

**ASSESSMENT OF GENETIC DIVERSITY OF SHOOT FLY-
RESISTANT SOURCES AND TRANSFER QTLs ASSOCIATED
WITH SHOOT FLY RESISTANCE INTO FORAGE
SORGHUMS (*Sorghum bicolor* L.)**

Thesis

**Submitted to Punjab Agricultural University
In partial fulfilment of the requirements
for degree of**

**DOCTOR OF PHILOSOPHY
in
PLANT BREEDING AND GENETICS
(Minor Subject: Biotechnology)**

By

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CERTIFICATE I

This is to certify that the dissertation entitled, “**ASSESSMENT OF GENETIC DIVERSITY OF SHOOT FLY RESISTANT SOURCES AND TRANSFER QTLs ASSOCIATED WITH SHOOT FLY RESISTANCE INTO FORAGE SORGHUMS (*Sorghum bicolor* L.)**” submitted for the degree of **Doctor of Philosophy** in the subject of **Plant Breeding and Genetics** (Minor subject: **Biotechnology**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Naveen Arora (L-2013-A-36-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE II

This is to certify that the dissertation entitled “**ASSESSMENT OF GENETIC DIVERSITY OF SHOOT FLY RESISTANT SOURCES AND TRANSFER QTLs ASSOCIATED WITH SHOOT FLY RESISTANCE INTO FORAGE SORGHUMS (*Sorghum bicolor* L.)**” submitted by **Naveen Arora (L-2013-A-36-D)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Ph.D.** in the subject of **Plant Breeding and Genetics** (Minor subject: **Biotechnology**) has been approved by the Student’s Advisory Committee along with External Examiner after an oral examination on the same.

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ABSTRACT

The present study consisted of two different sets of genotypes evaluated for variable reaction to shoot fly under field conditions. The first set consisted of a diverse collection of 53 different germplasm lines and the second set consisted of 30 genotypes including 10 cytoplasmic male sterile lines, along with their respective maintainers and 10 restorer lines. On the basis of morphological evaluation under field conditions, the genotypes varied significantly for all the traits under study. ICSA/B 487, ICSA/B 14037, ICSA/B 467, ICSV 93046 and ICSV 700 exhibited high glossiness, pigmented leaf sheath, lower number of eggs and suffered lesser incidence of deadhearts and exhibited resistant reaction to shoot fly while ICSA/B 458, ICSV 25337 and ICSV 25292 exhibited moderate resistance to the shoot fly damage and ICSV- 25280, 25292, 25303, 25316, BTx 623, SSV 74 and Swarna were susceptible to shoot fly. In the germplasm lines significant variation was found for most of the traits except the leaf sheath pigmentation, number of plants with shoot fly eggs and number of shoot fly eggs per plant. Significant positive and negative correlations were found among the important traits for shoot fly resistance like glossy score, leaf sheath pigmentation, shoot fly deadhearts, total number of eggs, number of plants with eggs and trichome density. The inheritance studies based on Line X Tester analysis, revealed that shoot fly resistance is a complex trait and crosses involving resistant parents yielded resistant lines. The lines ICSA 458, ICSA 467, ICSA 487, ICSA 14024 and ICSA 14037 and testers ICSV 700, ICSV 93046, S-35, ICSV 25292 and ICSV 25337 had significant SCA and GCA effects for the traits important for shoot fly resistance. The estimation of biochemical components, revealed that the genotypes exhibiting moderate (ICSA 84, ICSB 52, ICSB 14024) and high (ICSA 467, and ICSB 487) resistance to shoot fly recorded lesser content of total soluble sugars, proteins, higher content of tannins and there was no significant association found for phenol content. From the HPLC analysis of methanol extracts of leaf samples a total of 25 different peaks with 12 known compounds were recorded. Diversity analysis based on GBS based SNP markers grouped the germplasm and parental lines into three major clusters consisting of resistant, moderately resistant and susceptible genotypes. Population structure was analysed using principle component analysis. The first three PC were sufficient enough to explain maximum diversity within population structure. For Genome Wide Association Studies, first three PCs were used as a covariate for which, significant association of SNPs were found for glossy score on chromosome 3, 9, 10 and for percent plants with shoot fly eggs on chromosome 2. For leaf sheath pigmentation, SNPs on all the chromosomes were significantly associated. Similarly, multiple SNPs were associated for percent tiller with shoot fly deadhearts on chromosomes 2,3,4,6,7 and 9. SNPs for plant vigour score were significantly associated with chromosomes 1,2,3 and 6.

Keywords: Sorghum, genetic diversity, shoot fly resistance, deadhearts, HPLC, GBS, SNPs.

Signature of Major Advisor

Signature of the student

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ਸਾਰਾਂਸ਼

ਮੌਜੂਦਾ ਅਧਿਐਨ ਵਿਚ ਦੋ ਵੱਖਰੇ ਜੈਨੇਟਿਕਸ ਸੈੱਟ ਦਾ ਫੀਲਡ ਦੀਆਂ ਹਾਲਤਾਂ ਵਿਚ ਸ਼ਾਖ ਦੀ ਮੱਖੀ ਲਈ ਵੱਖ-ਵੱਖ ਪ੍ਰਤੀਕ੍ਰਿਆ ਲਈ ਮੁਲਾਂਕਣ ਕੀਤਾ ਗਿਆ। ਪਹਿਲੇ ਸੈੱਟ ਵਿੱਚ ਇੱਕ ਵੱਖਰੇ ਸੰਗ੍ਰਿਹ ਦੀਆਂ 53 ਜਰਮਪਲਾਜਮ ਲਾਈਨਾਂ ਸ਼ਾਮਲ ਸਨ ਅਤੇ ਦੂਜੇ ਸੈੱਟ ਵਿੱਚ 30 ਜੈਨੇਟਿਕਸ ਜਿਨਾਂ ਵਿੱਚ 10 ਸਾਇਟੋਪਲਾਸਮਿਕ ਨਰ-ਨਿਰਲੇਪ ਲਾਈਨਸ, ਉਨ੍ਹਾਂ ਦੇ ਸੰਬੰਧਿਤ ਦੇਖਭਾਲਕਰਤਾ ਸਮੇਤ 10 ਰੀਸਟੋਰਰ ਲਾਈਨਾਂ ਸ਼ਾਮਲ ਸਨ। ਫੀਲਡ ਹਾਲਤਾਂ ਦੇ ਅਧੀਨ, ਰੂਪ-ਵਿਗਿਆਨਿਕ ਮੁਲਾਂਕਣ ਦੇ ਆਧਾਰ ਤਹਿਤ, ਜੈਨੋਟਾਈਪਸ, ਅਧਿਐਨ ਅਧੀਨ ਸਾਰੇ ਗੁਣਾਂ ਲਈ ਮਹੱਤਵਪੂਰਣ ਤੌਰ ਤੇ ਭਿੰਨ ਸਨ। ICSA/B 487, ICSA/B 14037, ICSA/B 467, ICSV 93046 'ਤੇ ICSV 700 ਨੇ ਉੱਚ ਗਲੇਸੀਪਨ, ਪੱਤਿਆਂ ਦੀ ਰੰਗਦਾਰ ਮਿਆਨ, ਅਡਿਆ ਦੀ ਘੱਟ ਗਿਣਤੀ, ਘੱਟ ਡੈਂਡਰਾਰਟਸ ਦੀ ਘਟਨਾ 'ਤੇ ਸ਼ਾਖ ਦੀ ਮੱਖੀ ਵਾਸਤੇ ਪ੍ਰਤੀਰੋਧਕ ਪ੍ਰਤੀਕ੍ਰਿਆ ਦਰਸਾਈ, ਜਦਕਿ ICSA/B 458, ICSV 25337 'ਤੇ ICSV 25292 ਨੇ ਦਰਮਿਆਨੀ ਪ੍ਰਤੀਰੋਧਕ ਪ੍ਰਤੀਕ੍ਰਿਆ ਅਤੇ ICSV- 25280, 25292, 25303, 25316, BTx 623, SSV 74 'ਤੇ Swarna ਨੇ ਸੰਵੇਦਨਸ਼ੀਲਤਾ ਪ੍ਰਦਰਸ਼ਿਤ ਕੀਤੀ। ਜਰਮਪਲਾਜਮ ਲਾਈਨਾਂ ਰੰਗਦਾਰ ਪੱਤਾ ਮਿਆਨ, ਸ਼ਾਖ ਦੀ ਮੱਖੀ ਦੇ ਅੰਡੇ ਵਾਲੇ ਪੌਦਿਆਂ ਦੀ ਗਿਣਤੀ 'ਤੇ ਪ੍ਰਤੀ ਪੌਦਾ ਸ਼ਾਖ ਦੀ ਮੱਖੀ ਦੇ ਅੰਡੇ ਦੀ ਗਿਣਤੀ ਨੂੰ ਛੱਡ ਕੇ, ਜ਼ਿਆਦਾਤਰ ਵਿਸ਼ੇਸ਼ਤਾਵਾਂ ਲਈ ਮਹੱਤਵਪੂਰਣ ਪਰਿਵਰਤਨ ਲੱਭਿਆ ਗਿਆ ਸੀ। ਸ਼ਾਖ ਦੀ ਮੱਖੀ ਪ੍ਰਤੀਰੋਧ ਲਈ ਮਹੱਤਵਪੂਰਣ ਗੁਣਾਂ ਵਿੱਚੋਂ, ਜਿਵੇਂ ਗਲੇਸੀ ਸਕੋਰ, ਪੱਤਿਆਂ ਦੀ ਰੰਗਦਾਰ ਮਿਆਨ, ਡੈਂਡਰਾਰਟਸ, ਅੰਡੇ ਦੀ ਕੁੱਲ ਗਿਣਤੀ, ਅੰਡੇ ਵਾਲੇ ਪੌਦਿਆਂ ਦੀ ਗਿਣਤੀ 'ਤੇ ਟ੍ਰਾਈਕੋਮ ਘਣਤਾ ਵਿੱਚ ਮਹੱਤਵਪੂਰਨ ਸਕਾਰਾਤਮਕ ਅਤੇ ਨਕਾਰਾਤਮਕ ਸੰਬੰਧ ਮਿਲੇ। ਲਾਈਨ ਅਤੇ ਟੈਸਟਰ 'ਤੇ ਆਧਾਰਿਤ ਵਿਰਾਸਤ ਅਧਿਐਨ ਨੇ ਪ੍ਰਗਟ ਕੀਤਾ ਹੈ ਕਿ ਸ਼ਾਖ ਦੀ ਮੱਖੀ ਪ੍ਰਤੀਰੋਧ ਇੱਕ ਜਟਿਲ ਵਿਸ਼ੇਸ਼ਤਾ ਹੈ ਅਤੇ ਰੇਧਕ ਲਾਈਨਾਂ ਨੂੰ ਸ਼ਾਮਲ ਕਰਕੇ ਰੇਧਕ ਮਾਪੇ ਪੈਦਾ ਕੀਤੇ ਜਾ ਸਕਦੇ ਹਨ। ਲਾਈਨਸ, ICSA 458, ICSA 467, ICSA 487, ICSA 14024 'ਤੇ ICSA 14037 ਅਤੇ ਟੈਸਟਰਸ, ICSV 700, ICSV 93046, S-35, ICSV 25292 'ਤੇ ICSV 25337 ਵਿੱਚ ਸ਼ਾਖ ਦੀ ਮੱਖੀ ਪ੍ਰਤੀਰੋਧ ਲਈ ਮਹੱਤਵਪੂਰਣ ਗੁਣਾਂ ਵਾਸਤੇ ਮਹੱਤਵਪੂਰਨ SCA ਅਤੇ GCA ਪ੍ਰਭਾਵ ਪਾਏ ਗਏ ਸਨ। ਬਾਇਓ-ਕੈਮੀਕਲ ਭਾਗਾਂ ਦਾ ਅੰਦਾਜ਼ਾ ਲਗਾਇਆ ਗਿਆ ਅਤੇ ਇਹ ਖੁਲਾਸਾ ਕੀਤਾ ਗਿਆ ਕਿ ਸ਼ਾਖ ਦੀ ਮੱਖੀ ਨੂੰ ਮੱਧਮ (ICSA 84, ICSB 52, ICSB 14024) ਅਤੇ ਉੱਚ ਪ੍ਰਤੀਰੋਧ (ICSA 467, 'ਤੇ ICSB 487) ਦਿਖਾਉਣ ਵਾਲੇ ਜੈਨੋਟਾਈਪਸ ਵਿੱਚ ਘੱਟ, ਕੁਲ-ਘੁਲਕੀ ਸ਼ੱਕਰਾਂ 'ਤੇ ਪ੍ਰੋਟੀਨ ਦੀ ਸਮੱਗਰੀ ਅਤੇ ਟੈਨਿਨਸ ਦੀ ਉੱਚ ਸਮੱਗਰੀ ਨੂੰ ਰਿਕਾਰਡ ਕੀਤਾ, ਜਦਕਿ ਫਿਨੇਲ ਸਮੱਗਰੀ ਲਈ ਕੋਈ ਮਹੱਤਵਪੂਰਨ ਐਸੋਸੀਏਸ਼ਨ ਨਹੀਂ ਲੱਭੀ ਸੀ। ਪੱਤਿਆਂ ਦੇ ਮੈਥਾਨੋਲ ਐਕਸਟਰੈਕਟਸ ਦਾ ਐਚ.ਪੀ.ਐਲ.ਸੀ. ਵਿਸ਼ਲੇਸ਼ਣ ਕੀਤਾ ਗਿਆ ਜਿਸ ਵਿੱਚ ਕੁੱਲ 25 ਵੱਖੋ-ਵੱਖਰੇ ਪੀਕ ਮਿਲੇ ਜਿਸ ਵਿੱਚ 12 ਜਾਣੂ ਮਿਸ਼ਰਣ ਰਿਕਾਰਡ ਕੀਤੇ ਗਏ। ਜੀ.ਬੀ.ਐਸ. ਅਧਾਰਿਤ ਐਸ.ਐਨ.ਪੀ. ਮਾਰਕਰਾਂ ਦੇ ਅਧਾਰ ਤੇ ਡਾਇਵਰਸਿਟੀ ਦੇ ਵਿਸ਼ਲੇਸ਼ਣ ਨੇ ਜਰਮਪਲਾਜਮ ਅਤੇ ਮਾਪਿਆਂ ਦੀਆਂ ਲਾਈਨਾਂ ਨੂੰ ਤਿੰਨ ਮੁੱਖ ਕਲਸਟਰਾਂ ਵਿੱਚ ਵੰਡਿਆ ਜਿਸ ਵਿੱਚ ਰੇਧਕ, ਐਸਤ ਪ੍ਰਤੀਰੋਧੀ ਅਤੇ ਸੰਵੇਦਨਸ਼ੀਲ ਜੀਨੋਟਾਈਪਸ ਸ਼ਾਮਲ ਸਨ। ਆਬਾਦੀ ਦੇ ਢਾਂਚੇ ਦਾ ਸੈਧਾਂਤਿਕ ਕੰਪੋਨੈਂਟ ਭਾਗ ਵਿਸ਼ਲੇਸ਼ਣ ਕੀਤਾ ਗਿਆ ਸੀ ਜਿਸ ਵਿੱਚ ਇਹ ਲੱਭਿਆ ਗਿਆ ਕਿ ਪਹਿਲੇ ਤਿੰਨ ਪੀ.ਸੀ. ਜਨਸੰਖਿਆ ਦੇ ਢਾਂਚੇ ਵਿਚ ਵੱਧ ਤੋਂ ਵੱਧ ਭਿੰਨਤਾ ਨੂੰ ਵਿਆਖਿਆ ਕਰਨ ਲਈ ਕਾਫ਼ੀ ਸਨ। ਜੀਨੋਮ ਵਾਈਡ ਐਸੋਸੀਏਸ਼ਨ ਸਟੱਡੀਜ਼ ਲਈ, ਪਹਿਲੇ ਤਿੰਨ ਪੀ.ਸੀ. ਨੂੰ ਇੱਕ ਕੋਵਰੇਟ ਦੇ ਰੂਪ ਵਿੱਚ ਵਰਤਿਆ ਗਿਆ ਸੀ ਜਿਸ ਲਈ, ਕ੍ਰੋਮੋਸੋਮ 3, 9, 10 ਤੇ ਗਲੇਸੀ ਸਕੋਰ ਲਈ ਐਸ. ਐਨ. ਪੀ. ਅਤੇ ਕ੍ਰੋਮੋਸੋਮ 2 ਤੇ ਪ੍ਰਤੀਸ਼ਤ ਸ਼ਾਖ ਦੀ ਮੱਖੀ ਦੇ ਅੰਡੇ ਵਾਲੇ ਪੌਦਿਆਂ ਦੀ ਗਿਣਤੀ ਵਾਸਤੇ ਮਹੱਤਵਪੂਰਨ ਐਸੋਸੀਏਸ਼ਨ ਮਿਲਿਆ। ਪੱਤਿਆਂ ਦੀ ਰੰਗਦਾਰ ਮਿਆਨ ਲਈ, ਸਾਰੇ ਕ੍ਰੋਮੋਸੋਮਸ ਤੇ ਐਸ. ਐਨ. ਪੀ. ਮਹੱਤਵਪੂਰਨ ਤੌਰ ਤੇ ਜੁੜੇ ਹੋਏ ਸਨ। ਇਸੇ ਤਰ੍ਹਾਂ, ਪ੍ਰਤੀਸ਼ਤ ਟਿਲਰ ਵਾਸਤੇ ਮਲਟੀਪਲ ਐਸ. ਐਨ. ਪੀ. ਕ੍ਰੋਮੋਸੋਮਸ 2,3,4,6,7 ਤੇ 9 'ਤੇ ਜੁੜੇ ਹੋਏ ਸ। ਪੌਦਾ ਜੇਸ ਸਕੋਰ ਲਈ ਐਸ. ਐਨ. ਪੀ. ਕ੍ਰੋਮੋਸੋਮ 1,2,3 ਅਤੇ 6 ਦੇ ਨਾਲ ਮਹੱਤਵਪੂਰਣ ਢੰਗ ਨਾਲ ਜੁੜੇ ਹੋਏ ਸਨ।

