted laser desorption / ionizationtime of flight mass spectrometry of heteropoly-flavan-3-ols and glucosylated heteropolyflavans in sorghum (*Sorghum bicolor* (L.) Moench). *Journal of Agricultural and Food Chemistry* 51: 538-543.

126. Kumar L. S. 1999. DNA markers in plant improvement: An overview. *Biotechnology Advances* 17: 143-182.

127. Kumar S. and Singh R. 1996. Combing ability for shoot fly resistance in sorghum. *Crop Improvement* 23: 217-220.

128. Kumar S. and Singh R. 1998. Inheritance of tannin in relation to shoot fly resistance in sorghum. *Cereal Research Communications* 26: 271-273.

129. Kundu G. G. and Kishore P. 1970. Biology of the sorghum shoot fly, *Atherigona varia soccata* Rond. (Diptera: Anthomyiidae). *Indian Journal of Entomology* 32: 215-217.

130. Larsen A. L. 1967. Electrophoretic differences in seed proteins among varieties of soybean, *Glycine max* (L.) Merrill. *Crop Science* 7: 311-313

131. Levin D. A. 1973. The role of plant trichomes in plant defense. *The Quarterly Review of Biology* 48: 3-15.

132. Li SuJuan., Wu Y. Q., Liu A., Li S. and Han S. 2001. Studies on biochemical mechanism of resistance to *Rhopalosiphum padi* on wheat in milking stage. *Acta Phytophysiologica Sinica* 28: 331-334.

133. Lin Y. R., Shertz K. F. and Patreson A. H. 1995. Comparative analysis of QTL affecting plant height and maturity across the poaceae in reference to an interspecific sorghum population. *Genetics* 141: 391-411.

134. Lyamichev V., Brow M. A. D. and Dahlberg J. E. 1993. Structure-specific enonucleolytic cleavage of nuclic acids by eubacterial DNA polymerases. *Science* 260: 778-783.

135. Maiti R. K. 1980. Role of glossy trichome trait in sorghum crop improvement. In: *Annual Meeting, All India Sorghum Improvement Workshop*, 12-14 May, 1980, Coimbatore, Tamil Nadu, India. Pp. 1-14.

136. Maiti R. K. 1994a. The roles of morpho-physiological traits in shoot fly resistance in sorghum. *International Sorghum and Millets Newsletter* 35: 107-108.

137. Maiti R. K. and Bidinger F. R. 1979. A simple approach to the identification of shoo fly tolerance in sorghum. *Indian Journal of Plant Protection* 7: 135-140.

138. Maiti R. K. and Gibson R. W. 1983. Trichomes in segregating generations of sorghum matings, II. Associations with shoot fly resistance. *Crop Science* 23: 76-79

139. Maiti R. K., Heredia Rojas J. A. and Cazares Alonso N. P. 1994c. Electrophoretic analysis of protein profile of some glossy and nonglossy sorghum lines: a preliminary study. *Phyton Buenos Aires* 56: 105-107.

140. Maiti R. K., Rao K. V. and Swaminathan G. 1994b. Correlation studies between plant height and days to flowering to shoot fly (*Atherigona soccata* Rond.) resistance in glossy and nonglossy sorghum lines. *International Sorghum and Millets Newsletter* 35: 109-11.

141. Mate S. N., Phadanwis B. A. and Mehetre S. S. 1988. Studies on growth and physiological factors in relation to shoot fly attack on sorghum. *Indian Journal of Agricultural Research* 22: 81-84.

142. Matsuoka Y., Mitchell S. E., Kresovich S., Goodman M. and Doebley J. 2002. Microsatellites in *Zea* variability, patterns of mutations, and use for evolutionary studies. *Theoritical and Applied Genetics* 104: 436-450.

143. McDonough C. M., Rooney L. W. and Earp C. F. 1986. Structural characteristics of *Eleusinecoracana* (fingermillet) using scanning electron and fluorescence microscopy. *Food Microstructure* 5: 247-256.

144. Meksongsee B., Chawanapong M., Sangkasuwan U. and Poonyathaworn P. 1981. The biology and control of the sorghum shoot fly, *Atherigona soccata* Rondani, in Thailand. *Insect Science et Applicata* 2: 111-116.

145. Menkir A., Goldsbrough P. and Ejeta G. 1997. RAPD based assessment of genetic diversity in cultivated races of sorghum. *Crop Science* 37: 564-569.

146. Menz M., Klein R., Unruh N., Rooney W., Klein P. and Mullet J. 2004. Genetic diversity of public inbreds of sorghum determined by mapped AFLP and SSR markers. *Crop Science* 44: 1236-1244

147. Mishra B. K., Sontakke B. K. and Mohapatra H. 1990. Antibiosis mechanisms of resistance in rice varieties to yellow stem borer *Scirpophaga incertulas* Walker. *Indian Journal of Plant Protection* 18: 81-83.

148. Mishra N. C. and Misra B. C. 1993. Role of plant chemicals determining resistance in rice to white-backed planthopper *Sogatella furcifera*. *Environmental Ecology* 11: 88-91.

149. Mohan M., Nair S., Bhagwat A., Krishna T. G., Yano M., Bhatia C. R. and Sasaki T. 1997. Genome mapping, molecular markers and marker-assisted selection in the improvement of quantitative traits. *Molecular Breeding* 3: 87-103

150. Moholkar P. R. 1981. Investigations on the resistance to shoot fly, *Atherigona soccata* (Rondani) and Stem borer *Chilo partellus*

(Swinhoe) in sorghum, Ph.D. Thesis, Indian Agricultural Research Institute, New Delhi-110012.

151. Morden C. W., Doebley J. and Schertz K. F. 1989. Allozyme variation in Old World races of *Sorghum bicolor* (Poaceae). *American Journal of Botany* 76: 247-255.

152. Morgante M. and Olivieri A. M. 1993. PCR amplified microsatellites as markers in plant genetics. *Plant Journal* 3: 175-182.