ਸ਼ਬਦ: ਜੈਵਾਰ, ਜੈਨੇਟਿਕ ਵਿਭਿੰਨਤਾ, ਤਣਾ ਮੱਖੀ ਰੇਧਕ ਸਮਰੱਥਾ, ਡੈਂਡਰਾਰਟਸ, ਐਚ. ਪੀ. ਐਲ. ਸੀ., ਜੀ. ਬੀ. ਐਸ., ਐਸ. ਐਨ. ਪੀ.ਜ਼.

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CHAPTER – I

INTRODUCTION

Sorghum (*Sorghum bicolor* L.) is annual diploid, C_4 plant and an often-cross-pollinated crop species ($2n=20$) belonging to family, *Poaceae* and subfamily, *Panicoideae*. It is the fifth most important grain crop after wheat, rice, maize and barley. The name “sorghum” is derived from Italian term “sorgo”, which evolved from the Latin word “Syricum” (granum), meaning grain of Syria. The cultivated sorghum originated from a wild progenitor belonging to the subspecies *verticilliflorum* (Vavilov 1951).

As a versatile cereal crop, sorghum is widely grown for food, feed, fodder, forage and fuel in semi-arid tropics in Asia, Africa, the Americas and Australia. It is grown on an area of about 35.4 m ha in over 100 countries (FAO 2013). Based on utility traits like productivity (grain/ stover), relative height and sugar content in stalk (non-sweet/ sweet sorghums), sorghums are classified into grain sorghum, dual purpose (grain and fodder) sorghum, fodder sorghum, forage sorghum, and sweet stalk sorghum. The breeding strategy focuses on distinct traits and thus varies respectively for different sorghum types (Reddy *et al* 2012).

In India, it is grown in both rainy (June–October) and post-rainy (November–March) seasons. The post-rainy season crop is mainly grown for food grain (Reddy *et al.* 2009), while the rainy season crop serves the fodder crop. Recently, sorghum has gained greater significance due to the increasing demand for its use as poultry feed and for biofuel production, as sweet sorghums can be used for bioethanol production (Rao *et al.* 2009).

Forage sorghums are principally cultivated in Punjab, Haryana, Delhi, western and central Uttar Pradesh, and the adjoining areas of Rajasthan and Madhya Pradesh. In India it was grown on 5.65 mha area during 2016 (FAO 2016). Sorghum is the main fodder crop in Punjab during *Kharif* and was cultivated on 895 thousand hectares with a production of 803 lakh tonne and productivity of 719 quintal per hectare during 2016-17 (Anonymous, 2017). In general, forage sorghum is raised from April to August, supplying nutritious fodder to animals from June to November.

In the mixed crop-livestock systems, sorghum serves a good source of stover for the dairy and draught animals. Due to its quick growth, high yield and quality of biomass, sorghum is a good source of green fodder. Sorghum performs well in areas where maize and Napier grass have low yield potential. Having the same energy levels as maize and other cereals, it can replace maize for making silage, while freshly chopped crop is used as fodder for cows, goats, sheep, pigs and even chickens. Being coarse and fast growing in the warm season, fodder sorghum provides livestock feed in mid-summer (Juerg *et al.* 2009).

Despite of the high yield potential (up to 10 tons ha⁻¹), this could not be realized and the average sorghum grain yields on farmers' field in India hardly exceeds 500–800 kg ha⁻¹ (Sharma 1985). Sorghum production is severely constrained by several biotic and abiotic stresses. Sorghum, particularly rainy season crop is prone to various biotic constraints including shoot fly, stem borer, midge, grain mold, striga (primarily in Africa) (Sharma 1985; Thakur *et al.* 2006). Insect pests causes economic losses valued over US\$1 billion annually in the semi-arid tropics (Sharma *et al.* 2005). It is damaged by over 150 insect species, of which sorghum shoot fly, *Atherigona soccata* (Rondani) is a major pest in Asia, Africa and the Mediterranean Europe (Sharma, 1993).

Sorghum shoot fly, *A. soccata* damages the plant at the seedling stage (5 to 30 days after seedling emergence) producing typical deadheart symptoms, which is formed as a result of drying up of the central leaf when maggot cuts the growing point. The complete life cycle is completed in 15 to 18 days under favourable temperature (20-30°C) and relative humidity (>60%), (Srivastava, 1985). Sorghum shoot fly causes substantial damage in the late-sown crops due to the build-up of insect population on the early-sown crop, affecting the grain and fodder yields. In India, the losses in terms of yield, due to shoot fly may reach on an average to 90% and 45% in grain and forage sorghum respectively. (Sukhani and Jotwani, 1980; Jotwani, 1978). Minimization of the losses can be achieved by following appropriate cultural practices including appropriate planting time and chemical control of the insect pest (Sharma 1985), but their efficacy is dependent on the economic, edaphic and climatic factors such as costly chemicals beyond the reach of resource poor farmers and highly erratic rainfall in semi-arid tropics (SAT) shortening the sowing window (Sharma 1993).

The most economic and effective measure to reduce the extent of losses due to shoot fly damage is the use of cultural practices and host plant resistance (HPR) keeping the infestation below economic threshold levels. HPR being a highly complex trait is the final outcome of interaction among the multiple component traits imparting resistance to the genotype (Dhillon 2004). In case of sorghum shoot fly, a combination of traits imparts HPR including morphological, genetic and biochemical factors. Glossiness, trichome density on adaxial surface, leaf sheath pigmentation has been found to be associated with resistance to shoot fly incidence. (Kiranmayee *et al.*, 2016)

So, it becomes necessary to understand the gene action both in terms of nature and magnitude for gaining precise knowledge about the genetic potential of the breeding lines and utilizing the efficient breeding approach to breed resistant lines. The simplest way to increase productivity for forage and grain crop is the exploitation of heterosis breeding. This requires

focussed studies on combining ability and development of male sterile lines along with good combiners for the traits of interest (Prabhakar and Raut, 2010).

As the cultivated germplasm depicts low to moderate level of resistance to this insect pest, there is a need to widening the genetic base of the cultivated lines. Several genotypes with resistance to shoot fly have been identified (Jotwani 1978; Taneja & Leuschner 1985; Sharma *et al.* 2003). To broaden the genetic base for shoot fly resistance, there is a need for gaining and understanding different mechanisms of resistance to shoot fly in a wide array of shoot fly resistant/susceptible genotypes. Though sorghum germplasm is rich in variability for shoot fly resistance, the sources of resistance have several undesirable traits such as low grain and fodder yield, and poor nutritional quality and cross incompatibility, which limit their use in a breeding program. Also, the traits conferring resistance to shoot fly are complex and are controlled by several genes with a high GxE interaction (Riyazzuddin *et al.* 2015 a, b). Therefore, the identification of the genomic regions containing quantitative trait loci (QTLs) for resistance to shoot fly, and the linked markers will be useful in sorghum improvement through marker-assisted selection (MAS). Simultaneous identification of QTLs and phenotyping can be used to improve the efficiency of introgressing shoot fly resistant genes into the locally adapted cultivars.

Considering the importance of shoot fly resistance in sorghum, the present study entitled “Assessment of genetic diversity of shoot fly-resistant sources and transfer QTLs associated with shoot fly resistance into forage sorghums (*Sorghum bicolor* L.)” was undertaken to address the following objectives:

1. To assess morphological, biochemical, and genetic diversity among the shoot fly-resistant forage/sweet sorghums.
2. To study the inheritance of shoot fly resistance in forage/sweet sorghums.
3. To study the inheritance of QTLs linked to shoot fly resistance and component traits in F₁ hybrids of shoot fly resistant sweet sorghums and locally adapted cultivars.

CHAPTER-II

REVIEW OF LITERATURE

Over 150 insect species have been recorded as pests on sorghum, of which sorghum shoot fly, *Atherigona soccata* (Rond.), is one of the major pests affecting the grain and fodder yield (Sharma 1993). Annual losses due to insect pests in sorghum have been estimated to be over \$1089 million in the SAT (Sharma *et al* 2005). Shoot fly is a major problem in late-sown crops in regions or years with erratic rainfall. In forage sorghums, it causes on an average of 30-40% losses in forage yield, as most of the forage cultivars released till date are highly susceptible to this pest.

Cultural practices such as early and uniform sowing, biological control, insect-resistant cultivars, and need based application of insecticides can be used for pest management in sorghum. However, it is difficult to avoid insect damage in rain fed areas due to erratic rainfall and short sowing window. The insecticides are costly and beyond the reach of resource-poor farmers in the semi-arid tropics (Sharma 1993). Chemical control is quite effective, but the insecticide application is either not undertaken by the farmers or are applied after the insect has already damaged the crop. Host plant resistance could be efficiently utilized for minimizing the pest associated losses and forms an important component of insect pest management. Development of forage cultivars with shoot fly resistance is one of the most effective, economic and environmental friendly means of reducing the losses due to this pest. Moreover insect-resistant cultivars are compatible with other methods of pest control (Sharma *et al* 1997).

Aruna *et al* (2011) has stated that to tackle the increasing demand for the grain and fodder, sustained improvement using genetic manipulation imparting resistance against shoot fly is essential. They have suggested that resistance can be an effective mechanism in the integrated pest management approach and it would decrease the consumption of costly and environment harming chemical insecticides and providing eco-friendly, healthy and economic approach.

Among the present cultivated sorghum accessions, not even a single accession has been reported that confers absolute resistance against shoot fly and the level of available resistance level varies across cultivars. Moreover, being a quantitative trait, resistance to shoot fly is difficult to manipulate at genetic levels and poses hindrance due to complex insect-host-environment interaction. Resistance to insects results from the numerous heritable plant characters that enable plant to survive with minimum losses as compared to the susceptible plants lacking these characters (Sharma *et al* 1997). The review on the “Assessment of genetic diversity of shoot fly-resistant sources and transfer QTLs associated

with shoot fly resistance into forage sorghums (*Sorghum bicolor* L.)” is studied under the following subheadings:

2.1 Shoot fly resistance

Shoot fly resistance is a complex trait resulting from the interaction of number of interdependent characters providing resistance to sorghum plants (Dhillon 2004).

For a new variety or a cultivar to be released officially, the national program has to set a threshold resistance level due to high susceptibility of all the high yielding sorghum cultivars to the shoot fly. Several factors like narrow genetic variability, monocropping and ratooning practices has contributed significantly in imparting the sorghum shoot fly, a status of principal pest in India (Singh and Rana 1986). Though there is availability of the resistance sources in the wild relatives and other identified sources, yet only small success has been attained in improving resistance using conventional breeding. This has been attributed to the quantitative inheritance and crossing incompatibility among cultivated and wild sorghum genotypes by Satish *et al* (2009).

Tao *et al* (2003) have also attributed this slow pace and the labor-intensive work posing the insufficient acquired level of resistance, to the quantitative inheritance with strong environmental correlation, of resistance to various insect pest. Aruna and Padmaja (2009) has demonstrated with the help of classic genetic analysis using phenotypic data, that inheritance of sorghum shoot fly resistance is complex, quantitatively inherited along with strong influence of environment.

For evaluation of germplasm material and breeding lines against insect resistance, techniques like infester row, artificial infestation and various cage techniques has been standardize at ICRISAT. The interlard-fishmeal technique has been used to develop shoot fly resistant seed parents (Sharma *et al* 1992).

2.2 Diversity studies

The diversity information in the resistant sources and genetic relationships in the breeding material is of great importance for making proper strategies leading to crop improvement in terms of resistance to shoot fly.

Ganesamurthy *et al* (2010) conducted diversity analysis based on D^2 statistics which grouped 63 genotypes into 14 clusters. They suggested that using genotypes from distant clusters in the hybridization program could yield heterotic performance in F_1 and better spectrum of variability in filial generations. The diversity analysis conducted by Ganapathy *et al* (2012) among 82 rainy and post rainy sorghum genotypes from India based on a set of 35 SSR markers distributed across all linkage groups revealed a total of 198 alleles with an

average of 5.71 alleles per primer pair. PIC values for eighteen SSR primers were recorded to be more than 0.5 suggesting the potential of these markers.

Ramu *et al* (2013) used 40 EST-SSR markers to screen a reference set of sorghum genotypes for analyzing the genetic diversity. Out of the 360 total alleles detected, 9 alleles per marker were represented with polymorphic information content (PIC) value ranging between 0.1379-0.9483. Different clusters were formed based on the races and subraces, among which guinea race genotypes fell into two clusters representing Indian and west African guineas. Similarly, separate clusters were formed consisting the accessions of *Kafir* and *Guinea* margaritifera (Gma) races. They found very large genetic variance among the different accessions of this reference set and suggested wider utility of the reference set for various genotyping studies like allele mining and genome wide association mapping.

Chamarthi *et al* (2012a) assessed the genetic diversity of 15 sorghum genotypes varying for shoot fly resistance/susceptibility based on 93 sorghum simple sequence repeat (SSR) primer pairs. These markers were characterized for 15 morpho-biochemical traits associated with shoot fly resistance using the Principal Coordinate Analyses (PCoA) and cluster analyses based on dissimilarity matrices derived from allelic variation of SSR and the selected traits (based on Gower's distance). They reported the susceptible genotypes falling in a single cluster and proposed three resistant accessions viz., IS 1054, IS 1057 and IS 4664 diverging from the commonly used resistant donor viz. IS 18551 which can be used in sorghum improvement programs.

2.3 Inheritance of shoot fly resistance

In terms of classical genetics, resistance could be oligogenic, polygenic, or cytoplasmic and the resistance to sorghum shoot fly has been found to be inherited quantitatively with both additive and non-additive gene actions (Borikar and Chopde 1981b, Nimbalkar and Bapat 1992). Borikar and Chopde (1982) in a study reported significant additive (D) genetic component for dead heart (%) and number of eggs per plant along with the recessive nature of susceptibility to shoot fly under low insect population and dominant under high insect population along with the increase in magnitude of additive component as the shoot fly population increase. They also reported 15 to 25% resistance to be heritable under high to moderate infestation levels of shoot fly. Several workers have also reported the quantitative nature of inheritance (Agrawal and Abraham 1985) and the involvement of additive and non-additive gene actions for shoot fly resistance (Borikar and Chopde 1981b, Nimbalkar and Bapat 1992). There are reports suggesting additive gene action being more important than non-additive gene actions (Rao *et al* 1974 and Sharma *et al* 1977). In accordance with these reports studies of Balakotaiah *et al* (1975) also suggested the

predominant additive inheritance based on the normal frequency distribution of mortality rates. Sharma *et al* 1977 have reported a high value of broad-sense heritability (52-82%) for resistance to shoot fly.

Rana *et al* (1980) reported intermediate F_1 with partially dominant resistance under low to moderate infestation of shoot fly, and suggested the multi-genic control having additive gene effects. Tarumoto (1980) has reported the monogenic recessive control of leaf glossiness and its simple inheritance has been reported by Agrawal and House (1982). Similar results were proposed by Hallali *et al* (1982) and Maiti *et al* (1984) for leaf glossiness and trichome density representing independent inheritance and additive gene effect predominance for these two traits in imparting resistance to shoot fly. Dhillon *et al* (2006) have reported high value of the narrow sense heritability (60.5%) for leaf glossiness and a lower value (1.0%) for trichome density.

On the contrary control of resistance against shoot fly was reported to be controlled predominantly by non-additive gene action was suggested by Agrawal and Abraham (1985). Rao *et al* (1974) and Borikar and Chopde (1980) reported that shoot fly resistance exhibits partial dominance. Sharma *et al* (1977) and Borikar and Chopde (1980) suggested that resistance is outcome of accumulation of genes gradually, attributable to different generations exhibiting continuous variation. Several workers have reported the inheritance of various traits imparting resistance to shoot fly among which glossiness was reported to be having high heritability and controlled by a single gene (Tarumoto 1980) and exhibiting simple inheritance by Aggarwal and House (1982).

Therefore, it could be used as simple and reliable selection criteria for resistance Maiti and Gibson (1983) along with Omori *et al* (1983) suggested the use of simple selection criteria for resistance owing to the simple inheritance and single gene control with high heritability.

On the contrary Agrawal and Abraham (1985) reported the glossiness intensity was being governed by many genes having additive and non- additive effects. Inheritance of glossiness was studies by Tarumoto (1983) are also in agreement with these results which showed that glossiness is a polygenic controlled trait.

The inheritance of another important factor viz. trichome density that imparts reduced susceptibility to shoot fly has been reported to be recessive and monogenic by Gibson and Maiti (1983) who also proposed along with this that trichome density is a major but not the sole factor imparting resistance. Heritability for trichome density was observed to be 75% (Gibson and Maiti 1983) and it was proposed to be having both additive and non-additive gene effects by Halalli *et al* (1982).

Seedling height is also considered to be an important factor and affects the relative growth rate. The dwarf exotic sorghum lines have been reported to be susceptible to shoot fly attack and the late sown tall Indian genotypes have been reported to be resistant (Rao *et al* 1974). It has been considered to be an important trait for resistance to shoot fly and the additive component is determining the inheritance of seedling height (Sharma *et al* 1977, Borikar and Chopde 1982). Halalli *et al* (1983) has suggested moderate heritability for this trait. On the contrary to these studies some workers have reported non-additive gene action for this trait (Sharma *et al* 1977, Borikar and Chopde, 1981b).

Both additive and non-additive components of heritable variation have been reported for other traits like dead heart (%) were recorded for the (Borikar and Chopde 1981b, Halalli *et al* 1982; Biradar and Borikar 1985, Elbadavi *et al* 1997), ovipositional non- preference (Borikar and Chopde 1981b, Halalli *et al* 1982, Dabholkaret *al*1989) and for recovery resistance (Biradar *et al* 1986).