153. 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.

154. Mote U. N., Phadnis B. N. and Taley Y. M. 1979. Studies on some physiological factors of shoo fly resistance in sorghum. *Sorghum Newsletter* 22: 66-67.

155. Murthy D. S. and Kumar K. A. 1995. Traditional uses of sorghum and millets. In: Dendy. D. A. V. (ed), Sorghum and Millets: Chemistry and Technology. St. Paul, MN: American Association of Cereal Chemists. Pp 185-187.

156. Naik M. I., Hiremath I. G., Salimath P. M. and Mallapur C. P. 1994. Chemical analysis of sorghum ovaries and their interaction with sorghum midge. *International Sorghum and Millets Newsletter* 35: 108-109.

157. Narayana D. 1975. Characters contributing to sorghum shoot fly resistance. *Sorghum Newsletter* 18: 21-22.

158. Neupane F. P., Norris D. M. 1991. Sulfhydryl-reagent alteration of soybean resistance to the cabbage looper, Trichoplusia ni. *Entomologia Experimentalis et Applicata* 60: 239-245.

159. Nicholson R. L., Kollipara S. S., Vincent J. R., Lyons P. C. and Cadena-Gomez G. 1987. Phytoalexin synthesis by the sorghum mesocotyl in response to infection by pathogenic and nonpathogenic fungi. *Proceedings of Natural Academy of Sciences,* USA 84: 5520–5524.

160. Nip W. K. and Burns E. E. 1971. Pigment characterization in grain sorghum. II. White varieties. *Cereal Chemistry* 48: 74-80.

161. Noli E., Salvi S. and Tuberosa R. 1997. Comparative analysis of genetic relationships in barley based on RFLP and RAPD markers. *Genome* 40: 607-616.

162. Nwanze K. F., Nwilene F. E. and Reddy Y. V. R. 1998. Evidence of shoot fly, *Atherigone soccata* Rondani (Dipt., muscidae) oviposition response to sorghum seedlings volatiles. *Journal of Applied Entomology* 122: 591-594.

163. Nwanze K. F., Pring R. J., Sree P. S., Butler D. R., Reddy Y. V. R. and Soman P. 1992. Resistance in sorghum to the shoot fly, *Atherigona*

soccata: epicuticular wax and wetness of the central whorl leaf of young seedlings. *Annals of Applied Biology* 120: 373-382.

164. Nwanze K. F., Reddy Y. V. R. and Soman P. 1990a. The roles of leaf surface wetness in larval behavior of the sorghum shoot fly, *Atherigona soccata*. *Entomologia Experimentalis et Applicata* 56: 187-195.

165. Nwanze K. F., Sree P. S., Butler D. R., Reddy D. D. R., Reddy Y. V. R. and Soman P. 1990b. The dynamics of leaf surface wetness of sorghum seedlings in relation to resistance to the shoot fly, *Atherigona soccata*. *Entomologia Experimentalis et Applicata* 64: 151-160.

166. Ogwaro K and Kokwaro E. D. 1981. Development and morphology of the immature stages of the sorghum shoot fly, *Atherigona soccata* Rondani. *Insect Science and its Application* 1: 365-372.

167. Ogwaro K. 1978. Ovipositional behavior and host-plant preference of the sorghum shoot fly, *Atherigona soccata* (Diptera: *Anthomyiidae). Entomologia Experimentalis et Applicata* 23: 189-199.

168. Ogwaro K. 1978a. Observations on longevity and fecundity of the sorghum shoot fly, *Atherigona soccata* (Diptera: Anthomyiidae). *Entomologia Expermentalis et Applicata* 23: 131-138.

169. Ogwaro K. 1978b. Ovipositional behavior and host plant preference of the sorghum shoot fly, *Atherigona soccata* (Diptera: Anthomyiidae). *Entomologia Expermentalis et Applicata* 23: 189-199.

170. Oliviera A. C., Richter T. and Bennetzen J. L. 1996. Regional and racial specificities in sorghum germplasm assessed with DNA Markers. *Genome* 39: 579-587.

171. Omori, T., Agrawal B. L. and House L. R. 1983. Componential analysis of the factors influencing shoot fly resistance in sorghum (*Sorghum bicolor* (L.) Moench). Japan Agricultural Research Quarterly 17: 215-218.

172. Orita M., Iwahana H., Kanazawa H., Hatashi K. and Sekiya T. 1989. Detection of polymorphisms of human DNA by gel electophoresis as single strand conformation polymorphisms. *Proceedings of the National Academy of Sciences,* USA 86: 2766-2770.

173. Osuntogun B. O., Adewusi S. R. A., Ogundiwin J. O. and Nwasike C. C. 1989. Effect of cultivar, steeping, and malting on tannin, total polyphenol, and cyanide content of Nigerian sorghum. *Cereal Chemistry* 66: 87-89.

174. Painter R. H. 1958. Resistance of plants to insects. *Annual Review of Entomology* 3: 267-290

175. Pale E., Kouda Bonafos M., Mouhoussine N., Vanhaelen M., Vanhaelen Fastre R. and Ottinger R. 1997. 7-O-methylapigeninidin,

ananthocyanidin from *Sorghum Caudatum*. *Phytochemistry* 45: 1091-1092.

176. Pandey P. K., Singh R. and Shrotria P. K. 2005. Reversed-phase HPLC separation of phenolic acids in sorghum and their relation to shoot fly (*Atherigona soccata* Rond.) resistance. *Indian Journal of Entomology* 67: 170-174

177. Pare P. W. and Tumlinson J. H. 1996. Plant volatile signals in response to herbivore feeding. *Florida Entomologist* 79: 93-103

178. Patel G. M. and Sukhani T. R. 1990b. Biophysical plant characters associated with shoot fly resistance. *Indian Journal of Entomology* 52: 14-17.

179. Patil S. S., Narkhede B. N. and Barbate K. K. 2006. Effects of biochemical constituents with shoot fly resistance in sorghum. *Agricultural Science Digest* 26: 79-82.