Though both the additive and non-additive gene effects have been proposed to be important for inheritance of shoot fly resistance in sorghum, additive gene effects are suggested to be more important by Faujdar Singh (1997). Tao *et al* (2003) have described the resistance to various insect's pest having quantitative inheritance with strong environmental correlation. The general (GCA) and specific combining ability (SCA) estimates suggested that inheritance for oviposition non-preference, dead hearts, recovery resistance, and the morphological traits associated with resistance or susceptibility to *A. soccata* were governed by additive-type of gene action. Dhillon *et al* (2006) reported based on the inheritance studies and regression analysis that expression of leaf glossiness, trichomes, plants with eggs, and dead hearts in F₁ hybrids was governed by additive-type of gene action, and these can be used as morphological markers to select for resistance to sorghum shoot fly.

Aruna and Padmaja (2009) also demonstrated with the help of classic genetic analysis using phenotypic data, that inheritance of sorghum shoot fly resistance is complex, quantitatively inherited along with strong influence of environment. Resistance to shoot fly has been described to be constitutive and inducible with quantitative inheritance having major role of additive gene effects by Chamarthi *et al* (2012b).

2.4 Transfer of shoot fly resistance in locally adapted cultivars

The use of cytoplasmic-nuclear male-sterility (CMS) in *Sorghum bicolor* is credited to Stephens and Holland (1954) from crosses involving 'Day milo' with 'Kafir', and 'Milo' with 'Black hull kafir'. After which, a large number of hybrids have been developed using this cytoplasm (called A1 cytoplasm) and deployed on a magnificent scale worldwide. But several authors have reported cytoplasmic male-sterility as a potential danger to sustainability

of crop production (Sharma *et al* 2004, Dhillon *et al* 2005a). Sharma *et al* (1996, 2004) and Sharma (2001) have suggested the association of the CMS in sorghum with a hike in the susceptibility to sorghum midge, *Stenodiplosis sorghicola*, shoot fly, *Atherigona soccata* (Rondani) and sugarcane aphid, *Melanaphis sacchari* (Zehntner). Reddy *et al* (1997) have described some high yielding restorer lines identified at ICRISAT such as ICSRs 30, 103, 112, 160, 91026 and 93005 along with maintainers with high yield and shoot fly resistance such as ICSs 8, 9, 11, 30, 44 and 2968.

At present hybrids have occupied most of the area under cultivation so it becomes essential to identify and transfer the resistance genes against shoot fly into the CMS lines male sterile and restorer lines for developing resistant hybrids, but due to the lack of sufficient information on resistant and susceptible sterile, maintainer and restorer lines there is need of an understanding of the genetic makeup of the shoot fly resistant sources for designing efficient breeding programs necessary for rapid crop improvement (Sharma *et al* 1996, 2004; Sharma, 2006; Dhillon 2004).

Dhillon *et al* (2006) have suggested breeding for resistant male sterile and restorer lines based on their reports which suggested the relatively more resistance in the hybrids originating from such lines as compared to hybrid progenies of normal lines).

ICRISAT and partners over the years have undertaken extensive screening of the sorghum germplasm collections for resistance to key sorghum pests such as sorghum shoot fly, spotted stem borer, sorghum midge and head bugs and resistant lines for shoot fly and other pests have been identified. The donor parents have been chosen from the extensively screened material from germplasm material for resistance to shoot fly and many maintainer and restorer lines have developed at ICRISAT and partner locations (Sharma *et al* 2008). Using resistant sources having desirable agronomic background (ICSV 702, ICSH 705, ICSV 708, PS 21318, PS 307151 and PS 35805), as well as other sources (IS 18551) Reddy *et al* (2005) followed a trait-based pedigree breeding approach and developed a large number of shoot fly resistant seed parents for both the rainy season (ICSA/B-409 to ICSA/B-436) and post rainy season (ICSA/B-437 to ICSA/B-463). More recently, new sources of resistance (IS 923, IS 1057, IS 1071, IS 1082, IS 1096, IS 2394, IS 4663, IS 5072, IS 18369, IS 4664, IS 5470 and IS 5636) have been used in the development of shoot fly resistant hybrid parents (Reddy *et al* 2010).

2.5 Combining ability studies

To cope up with the increasing demand of fodder, it is necessary to improve the fodder sorghum yield with nutritionally superior qualities in order to obtain better animal performance. Heterosis can be exploited for the improvement of sorghum productivity. For

this the combining ability studies might be useful in selection of parents for hybrid production (Prakash *et al* 2010). The estimates of combining ability along with the magnitude and nature of heterosis for fodder yield along with certain quality traits of forage sorghum are very important for improvement of the hybrids. For developing high yielding varieties/hybrids through hybridization, the choice of the right type of parents is of paramount importance, hence the importance of testing parents for their combining ability. Further, for the system of breeding to be employed, the knowledge of gene action is of immense value which varies depending on the genetic variation as well as genetic divergence in the material. The information on these aspects is limited in forage sorghum (Akabari and Parmar, 2014).

Knowing general (GCA) and specific (SCA) combining ability effects of genetic materials is of practical value in breeding programs. Both components play an important role in selecting superior parents for hybrid combinations (Duvick 1999) has stated that GCA and SCA are of extreme importance for selection of superior parents, and according to Baker (1978) to measure the nature of gene action involved in quantitative traits, it represents a powerful method.

The combining ability analysis helps in understanding the various types of gene actions involved in inheritance of the quantitative traits. (Mohammed 2007, Mohammed and Talib 2008). Various types of mating designs are used for estimating the combining ability and Line x tester mating design is one of most commonly used for generation of material for estimation of combining ability effects which helps in getting the basic information about the genetic potential of parents to be used in hybrid seed production (Prakash *et al* 2010). GCA effects represent the fixable component of genetic variance and are important to develop superior genotypes. SCA represents the non-fixable component of genetic variation, it is important to provide information on hybrid performance (Mohammed 2009).

Studies conducted by Ross *et al* (1979) on plant height, days to bloom, forage yield, percent dry matter (DM), percent protein, and in vitro dry matter disappearance (IVDMD) revealed significant differences among hybrids averaged over females or males in different experiments, and positive correlation of forage yield with tall height and late maturity were found along with negative correlation with dry matter, protein and IVDMD.

Singhanian (1980) stated that for the grain yield SCA is more important while for other traits like plant height, days to bloom, panicle length, number of grains per panicle and hundred grain weight; the GCA is more important. Increase in number of seeds per panicle in taller and early maturing hybrids lead to yield superiority.

Mohammed *et al* (2008) has described about the little efforts made for the development of improved forage cultivars and the first fully devoted forage improvement

program in the country initiated in start of this century which was focused on development of locally adapted sorghum hybrids for fodder purpose.

Kenga *et al* (2004) studied combining ability for different traits like grain yield, days to anthesis, plant height, inflorescence length, threshing percentage, and seed mass using the line–tester method of analysis and found that general combining ability (GCA) and specific combining ability (SCA) effects were highly significant for all the traits except for the inflorescence length for which SCA was found to be non-significant. Studying the ratio of general combining ability to specific combining ability they have stated non-additive gene action being predominant for most of the traits.

Makanda *et al* (2010) have also reported were positive and significant correlation of grain yield with its component traits like head length and number of leaves plant⁻¹, and suggested that these traits can impart high yield with the increase in number of leaves and head size. GCA and SCA for all the traits being significant they have stated that both additive and non-additive gene effects were important.

2.6 QTL's for Shoot fly Resistance

Marker-assisted selection (MAS) can assist in extensive and efficient genetic manipulation for QTLs identified for the various biotic stresses in Sorghum. The QTLs so identified would help in better understanding the inheritance of the traits governed by these thereby clarifying the relationships between QTLs and their corresponding candidate genes for the basis for MAS of these traits (Aruna *et al* 2011). The various invented statistical models and marker techniques of QTLs, have allowed the identification of various QTLs governing resistance towards different pest by different workers like, shoot fly (Satish *et al* 2009), green bug (Agrama *et al* 2002; Nagaraj *et al* 2005; Wu and Huang 2008 and midge (Tao *et al* 2003). Devi *et al* (2013) has suggested that the quantitative traits can be improved by the use of molecular markers. Once major QTLs are detected they can be transferred into desirable parents through marker assisted backcrossing (MABC) can be used as an efficient tool for transferring the major QTLs, once detected target loci and recipient genome can be selected using foreground and background markers respectively. They have introgressed QTLs associated with leaf glossiness into an elite parent NTJ2 using MABC by validating foreground and background markers for the glossiness.

29 QTLs were found using replicated phenotypic data sets from four test environments and considering five component traits in four environments by multiple QTL mapping (MQM) in a RIL population derived from 296B × IS18551 cross (Satish *et al*, 2009). Aruna *et al* (2011) using the same approach found 25 QTLs for shoot fly resistance and the associated traits. They used 210 RIL population of the 27B (susceptible) X IS2122 (resistant)

cross and in three environments. The associated traits studied were; dead heart percentage, glossiness, and seedling vigor and trichome density in both the studies. Satish *et al* (2009) mapped the genes segregating for trichome morphology as morphological marker on SBI-10 linkage group between five important components of shoot fly resistance QTLs and suggesting that trichome density and morphology plays an important role in providing resistance against shoot fly. These studies were based on the observed trichome morphology in the RILs where each of the RIL showed presence of either blunt or pointed trichomes which also suggested the monogenic inheritance of the concerned trait.

Dhillon *et al* (2005) and Anandan *et al* (2009) also reported that the various mechanisms or traits imparting resistance to shoot fly resistance can be used as markers for selection of the resistant material. Apotikar *et al* (2011) using the approaches like composite interval mapping and multi-trait composite interval mapping identified pleiotropic QTLs which govern the traits like glossiness, oviposition and deadheart formation. They mapped a set of 14 SSR markers on two linkage groups A and C and such linked markers can be used in improvement programs focussing on multiple trait enhancement simultaneously. Similarly, MAS has been used to introgress the shoot fly resistant governing QTLs like trichome density and glossiness using SSR markers by backcross breeding methodology.

Kiranmayee *et al* (2016) identified and validated QTLs for two important traits imparting resistance to shoot fly viz. trichome density and glossiness. They used F₂ and derived populations and mapped the QTLs on long arm of chromosome 10 for both the traits. The QTLs were found to be showing loose linkage accounting for variation ranging from 2.29-11.37% with LOD score between 2.03 to 24.73. They were able to shorten the physical distance from 4 and 2 Mb to 2 Mb and 800 kb.

CHAPTER – III

MATERIAL AND METHODS

The present research work was carried out at the experimental area of the Forage and millet section Department of Plant Breeding & Genetics, Punjab Agricultural University (PAU), Ludhiana and International Crop Research Institute for Semi-arid Tropics (ICRISAT), Patancheru, Telangana. In PAU, the crop was sown during the rainy season of 2016 and 2017, while at ICRISAT, the experiment was conducted during the rainy and post rainy seasons of 2016 & 2017.

3. Experimental material

The research material consisted of two different set of genotypes which were evaluated for the susceptible and resistance reaction to shoot fly under field conditions. The first set consisted of a diverse collection of 53 different germplasm lines (Table 3.1) selected on the basis of availability of sequencing data of Genotyping by sequencing (GBS)-based SNP markers. This set of germplasm lines were screened for reaction to shoot fly in a randomized complete block design (RCBD) in two rows (2m in length) with a spacing of 75 cm X 10 cm in two replications during the rainy season in 2016 at ICRISAT. The second set consisted of 30 genotypes (Table 3.2) including 10 cytoplasmic male sterile lines, along with their respective maintainers and 10 restorer lines. This set was evaluated for its reaction to shoot fly during the rainy season of 2016 & 2017 at two locations viz. PAU and ICRISAT and during post rainy seasons of 2016 and 2017 at ICRISAT. The interlard fish meal technique (Fig. 3.1) was used to maintain uniform insect pressure so as to create optimum environment for screening against shoot fly. Along with this, the same set was sown under controlled condition with use of chemical control measures (carbofuran granules and cypermethrin spray) to produce all possible hybrid seed combinations in Line X Tester fashion at ICRISAT during post rainy season of 2016. The hybrid combination thus generated were sown in replicated trial to evaluate the hybrids for reaction to shoot fly so as to identify better hybrids and gaining information on the inheritance of traits governing resistance to shoot fly. Along with this hybrid evaluation trial, a replicated trial of the same hybrids, was conducted under controlled condition to evaluate the agronomic and morphological performances of these hybrids.

The test material was also evaluated for the identification of biochemical components associated with resistance or susceptibility to shoot fly. For this the test material was sown in field conditions and shoot fly pressure was maintained using interlard fish meal (Soto, 1974).

3.1. Morphological observations:

3.1.1. The two important shoot fly damage parameters are shoot fly eggs and deadhearts formation (Fig. 3.2). The data was recorded for these traits by counting the number of

shoot fly eggs on the lower surface of leaves, number of plants with shoot fly eggs and shoot fly deadhearts at 14 and 28 days after seedling emergence (DAE).

3.1.2. Data was also recorded for the traits like glossy score, leaf sheath pigmentation and trichome density. Glossy score was observed visually in early morning (when light reflection from the surface of leaf is maximum) at fifth leaf stage of 10-13 days old seedlings. Scoring was done on a scale of 1-5 as defined by Sharma and Nwanze (1997) where 5 represents non-glossy and 1 represents highly glossy. Leaf sheath pigmentation was also recorded visually and scoring was given on the basis of 1-5 scale where 1 being pigmented and 5 being non-pigmented. The trichome density on adaxial and abaxial leaf surface was recorded from a 2.5 cm centre portion of the leaf sample collected at 12-14 DAE (Maiti and Bidinger, 1979). For collection of leaf samples 3-5 seedlings were selected randomly from the field and kept in the glass vials of 10 ml, containing acetic acid and alcohol solution (2:1). These vials were kept for 24 hrs for dissolving and clearing the chlorophyll from leaves, after which the leaves were transferred into a preservative solution of lactic acid (90%). For counting the trichomes, the sections of leaves were mounted on glass slide and a drop of lactic acid was put on it. The trichomes were seen under 10X magnification and trichomes counted were expressed as number of trichomes under 10X microscopic field.

Table 3.1: Germplasm lines

S No	Entry	S No	Entry	S No	Entry
1	IS 1044	19	IS 18677	37	IS 4646
2	IS 1051	20	IS 18698	38	IS 4757
3	IS 1054	21	IS 19512	39	IS 4995
4	IS 10712	22	IS 19955	40	IS 5469
5	IS 1104	23	IS 20024	41	IS 5470
6	IS 12308	24	IS 20643	42	IS 5480
7	IS 13100	25	IS 20740	43	IS 5490
8	IS 14108	26	IS 2122	44	IS 5566
9	IS 14334	27	IS 2123	45	IS 5604
10	IS 15107	28	IS 21443	46	IS 5622
11	IS 17610	29	IS 21444	47	IS 6566
12	IS 17645	30	IS 2146	48	IS 7005
13	IS 17726	31	IS 21871	49	IS 8151
14	IS 18368	32	IS 21879	50	IS 8549
15	IS 18551	33	IS 21881	51	IS 8671
16	IS 18563	34	IS 2205	52	IS 8891
17	IS 18579	35	IS 2312	53	IS 9807
18	IS 18662	36	IS 27329		



Fig. 3.1: Interlard fish meal technique

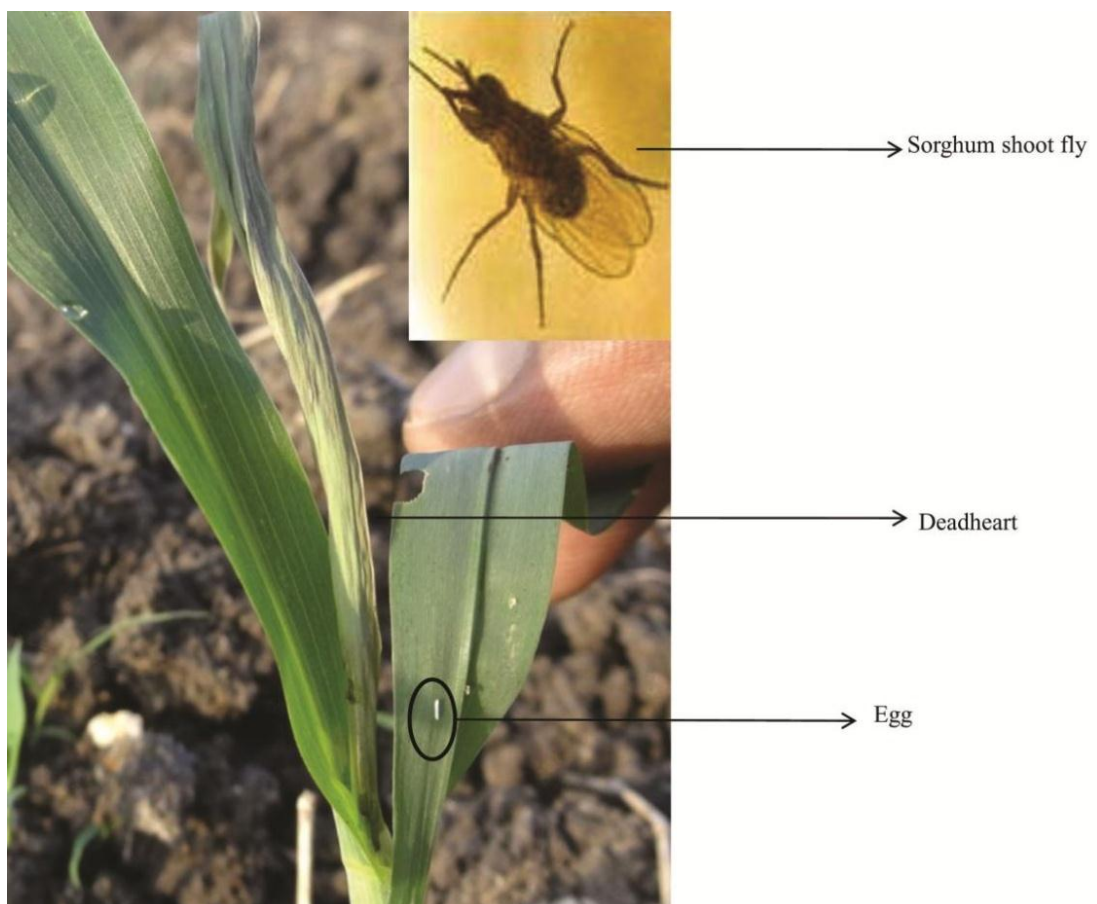


Fig. 3.2: Sorghum seedling with shoot fly egg and deadheart

Table 3.2: Parental lines with pedigree

A/B Lines		
S No	Genotype	Pedigree
1	ICSA/B 14013	(ICSB 38 x SSV 84-6-3)-1-2
2	ICSA/B 14024	(ICSB 344 x SSV 84-5-1)-2-1-1
3	ICSA/B 14027	ICSV 93046 x ICSB 88010)-1-3-2-1-1-1-1-1
4	ICSA/B 487	(ICSB 102 x ICSV 700) 5-2-4-2-1-2
5	ICSA/B 458	(ISS 18432 x ICSB 6) 11-1-1-2-2
6	ICSA/B 323	(IS 29016 x icsb 26)2
7	ICSA/B 14037	[[SPV 462 x (ICSB 102 x PS 28060-2)-1-2-2-1-5-3] x296 B]-2-1-1-1-1-1 x ICSB 451)-3-1-2-3-1-1-1
8	ICSA/B 467	[(ICSB 11 x ICSV 700) XPS 19349 B) x ICSB 13] 4-1
9	ICSA/B 84	296 B
10	ICSA/B 52	Ind. Syn. 422-1
R Lines		
1	ICSV 25280	(ICSV 93046 X SSV84)-7-2-1-3
2	ICSV 25303	(IS 19273 x SSV 84)-2-2-1-1-2-1-1
3	ICSV 25337	(ICSR 93034 x SSV 74-5-1)-1
4	ICSV 93046	(ICSV 700 x ICSV 714)-20-1-1-1-1-1-1 x UChV2 x Bulk Y-55)-1-5-1)-5-2-5-1-1)]-9-1-3-1-1-
5	SSV 74	Selection from 23558 (PAB 74)- Zera-zera landrace, Ethiopia)
6	ICSV 700	(IS 1082 x SC 108-3)-1-1-1-1-1
7	Btx 623	BTx3197/SC170 -6-4
8	S 35	IS 36556
9	ICSV 25316	(DSV 4 x SSV 84)-1-2-2-3-2-3-4
10	ICSV 25292	(DSV 4 x SSV 84)-1-1-1-1-2-1-2
11	IS 18551	Resistant check
12	Swarna	Susceptible check

3.2. Biochemical components associated with resistance to sorghum shoot fly (*A. soccata*)

3.2.1. Biochemical constituents of sorghum genotypes

For the estimation of the biochemical constituents, the test genotypes were planted in glass house area. The samples were collected at 5th leaf stage at 12-14 DAE for the estimation of total soluble sugars, total phenols, protein concentration and tannins. The samples were also subjected to HPLC fingerprints for studying the flavonoids associated with the reaction to shoot fly incidence.

3.2.2. Lyophilization and grinding of the samples

The plant samples were taken at 5th leaf stage (12-14 DAE) and subjected to lyophilization, i.e. vacuum freeze drying to keep the biochemical constituents intact. The samples were placed in the lyophilizer (ThermoSavant) tray and freeze drying was started with a temperature of -50°C and vacuum pressure of 350 mbar. The samples were subjected to lyophilization for 24-48h. The dried samples were grounded to form powder and stored in desiccators.

3.2.3. Estimation of Total soluble sugars

Sugars constitute an important component of storage and structural backbone of the plants. They exist as simple sugars and polysaccharides. The total soluble sugars can be estimated by acid hydrolysis of polysaccharides, leading to simple sugars by Anthrone reagent method (Hedge and Hofreiter 1962). For this, the powdered plant samples (100 mg each) were taken in the boiling test tubes and 5.0 ml of 2.5 N HCl was added. These test tubes were kept in the boiling water bath for 3 h. The test tubes were then cooled down to room temperature, and sodium carbonate (Na₂CO₃) was added until the effervescence ceased. The total volume was made up to 100 ml, and it was then centrifuged. The supernatant was collected in aliquots and anthrone reagent was added after which it was subjected to boiling again for 8 minutes. The conc. H₂SO₄ present in the Anthrone reagent reacts with carbohydrates causing dehydration to form furfural. Furfural so formed, condenses with the Anthrone reagent to form a blue-green colored complex, this was measured in the spectrophotometer at 630 nm. A standard glucose solution was prepared and using known concentrations of glucose, a standard curve was prepared. From the standard graph thus generated, the concentration of the sugars present in the sample was estimated by using the following formula:

$$\begin{aligned} & \text{Amount of carbohydrate present in the sample (\%mg)} \\ &= \frac{\text{Sugar value from the graph (mg)}}{\text{Aliquot sample (0.5 or 1 ml)}} \times \frac{\text{Total weight of extract (ml)}}{\text{Weight of sample utilised (mg)}} \times 100 \end{aligned}$$

3.2.4. Estimation of proteins by Lowry's method

Lowry's method (Lowry *et al* 1951) was used to estimate the protein content in the test genotypes. For this, 500 mg of lyophilized plant material was grounded in a mortar and pestle using 5 ml of buffer. The grounded material was centrifuged and supernatant was taken for protein estimation. The supernatant was pipetted out in different aliquots (0.1 ml) and final volume was made up to 1 ml by adding distilled water. To this 5 ml of solution C was added and mixed well and was incubated at room temperature for 10 minutes. Then 0.5

ml of Folin Ciocalteu Reagent (FCR) was added, and after mixing it well it was incubated at room temperature in dark for 30 minutes. The solution in the test tubes developed color due to reaction of alkaline copper in FCR with the proteins and it was read calorimetrically in spectrophotometer at 660 nm. Using the known concentrations of the bovine serum albumin (BSA) solution, a standard graph was prepared. The concentration of protein present in the test genotypes was calculated using the standard graph, and the results were expressed as mg/g or mg/100 g sample or percentage.