180. Peelman L. J., Mortiaux F., Van Zeveren A., Dansercoer A., Mommens G., Coopman F., Bouquet Y., Burney A., Renaville G. and Portetelle D. 1998. Evaluation of the genetic variability of 23 bovine microsatellite markers in four Belgian cattle breed: *Animal Genetics* 29: 161-167.

181. Pejic I. P., Ajmone M., Morgante M., Kozumplick V., Castiglioni P., Taramino G. and Motto M. 1998. Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. *Theoretical and Applied Genetics* 97: 1248-1255.

182. Pereira M. G., Lee M., Bramel Cox P., Woodman W., Doebley H. J. and Whitkus R. 1994. Construction of RFLP map in sorghum and comparative mapping in maize. *Genome* 37: 236-243.

183. Powell W., Machray G. C. and Provan J. 1996a. Polymorphism revealed by simple sequence repeats. *Trends in Plant Science* 7: 215-222.

184. Powell W., Morgante M., Andre C., Hanafey M., Vogel J., Tingey S. and Rafalski A. 1996b. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding* 2: 225-238

185. Pradhan S. 1971. Investigations on insect pests of sorghum and millets (1965-70). *Final Technical Report, PL 480 project grant no. FG. In-227, Project No. A7-ENT-31*, Division of Entomology, Indian Agricultural Research Institute, New Delhi.

186. Price M. L., Van Scoyoc S. and Butler L. G. 1978. A critical evaluation of the vanillin reaction-an assay for tannins in sorghum grain. *Journal of Agriculture and Food Chemistry* 26: 1214-1218.

187. Priyavratha Rao and Narasimha Rao D. V. 1956. Studies on the sorghum shoot-borer-fly *Atherigona indica* Malloch (Anthomyiidae: Diptera) at Siruguppa. *Mysore Agriculture Journal* 31: 158-174.

188. Putnam A. R. and Tang C. S. 1986. The science of Allelopathy, John Wiley & Sons, New york. Rooney and Serna-Saldivan 2000, sorghum. Pages 149-175 in hand book of cereal science and

technology. Second edition (Kulp K. and ponte J., eds.). New York, USA. Marcel-Dekker, Inc.

189. Rai S. and Jotwani M.G. 1977. Estimation of losses at various levels of shoot fly infestation. *Entomologists' Newsletter* 7: 15-16.

190. Raina A. K. 1981. Movement, feeding behaviouar and growth of larvae of the sorghum shoot fly, *Atherogona soccata*. *Insect Science and its Application* 22: 77-81.

191. Raina A. K. 1981b. Movement, feeding behavior and growth of larvae of the sorghum shoot fly, *Atherigona soccata*. *Insect Science et Applicata* 2: 77-81.

192. Raina A. K. 1982a. Daily rhythms in the sorghum shootfly, *Atherigona soccata*: Oviposition, egg hatch and adult eclosion. *Physiological Entomology* 7: 65-70.

193. Raina A. K. 1982b. Fecundity and oviposition behavior of the sorghum shoot fly, *Atherigona soccata*. *Entomologia Expermentalis et Applicata* 31: 381-385.

194. Raina A. K. 1985. Mechanisms of resistance to shoot fly in sorghum: a review. In: *proceedings, International Sorghum Entomology Workshop,* 15-21 July 1984, Texas A&M University, College Station, Texas, U.S.A. (Kumble, V., ed.): International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India. Pp. 131-136.

195. Raina A. K., Thindwa H. Z., Othieno S. M. and Corkhill R.T. 1981. Resistance in sorghum to sorghum shoot fly: Larval development and adult longevity and fecundity on selected cultivars. *Insect Science and its Application* 2: 99-103.

196. Raina A. K., Thindwa H. Z., Othieno S. M. and Douglass L. W. 1984. Resistance in sorghum to shoot fly (Diptera: Muscidae) and oviposition on selected cultivars. *Journal of Economic Entomology* 77: 648- 651.

197. Rana B. S., Singh B. U. and Rao N. G. P. 1985. Breeding for shoot fly and stem borer resistance in sorghum. 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 502 324, Andhra Pradesh, India, pp. 347-359

198. Rana B. S., Tripathi D. P., Balakotaiah K., Damodar R. and Rao N. G. P. 1975. Genetic analysis of some exotic x Indian crosses in sorghum selection for shoot fly resistance. *Indian Journal of Genetics* 35: 350-355.

199. Rao C. N. and Panwar V. P. S. 2001. Morphological plant factors affecting resistance to *Atherigona* spp. in maize. *Indian Journal of Genetics* 61: 314-317.

200. Rao C. N. and Panwar V. P. S. 2002. Biochemical plant factors affecting resistance to *Chilo partellus* (Swinhoe) in maize. *Annals 0f Plant Protection Sciences* 10: 28-30.

201. Rao S. S., Basheeruddin M. and Sahib K. H. 2000. Correlation studies between the plant characters and shoot fly resistance in sorghum. *Crop Research* 19: 366-367.

202. Ratnavathi C. V. and Sashidhar R. B. 1998. Microassay for the quantitation of protein precipitable polyphenols: use of bovine serum albumin-benzidine conjugate as a protein probe. *Food Chemistry* 61: 337-380.

203. Reddy K. L. 2003. Effect of plant volatiles extracted as steam distillates from selected rice cultures against *Nilaparvata lugens* (Stal.). *Insect Environment* 9: 132-134.

204. Reddy K. V. S. and Davies J. C. 1979. Pests of sorghum and pearl millet, and their parasites and predators, recorded at ICRISAT Center, India up to August 1979. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh. 23 pp.

205. Reddy K. V. S., SkinnerII J. D. and Davies J. C. 1981. Attractants for *Atherigona* spp. including the sorghum shoot fly, *Atherigona soccata* Rond. (Muscidae: Diptera). *Insect Science and its Application* 2: 83-86.

206. Rice E. L. 1984. Allelopathy. 2nd ed. Orlando, Florida, USA, Academic Press.

207. Rivnay E. 1960. Field crop pests in the near East (Pests of graminaceous and leguminous crops and stored products). *Monograph Biology Hassadeh, Israel* 10: 202-204.

208. Robert J. H., Glenn W. C. jr., Samuel D. S. and James A. R. 1992. Comparison of the volatile compounds from several commercial plum cultivars. *Journal of the Science of Food and Agriculture* 60: 21-23

209. Roberts W. K. and Selitrennikoff C. P. 1986. Isolation and partial characterization of two sorghum antifungal proteins from barley. *Biochemica Biophysica Acta* 880: 161-170

210. Roberts W. K. and Selitrennikoff C. P. 1988. Plant and bacterial citinases differ in antifungal activity. *Journal of General Microbiology* 134: 1771-1778.

211. Roberts W. K. and Selitrennikoff C. P. 1990. Zeamatin an antifungal protein from maize with membrane permeabilising activity. *Journal of General Microbiology* 136: 1771-1778

212. Roder M. S., Korzun V., Wendenhake K., Plaschke J., Tixier M. H., Leroy P. and Ganal M. W. 1998. Microsatellite map of wheat. *Genetics* 149: 2003-2007.

213. Romero Severson J., Smith J. S. C., Ziegle J., Hauser J., Joe L. and Hookstra G. 2001. Pedigree analysis and haplotype sharing within diverse groups of *Zea mays* L. inbreds. *Theoretical and Applied Genetics* 103: 567-574.

214. Russell J. R., Fuller J. D., Macaulay M., Hatz B. G., Jahoor A., Powell W. and Waugh R. 1997. Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. *Theoretical and Applied Genetics* 95: 714-722.

215. Saghai Maroof M. A., Biyashev R. B., Yang G. P., Zhang Q. and Allard R. W. 1994. Extraordinarily polymorphic microsatellite DNA in barley species diversity, chromosomal location and population dynamics. *Proceedings of the National Academy of Sciences, USA* 91: 5466-5470.

216. Saghai Maroof M. A., Soliman K. M., Jorgensen R. A. and Allard R. W. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences, USA* 81: 8014-8018.

217. Sahrawat K. L., Ravi Kumar G and Rao J. K. 2002. Evaluation of triacid and dry ashing procedures for determining potassium, calcium, magnesium, iron, zinc, manganese, and copper in plant materials. *Communication Soil Science Plant Analysis* 33: 95-102.

218. Sarkar G., Cassady J., Bottema C. D. K. and Sommer S. S. 1990. Characterization of polymerase chain reaction amplification of specific alleles. *Analytical Biochemistry* 186: 64-68.

219. Sastry L. V. S., Paulis J. W., Bietz J. A. and Wall J. S. 1986. Genetic variation of storage proteins in sorghum grain: Studies by isoelectric

focusing and high-performance liquid chromatography. *Cereal chemistry* 63: 420-427.

220. Schloss S. J., Mitchell S. E., White G. M., Kukatla R., Bowers J. E., Paterson A. H. and Kresovich S. 2002. Characterization of RFLP probe sequences for gene discovery and SSR development in *Sorghum bicolor* (L.) Moench. *Theoretical and Applied Genetics* 105: 912-920

221. Schoonhoven L. M. 1968. Chemosensory basis of host plant selection. *Annual Review of Entomology* 13: 115-136.

222. Scotti I., Magni F., Fink R., Powell W., Binelli G. and Hedley P. E. 2000. Microsatellite repeats are not randomly distributed within Norway spruce (*Picea abies* K.) expressed sequences. *Genome* 43: 41-46.

223. Seitz L. M. 2004. Effect of plant type (purplevs.tan) and mold invasion on concentrations of 3 deoxyanthocyanidins in sorghum grain. A. A. C. C. Annual Meeting Abstracts. URL:/http://www.aaccnet.org/meetings /2004/abstracts/a04ma384.html.

224. Sekhon S. S. and Kanta U. 1994. Mechanisms and bases of resistance in maize to spotted stem borer. Insect resistant maize: recent advances and utilization. Proceedings of an International Symposium held at the International Maize and Wheat Improvement Center, 27 November-3 December, 1994, 1997: 106-111.

225. Senior M. L. and Heun M. 1993. Mapping micro satellites and polymerase chain conformation of targeted repeats using a CT primer. *Genome* 36: 884-889.

226. Sharma G. C., Jotwani M. G., Rana B. S. and Rao N. G. P. 1977. Resistance to the sorghum shoot fly, *Atherigona soccata* (Rond.) and its genetic analysis. *Journal of Entomological Research* 1: 1-12.

227. Sharma H. C. 1985. Strategies for pest control in sorghum in India. *Tropical Pest Management* 31: 167-185.

228. Sharma H. C. and Norris D. M. 1991. Chemical basis of resistance in soybeen to cabbage looper, *Trichoplusia ni. Journal of Science of Food and Agriculture* 55: 353 - 364.

229. Sharma H. C. and Nwanze K. F. 1997. Mechanisms of resistance to insects in sorghum. *Information Bulletin* No. 45, International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India. 47 pp.

230. Sharma H. C., Dhillon M. K. and Reddy B. V. S. 2006. Expression of resistance to *Atherigona soccata* in F1 hybrids involving shoot fly resistant and susceptible cytoplasmic male-sterile and restorer lines of sorghum. *Plant Breeding* 125: 473-477

231. Sharma H. C., Reddy B. V. S., Dhillon M. K., Venkateswaran K., Singh B. U., Pampapathy G., Folkertsma R. T., Hash C. T. and Sharma K. K. 2005. Host Plant resistance to insects in sorghum:

present status and need for future research. *International Sorghum and Millets Newsletter* 46: 36-42.

232. 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 502324, Andhra Pradesh, India. 177 pp.

233. 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 (ICRISAT), Patancheru 502 324, A.P, India. 48 pp.