3.2.5. Estimation of total phenols

Total phenols were estimated by using the method defined by Bray and Thorpe (1954). A 500 mg of the lyophilised leaf sample was grounded and homogenized with 80% ethanol in a mortar and pestle. The homogenate was centrifuged at 10,000 rpm for 20 minutes, and supernatant was taken and residue was reextracted with five times the volume with 80% ethanol. The supernatants were collected, pooled and evaporated to dryness using rotavapor. The residue thus left was dissolved in a known volume of distilled water (5 ml). Pipetted out the different aliquots of the sample into the test tubes, and final volume was made up to 3 ml with distilled water and 0.5 ml of FCR was added. After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added to each test tube. After mixing thoroughly, the test tubes were placed in hot water bath for one minute. The test tubes were cooled to room temperature and absorbance was recorded at 650 nm. Different concentrations of catechol were used to prepare the standard curve. This standard curve was used to estimate the concentration of the phenols in the test samples which was then expressed as mg phenols/100 mg material.

3.2.6. Estimation of tannins by vanillin hydrochloride method

For the estimation of tannins, vanillin hydrochloride method (Robert 1971) was used. In this method, a 100 mg of lyophilised plant material of test genotypes was extracted in 50 ml of methanol which were then placed on rotary shaker for continuous swirling for 20 - 28 h. After 28 h, the contents were centrifuged and the supernatant was collected. Different aliquots of the samples were pipetted out and 5 ml of vanillin hydrochloride reagent was added. Vanillin reagent reacts with any phenol containing unsaturated resorcinol or phloroglucinol nucleus, resulting in formation of a colored substituted product. After mixing the contents, were incubated at room temperature for 20 min. The absorbance was recorded in spectrophotometer at 550 nm. For the preparation of a standard graph, known concentration of catechin were used and this was used to estimate the amount of catechin present in the solution. The tannin content in the sample was expressed in terms of catechin equivalents.

3.2.7 HPLC fingerprints of sorghum flavonoids:

3.2.7.1 Extraction of phenols

For this slight modification were made to the methodology described by Hahn *et al* (1983) for extraction of phenols. The lyophilized sorghum samples were homogenized in mortar and pestle after grounding in 5 ml of methanol. The homogenized samples were then subjected to sonication for 30 minutes. After this, the samples were centrifuged at 5000 rpm for 10 minutes and supernatant was collected. It was partitioned in a separation funnel using 5 ml of hexane and this step was repeated three times. The methanol extracts obtained after separation were transferred to the rotavapor for drying. When the extract was reduced near to dryness it was re-dissolved in 3 ml of HPLC grade methanol.

3.2.7.2 Preparation of samples for HPLC analysis

Millipore filters with a pore size of .45 μm were used to filter the methanol and then the filtered extracts were transferred to HPLC vials for separation.

3.2.7.3 HPLC procedure/protocol

For obtaining the chromatograms, the samples including standards of a known volume (20 μl) were chromatographed singly and in mixtures using a Waters Sunfire C₁₈ column (4.6 x 250 mm) having pore size of 5 μm . 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-acetonitrile (B) was used for separation.

The protocol for the separation of the compounds was as follows:

Running time	2% Acetic acid (A%)	Acetic acid-acetonitrile (B%)
0.00	95.00	5.00
10.00	95.00	5.00
17.50	85.00	15.00
31.00	85.00	15.00
41.00	50.00	50.00
45.00	50.00	50.00
50.00	85.00	15.00
55.00	95.00	5.00

3.3 Combining ability studies

Inheritance studies of traits governing resistance/susceptible reaction to shoot fly included evaluation of hybrid combinations generated from the set of 30 genotypes exhibiting variable reaction to shoot fly. The CMS genotypes used as lines and the restorer genotypes used as testers were planted during the post rainy season of 2016 and crossed in a line x tester fashion to generate 100 hybrids. The hybrid combinations were planted in three replications during the rainy season of 2017 at ICRISAT. The observations were recorded for the traits like glossy score, leaf sheath pigmentation, total number of plants, seedling height and trichome density at both adaxial and abaxial surface of leaves. The traits like shoot fly deadhearts, total number of shoot fly eggs, plants with shoot fly eggs were recorded at 14 and 28 days after emergence (DAE). The data thus generated was subjected to the statistical software SPAR 2.0 for LXT analysis.

3.4 Assessment of genetic and molecular diversity

3.4.1 For molecular analysis the DNA was isolated from the test genotypes grown in the glass house area, using the CTAB methodology (Mace *et al*, 2003) with some modifications. The procedure is explained below:

3.4.2 DNA extraction: For DNA extraction 2-3 steel of 4mm diameter balls which were kept at -20°C for about 30 minutes were added to the 96 well extraction plate with the strip caps (Marsh Biomarket, USA) that was kept in ice box. The leaf samples of the test genotypes collected from the glasshouse area were chopped down into small pieces and from this a small amount or around 30mg was taken into the extraction tubes. To these tubes the preheated CTAB buffer (3%) measuring 450 μl was added and these samples were subjected to grinding in GenoGrinder (Spex Certiprep, USA) at the rate of 500 strokes/minute for around 2 minutes. This procedure was repeated until the sample was grounded sufficiently and care was taken that there was no leakage and the leaf samples were properly mixed with the buffer. The box with the samples was taken out of the grinder and placed in a locking device which was then incubated at 65°C for a short span of 10 minutes.

3.4.3 To these samples a mixture of chloroform: isoamyl alcohol (24:1) was added and mixed properly. Then the tubes were subjected to centrifugation @ 6200 rpm for a time period of 10 minutes in the centrifuge. The aqueous layer was sucked out with a micropipette and transferred into fresh tubes (Marsh Biomarket). To these tubes chilled isopropanol (200 μl) was added and mixed properly. The tubes were then kept in the -20°C for 10 minutes and then taken out and again centrifuged @6200 rpm for 15 minutes. The

supernatant was collected and discarded and the pellets at the bottom of the tube were dried. These pellets were then subjected to RNase A treatment by dissolving in 200 µl of low salt T buffer mixed with 3 µl of RNase A. This solution was kept overnight at room temperature. Next day, to this solution 200 µl of phenol: chloroform: isoamyl alcohol (25: 24: 1) was added and mixed well. These were then centrifuged @ 5000 rpm for 10 minutes and the supernatant containing aqueous phase was collected and transferred into fresh tubes. Again, to these tubes, 200 µl of chloroform: isoamyl alcohol (24: 1) was added, mixed well and centrifuged at 5000 rpm for 10 minutes and the supernatant was collected into fresh tubes. To these tubes a mixture of 15 µl 3.0 M sodium acetate (pH 5.2) and 300 µl chilled absolute alcohol was added and then incubated at -20°C for 5 minutes. Then these tubes were centrifuged @ 6200 rpm for 15 minutes and the supernatant was discarded carefully keeping the pellet intact at the bottom of the tube. To these tubes, 200 µl of 70% ethanol was added and again centrifuged @ 5000 rpm for 5 minutes. The supernatant was discarded again and the pellet was air dried for an hour, after which it was re-suspended in 100 µl of T E buffer and was allowed to dissolve properly overnight at room temperature and after that the DNA samples were stored at 4°C.

3.4.4 Quantification and normalization of DNA: The DNA was checked for the quality using gel electrophoresis with 0.8% agarose gels stained with ethidium bromide. The sample for loading was prepared by mixing 3 µl distilled water + 1 µl orange dye + 1 µl DNA sample and loaded into the wells and subjected to electrophoresis at 100 V for 5-10 minutes. Once the run was complete, the gel was visualized under the UV light. A distinguished band represents the good quality DNA the samples with low quality of DNA were re-extracted. For the quantification purpose the DNA was diluted and compared with the standard concentration λ DNA molecular weight markers and on the basis of visual comparison the DNA was normalized to 2.5 ng/µl concentration.

3.4.5 Genotyping by sequencing (GBS) and diversity analysis: The GBS data of 53 sorghum germplasm lines was obtained from Centre of Excellence in Genomics (CEG), ICRISAT. GBS was done using the protocol given by Elshire *et al* (2011). GBS-SNP data was used to analyse diversity among sorghum germplasm lines. Diversity analysis was conducted using the statistical software DARwin 6.

3.4.6 Genome wide association studies (GWAS): GWAS was done using FarmCPU (Liu *et al* 2016) in R (R core team, 2013) to identify the QTLs associated with shoot fly resistance variables. Briefly FarmCPU iteratively uses fixed and random effect models

for powerful and effective GWAS. Probability (p) values of FarmCPU were obtained at 0.05 threshold output. Manhattan plots showing association of SNPs with shoot fly resistance variables were obtained from the output of FarmCPU.

CHAPTER IV

RESULTS AND DISCUSSION

The sorghum shoot-fly lays eggs on the sorghum seedlings of 7-10 days old, after emergence. Although the duration of the egg stage is very short, but the life cycle of the fly also being short leads to a number of generations feeding on the seedlings and causing huge loss to yield and production of sorghum. Various control measures have been used to check the shoot fly infestation among which the chemical control is the most effective, but the resource poor farmers of semi-arid tropics cannot afford the costly chemicals and short sowing window does not allow them to go for early planting to avoid the damage from this insect. So, host plant resistance (HPR) and use of cultural practises is the most economic and effective measure to reduce the extent of losses due to shoot fly damage by keeping the infestation below economic threshold levels. HPR being a highly complex trait is the final outcome of interaction among the multiple component traits imparting resistance to the plant (Dhillon 2004). Various morphological, genetic and biochemical traits in combination yield to the resistance against sorghum shoot fly. The available cultivated material exhibits moderate to low levels of resistance and there is a need to explore the sorghum germplasm seeking diversity for the traits imparting resistance to sorghum shoot fly. These can be utilized to understand the diverse mechanisms of resistance to shoot fly in a wide array of lines exhibiting variable reaction to shoot fly incidence. The various morphological characters and biochemical compounds which are responsible for attracting or repelling the shoot fly, can be studied and their desirable combinations can be utilized in the breeding programs. Also, with the advancement in the molecular breeding technologies, it has become relatively easier to identify the genomic regions governing the different traits and thusly making the use of the germplasm material relatively easier. The GBS based SNP markers can be used to associate various genomic regions to the trait of interest and transferring such regions efficiently. The present investigations were conducted with the same motive of assessing diversity in a diverse set of germplasm lines which included morphological and molecular evaluation followed by association of the genomic regions to the traits imparting resistance against shoot fly. Along with this, a diverse set of 20 genotypes was also used to study diversity based on the morphological, biochemical components and molecular markers for the traits associated with shoot fly resistance. The same set was also used to study the combining ability effects by generating hybrid combinations in LXT fashion. The hybrids were also evaluated so as to identify better hybrids in terms of shoot fly resistance. The results of the studies conducted are discussed below.