234. Sharma H. C., Vidyasagar P. and Leuschner K. 1990. Componental analysis of the factors influencing resistance to sorghum midge, *Contarinia sorghicola* Coq. *Insect Science and its Application* 11: 889-898.

235. Sharma H. C., Vidyasagar P. and Subramanian V. 1993. Antibiotic component of resistance in sorghum to sorghum midge, *Contarinia sorghicola*. *Annals of Applied Biology* 123: 469-483

236. Shi ZhongLiang., Qiu SongYing., Ma AiPing., Xu GangYuan., Wu JiPing and Lu LaHu. 2002. Studies on mechanisms of biochemical resistance to blossom midge (*Sitodiplosis mosellana* Gelin) in wheat. *Journal of Triticeae Crops* 22: 63-65.

237. Shibamoto T., Horiuchi M. and Umano K. 2007. Composition of the young green barley and wheat leaves. *Journal of Essential Oil Research* 19: 134-137.

238. Shivankar V. I., Ram S. and Gupta M. P. 1989. Tolerances in some sorghum germplasm to shoot fly (*Atherigona soccata* Rondani). *Indian Journal of Entomology* 51: 593-596.

239. Simmonds M. S. J. 2001. Importance of flavonoids in insect-plant interactions: feeding and oviposition. *Phytochemistry* 56: 245-252.

240. Singh B. U. and Rana B. S. 1986. Resistance in sorghum to the shoot fly, *Atherigona soccata* Rondani. *Insect Science and its Application* 7: 577-587.

241. Singh B. U., Padmaja P. G. and Seetharama N. 2004. Stability of biochemical constituents and their relationships with resistance to shoot fly, *Atherigona soccata* (Rondani) in seedling sorghum. *Euphytica* 136: 279-289.

242. Singh B. U., Rana B. S. and Rao N. G. P. 1981. Host plant resistance to mite (*Oligonychus Indicus* H.) and its relationship with shoot fly (*Atherigona soccata* Rond.) resistance in sorghum. *Journal of Entomological Research* 5: 25-30.

243. Singh R. and Narayana K. L. 1978. Influence of different varieties of sorghum on the biology of sorghum shoot fly. *Indian Journal of Agricultural Sciences* 48: 8-12.

244. Singh S. P. 1998. Field efficacy of some bio-pesticides against shoot fly and stem borer in forage sorghum. *Forage Research* 24: 177-178.

245. Singh S. P. and Jotwani M. G. 1980. Mechanisam of reisistance in sorghum shoot fly. III. Biochemical basis of resistance. *Indian Journal of Entomology* 42: 551-566.

246. Singh S. P. and Jotwani M. G. 1980a. Mechanisms of resistance in sorghum to shoot fly I. Ovipositional non-preference. *Indian Journal of Entomology* 42: 353-360.

247. Singh S. P. and Jotwani M. G. 1980b. Mechanisms of resistance in sorghum to shoot fly II. Antibiosis. *Indian Journal of Entomology* 42: 240-247.

248. Singh S. P. and Jotwani M. G. 1980d. Mechanisms of resistance in sorghum to shoot fly IV. Role of morphological characters of seedlings. *Indian Journal of Entomology* 42: 806-808.

249. Sivaramakrishnan S., Soman P., Nwanze K. F., Reddy Y. V. R. and Butler D. R. 1994. Resistance in sorghum to shoot fly, *Atherigona soccata*: Evidence for the source of leaf surface wetness. *Annals of Applied Biology* 125: 93-96.

250. Smith J. S. C. 1994. RP-HPLC for varietal identification in cereals and legumes: Sorghum. Pages 201-205 in: high performance liquid chromatography of cereal and legume proteins. J. E. Kruger and J. A. Biez, eds. American Association Cereal Chemistry: St. Paul, MN.

251. Smith J. S. C., Kresovich S., Hopkins M. S., Mitchell S. E., Dean R. E., Woodman W. L., Lee M. and Porter K. 2000. Genetic diversity among elite sorghum inbred lines assessed with simple sequence repeats. *Crop Science* 40: 226-232.

252. Soman P., Nwanze K. F., Laryea K. B., Butler D. R. and Reddy Y. V. R. 1994. Leaf surface wetness in sorghum and resistance to shoot fly, *Atherigona soccata*: role of soil and plant water potentials. *Annals of Applied Biology* 124: 97-108.

253. Soto P. E. 1972. Mass rearing of sorghum shoot fly and screening for host plant resistance under greenhouse conditions. In: *Control of Sorghum Shoot Fly* (Jotwani, M.G. and Young, W.R. eds.). New Delhi, India: Oxford & IBH. Pp. 137-138.

254. Soto P. E. 1974. Ovipositional preference and antibiosis in relation to resistance to sorghum shoot fly. *Journal of Economic Entomology* 67: 265-267.

255. Soto P. E. and Laxminarayan K. 1971. A method for rearing sorghum shoot fly. *Journal of Economic Entomology* 64: 553.

256. Southern E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* 98: 503-517.

257. Sree P. S., Butler D. R. and Nwanze K. F. 1992. Morphological factors associated with leaf surface wetness. In: *Cereal Program*

Annual Report 1992, International Crops Research Institute for the Semi-Arid Tropics, Patancheru, 502 324, Andhra Pradesh, India.

258. Sree P. S., Nwanze K. F., Butler D. R., Reddy D. D. R. and Reddy Y. V. R. 1994. Morphological factors of the central whorl leaf associated with leaf surface wetness and resistance in sorghum to shoot fly, *Atherigona soccata*. *Annals of Applied Biology* 125: 467-476.

259. SubbaRao M. V. S. S. T. and Muralikrishna G. 2002. Evaluation of the antioxidant properties of free and bound phenolic acids from native and malted fingermillet (Ragi, *Eleusine coracana* 15). *Journal of Agricultural and Food Chemistry* 50: 889-892.

260. Subudhi P. K., Rosenow D. T. and Nguyen H. T. 2000. Quantitative trait loci for the stay green trait in sorghum (*Sorghum bicolor* L. Moench): consistency across genetic backgrounds and environments. *Theoretical and Applied Genetics* 101: 733-741.

261. Sunitha Kumari R., Chandrashekar A. and Frederiksen R. A. 1996. Levels of three antifungal proteins during development, germination and in response to fungal infection in grain sorghum. *African Crop Science Journal* 4: 79-87

262. Swarup V. and Chagale D. S. 1962. A preliminary study on resistance to stem borer *Chilo zonellus* (Swinhoe) infestation in sorghum (*Sorghum vulgare* pers) *Current Science* 31: 163-164.

263. Taneja S. L. and Leuschner K. 1985. Resistance screening and mechanisms of resistance in sorghum to shoot fly. In: *Proceedings,*

International Sorghum Entomology Workshop, 15-21 July, 1984, Texas A&M University, College Station, Texas, USA (Kumble, V. ed.): International Crops Research Institute for the Semi- Arid Tropics, Patancheru 502324, Andhra Pradesh, India. Pp.115-129.

264. Taneja S. L., Seshu Reddy K. V. and Leuschner K. 1986. Monitoring of shoot fly population in sorghum. *Indian Journal of Plant Protection* 14: 29-36.

265. Tao Y. Z., Jordan D. R., Henzell R. G. and McIntyre C. L. 1998. Construction of an integrated sorghum genetic map in a sorghum RIL population by using probes from different sources and its alignment with other sorghum maps. *Australian Journal of Agricultural Research* 49: 729-736.

266. Tao Y., Manners J. M., Ludlow M. M. and Henzell R. G. 1993. DNA polymorphism in grain sorghum (*Sorghum bicolor* (L.) Moench). *Theoretical and Applied Genetics* 86: 679-688.

267. Taramino G. and Tingey S. 1996. Simple sequence repeats for germplasm analysis and mapping in maize. *Genome* 39: 277-287.

268. Taramino G., Tarchini R., Ferrario S., Lee M. and Pe M. E. 1997. Characterization and mapping of simple sequence repeats (SSRs) in *Sorghum bicolor*. *Theoretical and Applied Genetics* 95: 66-72.

269. Tarun Verma and Singh S. P. 2000. Morpho-physio plant characters associated with shoot fly resistance in sorghum. *Haryana Agricultural University Journal of Research* 30: 41-43.

270. Taylor J. N. and Schussler L. 1984. Sorghum cultivar verification by electrophoresis. *Journal of the Science of Food and Agriculture* 35: 1-8

271. Turlings T. C. J., Lengwiler U. B., Bernasconi M. L. and Wechsler D. 1998. Timing of induced volatile emissions in maize seedlings. *Planta* 207: 146-152.

272. Unnithan G. C. and Saxena K. N. 1990. Diversion of oviposition by *Atherigona soccata* (Diptera: Muscidae) to nonhost maize with sorghum seedling extract. *Environmental Entomology* 19: 1432-1437.

273. Uptmoor R., Wenzel W., Friedt W., Donaldson G., Ayisi K. and Ordon F. 2003. Comparative analysis on the genetic relatedness of *Sorghum bicolor* accessions from Southern Africa by RAPDs, AFLPs and SSRs. *Theoretical and Applied Genetics* 106: 1316-1325

274. Van Soest P. J and Robertson J. B. 1985. Analysis of Forage and Fibrous Foods–A Laboratory Manual for Animal Science 613. Cornell University, Ithaca, New York, USA.

275. Van wettstein D. and Chua N. H. 1987. Plant molecular biology, plenum press, New York.

276. Ventelon M., Deu M., Garsmeur O., Doligez A., Ghesquiere A., Lorieux M., Rami J. F., Glaszmann J. C. and Girvet L. 2001. Direct comparison between the genetic maps of sorghum and rice. *Theoretical and Applied Genetics* 102: 379-386.

277. Vidhyasekaran p. 1988. Physiology of disease resistance in plants, CRC press, Boca raton, FL.

278. Vierling R. A., Xiang Z., Joschi C. P., Gilbert M. L. and Nguyen H. T. 1994. Genetic diversity among elite sorghum lines revealed by restriction fragment length polymorphisms and random amplified polymorphic DNAs. *Theoretical and Applied Genetics* 87: 816-820

279. Vijayalakshmi K. 1993. Study of the interrelationship of important traits contributing to the resistance of shoot fly in *Sorghum bicolor* (L.) M.Sc. Thesis, Andhra Pradesh Agricultural University, Hyderabad, India.

280. Vos P., Hogers R., Bleeker M., Reijans M., Van De Lee T., Hornes M., Frijters A., Pot J., Kuiper M. and Zabeau M. 1995. AFLP: a new technique for DNA finger printing. *Nucleic Acids Research* 23: 4407-4414.

281. Waiss Jr. A. C., Chan B. G., Elliger C. A., Wiseman B. R., McMillian W. W., Widstrom N. W., Zuber M. S. and Keaster A. J. 1979. Maysin, a flavone glycoside from corn silks with antibiotic activity toward corn earworm. *Journal of Economic Entomology* 72: 256-258.

282. Waniska R. D., Poe J. H. and Bandyopadhyay. 1989. Effects of growth conditions on grain molding and phenols in sorghum caryopsis. *Journal of Cereal Science* 10: 217-225.

283. Watterson J. J. and Butler L. G. 1983. Occurrence of an unusual leucoanthocyanidin and absence of proanthocyanidins in sorghum leaves. *Journal of Agricultural and Food Chemistry* 31: 41-45.

284. Weising K., Bayermann B., Ramser J. and Kahl G. 1991. Plant DNA fingerprinting with radioactive and digoxigenated oligonucleotide probes complementary to simple repetitive DNA sequences. *Electrophoresis* 12: 159-169

285. Welsh J. and McClilland M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research* 8: 7213-7218.

286. Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A. and Tingey S.V. 1990. Oligonucleotide primers of arbitrary sequence amplify DNA polymorphisms which are useful as genetic markers. *Nucleic Acids Research* 18: 6531-6535.