4.1 Morphological evaluation of the parental lines

The parental lines were evaluated for the traits imparting resistance or susceptibility to sorghum shoot fly during rainy and post rainy from 2016 to 2017. The traits like glossy score, leaf-sheath pigmentation, total no. of plants, seedling height, number of shoot fly dead hearts (at 14 & 28 DAE), number of plants with SF eggs (at 14 & 28 DAE), total no. of SF eggs (at 14 & 28 DAE), trichome density at abaxial & adaxial surface of leaves and plant height (cm) were evaluated in relation to the susceptibility or resistance against sorghum shoot fly. In rainy, 2016 the test genotypes exhibited significant variation for all the traits except shoot fly deadhearts 28DAE (Appendix 1) while during post rainy, 2016 (Appendix 2) the genotypes were found to be significantly different for all the traits studied. Similarly, in 2017, the test genotypes varied significantly for most of the traits except shoot fly deadhearts (14DAE), number of plants with shoot fly eggs (14DAE) and total number of shoot fly eggs during the rainy period (Appendix 3). Whereas in the post rainy, 2017 (Appendix 4) the genotypes differed significantly for all the traits except, trichome density at adaxial and abaxial surface. The differential expression of different genotypes with respect to the traits is described below:

4.1.1 Glossy score

The scoring for leaf glossiness was given on the visual basis by observing the seedlings 10-12 DAE in the early morning hours. A scale of 1-5 scale was used as described by (Sharma, 1997) where 1 is glossy (shinning, erect and narrow leaf with light green colour) and 5 is non-glossy (drooping, broad, dull and dark brown leaves). In 2016, the glossy score ranged from 1 to 5 with an average value 3.52 (SE $\pm$ 0.34, CV 13.60) during the rainy season while it ranged from 1 to 5 with an average value 3.33 (SE $\pm$ 0.32, CV 13.60) during post rainy season. In 2017, it ranged from 1 to 4.5 with an average value 2.9 (SE $\pm$.40, CV 18.10) during rainy season whereas during post rainy season, the value ranged from 1 to 5 with an average value 3.34 (SE $\pm$ 0.30, CV 12.90). The genotypes IS 18551, ICSA/B 487, ICSA/B 467, ICSV 25280, ICSV 700 and ICSV 93046 were found to be glossy whereas genotypes Swarna, SSV 74, BTx 623, ICSA/B 52, ICSV 25292 and ICSA 323 non-glossy.

4.1.2 Leaf sheath pigmentation

The LSP was recorded visually 2-4 DAE on a scale of 1-5 (1- pigmented plumule with dark leaf sheath; 5- non-pigmented plumule with green leaf sheath) as described by Sharma (1997). The genotypes with pigmented leaf sheath generally harbours less shoot fly incidence as compared to non-pigmented (Gomashe *et al* 2010). The test genotypes varied significantly for LSP in the present study. In 2016, the LSP varied from 1 to 4 with a mean value of 3.51 (SE $\pm$ 0.34, CV 18.60) during the rainy season and from 1 to 4.5 with an average value of 3

(SE±0.48, CV 22.20) in the post rainy season. Similarly, in 2017, the value of LSP was recorded in the range of 1.5 to 3 with a mean value of 2.3 (SE±0.30, CV 18.90) and from 1.5 to 5 with a mean value of 3.56 (SE±0.33, CV 13.30) during rainy season. The genotypes (IS 18551, ICSA/B 487, ICSA/B 467, ICSV 93046, ICSA/B 458 and ICSA/B 14037) exhibiting moderate to high resistance against shoot fly had pigmented leaf sheath while the genotypes (Swarna, SSV 74, ICSA/B 52, BTx 623, ICSA/B 323 and ICSA/B 14024) had green leaf sheath.

4.1.3 Seedling height

The seedling height is also an important trait with respect to the shoot fly incidence as the genotypes which are more vigorous in growing, attain good seedling height in short period of time and thereby resisting the attack of shoot fly. The seedling height was measured at 14-18 DAE and the test genotypes showed significant variation for this trait during both the seasons. The seedling height ranged from 4 to 12.3 cm with IS 18551 and ICSA 323 recording maximum and minimum height respectively. The mean value of 6.23 (SE±0.19, CV 4.30) was recorded in the rainy season of 2016. For the post rainy season, the value ranged from 4.9 to 11.5 with an average value of 7.6 cm (SE±0.28, CV 5.30) with the genotypes SSV 74 and ICSA 458 having maximum and minimum height respectively. Similarly, in 2017, for the rainy season, the value ranged from 4 to 12.4 cm with a mean value of 6.1 cm (SE±4.40, CV 8.0). The max and minimum height was recorded for the genotype IS 18551 and ICSA 323 respectively. During the post rainy season, seedling height ranged from 5.2 to 11.4 cm with a mean value of 7.96 (SE± 0.35, CV 6.20) with genotypes IS 18551 and ICSB 458 having maximum and minimum height respectively.

4.1.4 Shoot fly deadhearts (SFDH)

The symptomatic dried central leaf whorl, due to shoot fly attack is known as deadheart (Fig. 4.1). It is a very important trait for screening against shoot fly resistance as the genotypes suffering less incidence of shoot fly deadhearts are resistant to shoot fly and this trait can be used as one of the morphological marker to identify the resistant sources for sorghum shoot fly (Dhillon *et al*, 2005). In the present study, the number of seedlings bearing deadhearts were counted at 14 DAE & 28 DAE. For the sake of representation, the deadheart data is represented in percentage which is calculated by dividing the number of seedlings showing deadhearts with the total number of seedlings [(seedlings with DH/total number of seedlings) X100]. The incidence of DH at 14 DAE, was found to be significantly different among the genotypes during both the seasons of 2016 and post rainy season of 2017. The shoot fly deadhearts at 14 DAE (SFDH) ranged from 3.5 to 26.5 (13.7 to 97.8%) and 1.5 to 18.5 (5.2 to 75.4 %) during rainy and post rainy seasons in 2016. Similarly, the data ranged from 3.5 to 24.0 (37.8 to 96.9 %) and 1.3 to 16.5 (6.1 to 66.9 %) during the rainy and post

rainy seasons in 2017 respectively. Deadhearts at 28 DAE (SFDH1) for the test genotypes varied significantly in both seasons during 2016 and 2017, while the percent shoot fly deadhearts during rainy season (2016) did not show any significant variation among the genotypes. The genotypes (IS 18551, ICSA/B 487, ICSA/B 467, ICSA/B 458, ICSA/B 14024, ICSA/B 14027, ICSA/B 14037, ICSV 700, ICSV 93046, ICSV 25280) suffering less shoot fly deadhearts exhibited resistant reaction to shoot fly.

4.1.5 Number of Plants with shoot fly eggs and total number of shoot fly eggs

The sorghum shoot-fly lays eggs on the under surface of leaves 7-10 days after seedling emergence. Although the duration of the egg stage is very short, but the life cycle of the fly also being short leads to a number of generations feeding on the seedlings and causing huge loss to yield and production of sorghum. So, the number of shoot fly eggs and plants bearing shoot fly eggs is very crucial for evaluating the resistance against shoot fly. In present studies, the total number of plants bearing shoot fly eggs and total number of shoot fly eggs in a plot were counted at 14 and 28 DAE. Significant variation was found for these traits among the test genotypes during rainy and post rainy seasons in 2016 while it was significant only in postrainy in 2017. The data ranged from 11 to 33.5 with 20.5 to 82 eggs and 3 to 24.5 plants bearing 4.5 to 30 eggs during the rainy and post rainy seasons (2016) respectively while from 5.5 to 22.5 plants with 13.5 to 58 eggs and 3.5 to 23.3 plants bearing 3.8 to 26.0 eggs in rainy and post rainy seasons (2017) respectively. The genotypes (ICSA/B 487, ICSA/B 84, ICSV 700, ICSV 93046, IS 18551, ICSA/B 458, ICSA/B 14037 and ICSV 25337) bearing the lesser number of eggs can be used in resistance breeding programs.

4.1.6 Trichome density

The trichome density at adaxial surface hinders the egg laying and larval movement underneath leaf surface and is associated with shoot fly resistance (Dhillon *et al* 2005b). In the present studies, the genotypes varied significantly for the trichome density during both the rainy (2016, 2017) only post rainy (2016). The genotypes (ICSA/B 487, ICSA/B 14027, ICSA/B 14037, IS 18551, ICSA 458, ICSB 52, ICSV 25316, ICSV 25280, ICSV 25337, ICSV 700 and ICSV 700) that exhibited the moderate to high resistance reaction against shoot fly also recorded high trichome density on both the leaf surfaces. Whereas some genotypes exhibiting moderate to low resistance to shoot fly recorded low density to absence of trichomes on both leaf surfaces.

4.1.7 Plant height

The plant height was recorded near the flowering stage and is an important trait in relation of forage yield. The seedlings escaping the shoot fly attack or exhibiting better recovery resistance attained substantial height in later stages of growth. In the present studies

the test genotypes showed significant differences for plant height during both seasons across years. Some genotypes (SSV 74, ICSV 25280 and ICSV 25292) exhibiting susceptible reaction to shoot fly also attained significant height in later stages. Such genotypes can also be explored in breeding programs for their combining ability studies for achieving better forage yield.

Analysis of variance across seasons (Table 4.1) revealed that in 2016, shoot fly deadhearts, plants with shoot fly eggs and total number of shoot fly eggs were found to be significantly differing among the test genotypes across both seasons while in 2017, except percent shoot fly deadhearts and trichome density at both surfaces, the genotypes varied significantly for all the traits across seasons.

For SFDH at 14 DAE, the genotypes, ICSB 458 (3.5), ICSB 487 (6), ICSA 14027 (6.5), ICSV 93046 (7), S 35 (7), ICSB 467 (7.5), ICSV 25280 (8), ICSV 700 (8) and ICSA 14024 (8.5) performed better, bearing lesser deadhearts at 14 DAE in comparison to the resistant check IS 18551 (8.5) during rainy season, while none of the test genotype out pars the resistant check IS 18551 (1.5) in post rainy season, but had significantly lesser deadhearts than the susceptible check. For the number of plants with shoot fly eggs, the genotypes ICSV 25292 (14), ICSB 323 (18), ICSA 14024 (18.5), ICSA 14027 (19), ICSV 700 (19), ICSA 14013 (21), ICSB 14027(21), ICSB 52 (21.5), ICSV 25303 (21.5), ICSA 14037 (22) and ICSV 93046 (22) recorded lower number of plants with SF eggs, as compared to the resistant check IS 18551(22). Only ICSB 487 (6.5) was at par with the resistant check IS 18551 (6.5) during post rainy season while other genotypes like ICSA 487 (6.5), ICSB 467 (7.5), ICSA 458 (9.5) and ICSB 458(11) performed better than susceptible check.

Similarly, the total number of eggs were found to be significantly different across seasons and followed the same pattern as plants with SF eggs. The genotypes, ICSB 14037 and ICSV 25292 recorded the minimum number of eggs (26.5) followed by ICSV 93046, ICSA 14024 and ICSV 25316 (27.5) and ICSA 467 (30.5) which performed out par than the resistant check IS 18551 (31.8) during the rainy season, 2016. While during the post rainy season, ICSA 467 (6.5) was at par with IS 18551 (6.5) and the genotypes ICSB 458 and 467 (7.5), ICSA 458 (9.5), ICSV 93046 (11) ICSA 467 and ICSB 487 (13) also performed significantly better than the susceptible check Swarna (43.50).

Overall in terms of resistance to shoot fly, the genotypes ICSA/B 487, ICSA/B 14037, ICSA/B 467, ICSV 93046 and ICSV 700 exhibited high glossiness, pigmented leaf sheath, lower number of eggs and suffered lesser incidence of deadhearts whereas the genotypes ICSA/B 458, ICSV 25337 and ICSV 25292 exhibited moderate resistance to the shoot fly damage.