287. Williams M. N. V., Pande N., Nair S., Mohan M. and Bennett J. 1991. Restriction fragment length polymorphism analysis of polymerase chain reaction products amplified from mapped loci of rice (*Oryza sativa* L.) genomic DNA. *Theoretical and Applied Genetics* 19: 506-536.

288. Woodhead S. and Bernays E. A. 1978. The chemical basis of resistance of *Sorghum bicolor* to attack by *Locusta migratoria*. *Entomologia Experimentalis et Applicata* 24: 123-144.

289. Woodhead S. and Cooper Driver. 1979. Phenolic acid and resistance to insect attack in *Sorghum bicolor*. *Biochemical Systematics and Ecology* 7: 301-310.

290. Woodhead S., padgham D. E. and Bernays E. A. 1980. Insect feeding on different sorghum cultivars in relation to cyanide and phenolic content. *Annals of Applied Biology* 95: 151-157.

291. Wu K. S. and Tanksley S. D. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. *Molecular Genetics and Genetics* 241: 225-235.

292. Wu X. and Prior R. L. 2005. Identification and characterization of anthocyanins by high-performance liquid chromatography electro sprayionization tandem massspectrometry in common foods in the United States : vegetables, nuts, and grains. *Journal of Agricultural and Food Chemistry* 53: 3101-3113.

293. Wyman A. R. and White R. 1980. A highly polymorphic locus in human DNA. *Proceedings of the National Academy of Sciences, USA* 77: 6754-6758.

294. Yang G. P., Saghai Maroof M. A., Xu C. G., Zhang Q. and Biyashev R. M. 1994 Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. *Molecular Genetics and Genetics* 245: 187-194.

295. Yang W., Oliviera A. C., de Godwin I., Schertz K. and Bennetzen J. L. 1996. Comparison of DNA marker technologies in characterizing plant genome diversity: variability in Chinese sorghums. *Crop Science* 36: 1669-1676.

296. Yin Jiao, Chen JuLian, Cao YaZhong, Li KeBin, Hu Yi and Sun JingRui. 2005. Wheat resistance induced by exogenous chemicals to the wheat aphid, *Sitobion avenae* (F.) and the oriental armyworm, *Mythimna separata* (Walker). *Acta Entomologica Sinica* 48: 718-724.

297. Zein el Abdin A. M. 1981. Review of sorghum shoot fly research in the Sudan. *Insect Science and its Application* 2: 55-58.

298. Zietkiewicz E., Rafalski A. and Labuda D. 1994. Genomic fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. *Genomics* 20: 176-183.

PUBLICATIONS

1. C Siva Kumar, H C Sharma, M Lakshmi Narasu and G Pampapathy. 2008. Mechanisms and diversity of resistance to shoot fly, *Atherigona soccata* in *Sorghum bicolor*. *Indian Journal of Plant Protection* (accepted, letter attached).

2. **Siva C Kumar,** Hari C Sharma, Lakshmi M Narasu and Mukesh K Dhillon. 2008. Physico-chemical mechanisms of resistance to shoot fly, *Atherigona soccata* (Rondani) (Diptera: Muscidae) in sorghum (*Sorghum bicolor* (L.) Moench). *Australian Journal of Entomology* (under review).

3. C. **Siva Kumar,** H. C. Sharma, M. Lakshmi Narasu and M. Peter Vijay. 2008. Qualitative and quantitative estimations of induced phenolic compounds from damaged and undamaged seedlings of sorghum by HPLC against shoot fly, *Atherigona soccata* resistance (under review).

4. C. Siva Kumar, H. C. Sharma and M. Lakshmi Narasu. 2008. Identification of leaf volatiles and germinated seed proteins from sorghum seedlings by GC-MS and HPLC against shoot fly, *Atherigona soccata* resistance (under review).

APPENDIX

Appendix 3.1 List of primers used for sorghum genetic diversity analysis.

S.no	Marker name	Label	Linkage group	Repet length	PCR protocol
1	gpsb067	F	SBI-08/H	(GT)10	M13
2	gpsb123	H	SBI-08/H	(CA)7+(GA)5	M13
3	mSbCIR223	H	SBI-02/B	(AC)6	M13
4	mSbCIR238	F	SBI-02/B	(AC)26	M13
5	mSbCIR240	H	SBI-08/H	(TG)9	M13
6	mSbCIR246	H	SBI-05/J	(CA)7.5	M13
7	mSbCIR248	H	SBI-10/G	(GT)7.5	M13
8	mSbCIR262	F	SBI-07/E	(CATG)3.25	M13
9	mSbCIR276	N	SBI-03/C	(AC)9	M13
10	mSbCIR283	N	SBI-07/E	(CT)8 (GT)8.5	M13
11	mSbCIR286	F	SBI-01/A	(AC)9	M13
12	mSbCIR300	F	SBI-05/J	(GT)9	M13
13	mSbCIR306	H	SBI-01/A	(GT)7	M13
14	mSbCIR329	H	SBI-10/G	(AC)8.5	M13
15	Sb4-72	N	SBI-09/F	(AG)16	M13
16	SbAGB02	N	SBI-05/J	(AG)35	M13
17	Xcup02	H	SBI-06/I	(GCA)6	M13
18	Xcup14	F	SBI-03/C	(AG)10	M13
19	Xcup53	H	SBI-01/A	(TTTA)5	M13
20	Xcup61	N	SBI-03/C	(CAG)7	M13
21	Xcup63	N	SBI-02/B	(GGATGC)4	M13
22	Xgap206	N	SBI-06/I	(AC)13/(AG)20	M13
23	Xgap84	H	SBI-02/B	(AG)14	M13
24	Xisep0310	H	SBI-02/B	(CCAAT)4	M13
25	Xtxp010	F	SBI-06/I	(CT)14	M13
26	Xtxp015	N	SBI-10/G	(TC)16	M13
27	Xtxp040	F	SBI-05/J	(GGA)7	M13
28	Xtxp057	H	SBI-09/F	(GT)21	M13
29	Xtxp114	F	SBI-03/C	(AGG)8	M13
30	Xtxp12	H	SBI-04/D	(CT)22	M13
31	Xtxp136	N	SBI-10/G	(GCA)5	M13
32	Xtxp141	N	SBI-07/E	(GA)23	M13
33	Xtxp145	H	SBI-09/F	(AG)22	M13
34	Xtxp21	F	SBI-04/D	(AG)18	M13
35	Xtxp265	F	SBI-09/F	(GAA)19	M13
36	Xtxp273	F	SBI-08/H	(TTG)20	M13
37	Xtxp278	H	SBI-05/J	(TTG)12	M13
38	Xtxp320	N	SBI-01/A	(AAG)20	M13
39	Xtxp321	F	SBI-08/H	(GT)4+(AT)6+(CT)21	M13
40	SbAGA01	F	SBI-10/G	(AG)33	5
41	Sb6-34=Xgap34	H	SBI-08/H	[(AC)/(CG)]15	5
42	gpsb148	N	SBI-07/E	CA	5
43	Xtxp6	F	SBI-06/I	(CT)33	4
44	Xisep0607	M13-H	SBI-10/G	AGA(4)	5
45	Xcup60	N	SBI-01/A	(CGGT)4	5
46	gpsb118	F	SBI-02/B	CA	5
47	Xisep0632	M13-H	SBI-08/H	CATG(4)	5
48	Xtxp354	N	SBI-08/H	(GA)21+(AAG)3	5

49	Xtxp88	F	SBI-01/A	(AG)31	4
50	Xisep0608	M13-H	SBI-04/D	AGA(4)	5
51	Xcup28	N	SBI-04/D	(TGAG)5	4
52	Xcup69	F	SBI-02/B	(ATGCG)4	5
53	Xisep0948	M13-H	SBI-04/D	TA(5)	5
54	Sb1-10=Xgap10	N	SBI-04/D	(AG)27	5
55	Xtxp262	F	SBI-O5/J	(GT)5	4
56	Xisep0228	M13-H	SBI-04/D	GAGG(3)	5
57	Xtxp47	N	SBI-08/H	(GT)8(GC)5+(GT)6	5
58	Xtxp75	F	SBI-O1/A	(TG)10	5
59	Xtxp287	H	SBI-09/F	(AAC)21	5
60	gpsb128	N	SBI-02/B	GT	5
61	gpsb089	F	SBI-O1/A	TG	5
62	Xisep1128	M13-H	SBI-09/F	AT(6)	5
63	Xtxp343	N	SBI-04/D	(AGT)21	5
64	Xcup52	F	SBI-07/E	(AATT)5	5
65	Xisep1014	M13-H	SBI-09/F	GT(5)	5
66	Xtxp304	N	SBI-02/B	(TCT)42	7
67	Xtxp23	F	SBI-05/J	(CT)19	7
68	Xisep1008	M13-H	SBI-09/F	CAG(7)	5
69	Xtxp339	N	SBI-09/F	(GGA)7	5
70	Xtxp210	F	SBI-08/H	(CT)10	7
71	Xisep0314	M13-H	SBI-10/G	GCC(4)	5
72	Xtxp31	N	SBI-03/C	(CT)25	4
73	Xisep0110	M13-H	SBI-09/F	CG(6)	5
74	Xtxp27	N	SBI-04/D	(AG)37	5
75	Sb1-1=Xgap01	F	SBI-10/G	(AG)16	5
76	Xtxp95	H	SBI-06/I	(GA)18(GC)4	5
77	Xtxp312	N	SBI-07/E	(CAA)26	5
78	Xtxp59	F	SBI-03/C	(GGA)5	4
79	SbAGE03	H	SBI-09/F	(AG)34GA(CA)4	5
80	Xtxp215	N	SBI-03/C	(CA)9	5
81	Xtxp20	F	SBI-10/G	(AG)21	5
82	Xtxp65	H	SBI-05/J	(ACC)4+(CCA)3CG(CT)8	5
83	gpsb017	N	SBI-05/J	CA	4
84	Xcup07	F	SBI-10/G	(CAA)8	7
85	Xcup62	H	SBI-01/A	(GAA)6	5
86	Xtxp327	N	SBI-04/D	(TAG)3+(GA)22	5
87	Xtxp340	F	SBI-01/A	(TAC)15	5
88	Sb6-342=Xgap342	H	SBI-07/E	(AC)25	4
89	gpsb027	N	SBI-10/G	CA	4
90	Xtxp289	F	SBI-09/F	(CTT)16+(AGG)6	5
91	Xisep0443	M13-H	SBI-06/I	GCA(7)	5
92	SbKAFGK1	F	SBI-05/J	(ACA)9	5
93	Xtxp295	H	SBI-07/E	(TC)19	7

Appendix 3.2 PCR protocols followed for assessing genetic diversity of sorghum.

Protocols	Primer (2pm/ul)	MgCl2 (25mM)	Buffer (10X)	*Taq* Enzyme (5U/ul)	dNTPS (2mM)	DNA (2.5ng/ul)	DDW	Total (µl)
4	0.5	0.3	0.5	0.03	0.50	0.5	2.7	5
5	0.5	0.4	0.5	0.02	0.25	1	2.3	5
7	1	0.4	0.5	0.02	0.38	0.5	2.2	5

Protocol with m13 primers									
M13 tail Forward (2pm/ul)	M13 Label (2pm/ul)	Reverse Primer (2pm/ul)	MgCl2 (25mM)	Buffer (10X)	*Taq* Enzyme (5U/ul)	dNTPS (2mM)	DNA (2.5ng/ ul)	DDW	Total (µl)
0.20	0.4	0.4	0.4	0.5	0.02	0.25	1	1.83	5