Table 4.1: Analysis of Variance across seasons

ANOVA	Range								h ²	σ ² g					
	2016 R		2016 PR		2017 R		2017 PR			2016		σ ² gxe	2017		σ ² gxe
	Min	Max	Min	Max	Min	Max	Min	Max		R	PR	RXPR	R	PR	RXPR
GS	1.0	5.0	1.0	5.0	1.0	4.5	1.0	5.0	0.34	9.63**	11.26**	1.360	6.71**	15.38**	2.4*
LSP	1.0	4.0	1.0	4.5	1.5	3.0	1.5	5.0	0.35	6.77**	4.07**	0.540	2.99*	8.9**	2.73**
TNP	14.0	36.5	13.5	33.0	6.0	33.5	10.3	31.5	0.27	2.97*	2.35**	1.300	2.85*	1.7*	2.11*
SFDH	3.5	26.5	1.5	18.5	3.5	24.0	1.3	16.5	0.25	5.04**	3.66**	1.53*	1.26	3.05**	1.93*
SFDH (%)	13.7	97.8	5.2	75.4	37.8	96.9	6.1	66.9	0.35	9.29**	4.11**	2.31*	1.20	4.78**	0.60
PWSFE	11.0	33.5	3.0	24.5	5.5	22.5	3.5	23.3	0.15	2.48*	6.1**	2.12*	1.30	3.91**	2.79**
NSFE	20.5	82.0	4.5	30.0	13.5	58.0	3.8	26.0	0.14	4.1**	6.57**	3.93*	1.52	6.42**	1.72*
SFDH 1	14.0	36.0	4.0	24.5	6.0	30.5	5.0	25.3	0.24	1.81*	2.56*	1.48*	2.95*	2.64*	3.19**
PSFDH	78.6	100.0	19.3	86.8	65.6	100.0	26.8	92.6	0.37	1.15	4.49**	2.3*	7.29**	3.38**	1.01
PWSFE 1	14.0	33.5	6.5	28.5	6.0	26.0	5.8	25.5	0.15	1.94*	2.71*	1.55*	1.79*	4.93**	3.46**
NSFE 1	26.5	87.0	6.5	43.5	16.5	47.0	6.3	35.8	0.13	5.03**	4.71**	3.74**	1.05	4.79**	1.78*
TD _{aba}	0.0	46.0	0.0	59.5	0.0	46.0	0.0	40.0	0.26	10.44**	18.79**	0.520	10.44**	1.00	0.62
TD _{ada}	0.0	72.0	0.0	79.5	0.0	73.5	0.0	57.9	0.29	20.38**	13.12**	0.450	27.36**	1.27	1.33

4.2 Morphological evaluation of germplasm lines

Similarly, like the parental evaluation, the 53 germplasm lines were evaluated for nine traits viz. total number of plants (TNP), glossy score (GS), leaf sheath pigmentation (LSP) plant vigour score (PVS), percent plants with shoot fly eggs (PPSFE), number of shoot fly eggs per plant (NSFEP), percent shoot fly deadhearts (PPSFDH), total no. of tillers (TL) and percent tillers with shoot fly deadhearts (PTSFDH) during the rainy season, 2016. From the analysis of variance (Table 4.2), significant variation was found among the lines for most of the traits except the leaf sheath pigmentation, number of plants with shoot fly eggs and number of shoot fly eggs per plant. The glossy score value ranged from 1 to 5 with a mean value of 3.59 and CV of 21.70 and SE of ± 0.55 , while vigour score value ranged from 1 to 3 with a mean value of 2.03 and the genetic variance for this trait was found to be 2.43 among genotypes at SE of ± 0.41 and CV of 28.20. Also, there was significant differences among these lines for the SFDH (%) which varied from 37.50 to 97.50 % with a mean value of 75.73 %. These germplasm lines also varied significantly for the trait of total number of tillers and the number of tillers with shoot fly deadhearts (%).

Table 4.2: ANOVA for the germplasm lines

Genotype	TNP	GS	PVS	LSP	PPSFE	NSFEPP	PPSFDH	TL	PTSFDH
IS 1044	20.50	4.00	2.50	2.00	92.74	2.18	73.33	45.00	53.33
IS 1051	17.50	4.50	2.00	1.50	100.00	2.09	79.90	77.50	57.17
IS 1054	17.50	3.50	2.50	2.00	94.12	1.94	70.92	16.00	65.83
IS 10712	14.50	4.50	2.00	1.00	69.52	1.65	72.86	81.00	55.28
IS 1104	18.50	2.50	2.00	2.00	100.00	2.00	75.88	39.00	55.35
IS 12308	18.00	3.50	1.00	2.00	94.44	2.19	61.11	68.00	39.97
IS 13100	13.50	3.50	2.00	2.00	85.00	3.52	87.06	93.50	33.76
IS 14108	17.50	4.50	2.50	1.00	100.00	3.01	94.44	50.50	68.95
IS 14334	17.50	4.50	2.50	3.00	97.22	3.23	94.12	75.50	55.09
IS 15107	18.50	5.00	3.00	1.00	97.37	2.77	91.81	118.50	67.70
IS 17610	18.00	5.00	3.00	1.00	100.00	2.20	91.25	37.00	65.20
IS 17645	20.00	5.00	3.00	1.50	100.00	2.15	95.00	82.00	58.71
IS 17726	20.00	2.50	2.00	2.50	100.00	1.65	80.00	26.50	76.80
IS 18368	15.50	3.50	3.00	2.50	94.74	2.82	55.92	23.00	67.25
IS 18551	20.00	2.50	1.50	1.50	95.24	2.41	63.28	51.00	54.51
IS 18563	15.00	4.50	2.00	1.00	100.00	1.28	80.00	33.00	60.19
IS 18579	18.50	3.00	2.00	1.50	92.11	1.82	70.32	45.50	59.49

Genotype	TNP	GS	PVS	LSP	PPSFE	NSFEPP	PPSFDH	TL	PTSFDH
IS 18662	17.50	3.50	1.50	1.50	100.00	1.86	77.96	37.50	45.00
IS 18677	19.00	4.50	2.00	2.50	100.00	1.45	89.44	45.50	55.37
IS 18698	19.50	5.00	2.50	1.00	89.87	1.55	92.50	71.50	48.25
IS 19512	22.00	4.50	2.50	1.00	95.42	2.75	82.08	67.50	50.08
IS 19955	20.00	4.50	2.00	3.00	100.00	2.00	95.00	95.00	51.22
IS 20024	18.50	3.50	2.50	2.00	94.44	1.64	89.04	85.50	71.46
IS 20643	18.50	5.00	2.50	1.00	100.00	2.63	74.12	86.00	40.41
IS 20740	19.00	5.00	2.50	1.50	97.83	3.01	84.64	42.00	75.93
IS 2122	19.00	1.00	1.00	1.00	91.67	2.71	49.72	52.00	43.74
IS 2123	19.50	1.50	1.00	1.50	97.50	2.15	69.21	37.50	56.13
IS 21443	20.00	4.50	2.50	3.00	92.50	1.68	92.50	132.50	46.61
IS 21444	21.50	5.00	3.00	3.00	100.00	1.64	87.39	109.00	47.87
IS 2146	20.50	1.50	1.00	2.00	80.60	1.52	53.69	56.00	49.33
IS 21871	20.00	5.00	3.00	3.00	100.00	2.48	97.50	67.00	63.47
IS 21879	18.00	4.00	3.00	3.00	89.38	2.04	61.25	47.50	64.31
IS 21881	20.00	4.50	2.50	3.00	92.50	2.20	82.50	61.00	66.11
IS 2205	19.00	2.00	2.00	1.50	95.00	1.55	57.78	61.50	43.88
IS 2312	18.00	2.50	2.00	2.00	88.24	1.22	60.68	42.00	46.07
IS 27329	18.50	5.00	3.00	3.00	100.00	2.69	97.06	102.50	61.40
IS 4646	21.50	1.50	1.50	2.00	97.50	1.46	71.63	58.00	56.49
IS 4757	19.50	4.50	2.50	2.00	100.00	2.28	87.11	55.00	42.19
IS 4995	20.00	4.00	1.50	1.00	92.50	2.15	47.50	62.00	58.87
IS 5469	20.00	3.50	1.50	1.50	92.50	1.93	77.50	51.00	51.90
IS 5470	20.00	1.50	1.50	1.50	82.50	1.40	47.50	63.00	44.09
IS 5480	20.00	2.00	1.00	2.00	100.00	1.88	37.50	58.00	50.96
IS 5490	17.50	3.00	1.50	2.50	94.17	2.05	70.00	34.50	43.18
IS 5566	20.00	2.50	1.50	1.50	87.50	1.73	67.50	43.50	48.08
IS 5604	19.00	1.50	1.50	1.50	81.58	1.40	55.26	60.00	62.79
IS 5622	18.50	3.00	1.50	1.00	94.44	1.81	64.33	51.00	56.19
IS 6566	21.00	2.50	1.50	1.50	92.50	1.68	72.95	65.50	48.11
IS 7005	19.50	4.50	2.50	1.00	92.11	2.08	74.08	97.50	40.11
IS 8151	19.50	4.50	1.50	1.00	100.00	2.55	92.11	76.00	76.89
IS 8549	18.00	3.00	2.00	1.00	92.50	1.88	73.75	66.50	48.58

Genotype	TNP	GS	PVS	LSP	PPSFE	NSFEPP	PPSFDH	TL	PTSFDH
IS 8671	18.50	4.50	1.50	1.50	97.06	2.64	63.09	104.00	52.95
IS 8891	19.50	3.00	1.00	1.00	100.00	2.06	84.47	90.00	40.53
IS 9807	14.00	3.50	1.50	1.00	52.17	48.46	96.25	79.76	48.45
Vr	0.80	4.57**	2.43**	0.98	0.93	0.99	1.59*	5.07**	1.63*
Mean	18.76	3.59	2.03	2.69	93.71	2.96	75.73	63.71	54.56
SE±	2.00	0.55	0.41	6.80	8.81	6.41	11.98	11.11	8.03
LSD (P 0.05)	5.66	1.57	1.15	19.29	25.00	18.19	34.01	31.52	22.79
CV (%)	15.00	21.70	28.20	35.75	13.30	30.50	22.40	24.70	20.80

******, and ***** Significant at Fpr value 0.01% and 0.05% respectively

4.3 Correlation studies

Pearson correlation coefficient for the traits related to shoot fly susceptibility/resistance was calculated using MS-excel using the observations recorded during rainy and postrainy season (Appendix 5-8). The glossy score (GS), leaf sheath pigmentation (LSP), number of shoot fly deadhearts (SFDH), percent shoot fly deadhearts (PSFDH), plants with shoot fly eggs (PWSFE), number of shoot fly eggs (TNE) and trichome density at abaxial (TD_{aba}) and adaxial (TD_{ada}) surface are the some of important traits which have been found to be associated with the shoot fly resistance.

4.3.1 Glossy score (GS)

Glossy score is majorly associated with the non-preference mechanism of resistance (Gomashe *et al*, 2010) and in the present studies it was found to be significantly and positively correlated with the number of SFDH (0.475**), and PSFDH (0.647**) in rainy 2016 while with SFDH (0.677**), PSFDH (0.643**), PWSFE (0.689**) and TNE (0.707**) in post rainy season. A significant and negative correlation of GS with the TD_{aba} (-0.436*), TD_{ada} (-0.506**) and plant height (-0.487**) was found in the rainy 2016 while with TD_{ada} (-0.420*) and plant height (-0.413*) in post rainy season. In the rainy 2017, the GS was found to be positively correlated with LSP (0.813**) and PSFDH (0.773**) whereas negatively correlated with TD_{ada} (-0.375*) and during post rainy it was significantly correlated with LSP (0.868**), SFDH (0.670**), PSFDH (0.708**), PWSFE (0.610**) and TNE (0.638**) along with negative correlation with TD_{aba} (-0.589**), TD_{aba} (-0.635**) and PH (-0.389*).

4.3.2 Leaf sheath pigmentation (LSP)

The coloured plumule of the leaf also has been found to be associated with shoot fly resistance. In the present studies the LSP was also found to be correlated with the shoot fly resistance and there was a positive correlation of LSP score was found with SFDH (0.461**)

and PSFDH (0.625**) whereas it was negatively correlated with the traits like TD_{aba} (-0.428*), TD_{ada} (-0.501**) and plant height (-0.439*) during rainy season in 2016. Similarly, it was found to be positively correlated with SFDH (0.635**), PSFDH (0.609**), PWSFE (0.707**) and TNE (0.699**) while negatively correlated with TD_{ada} (-0.378*) and Plant height (-0.412*) in the post rainy 2016. A similar trend was found during rainy and post rainy season in 2017 where LSP was found to be significantly correlated with the other shoot fly traits. LSP in combination with GS can be used as effective morphological markers for selecting the genotypes exhibiting resistance to shoot fly at the seedling stage itself (Dhillon, 2005).

4.3.3 Number of shoot fly deadhearts (SFDH) and percent shoot fly deadhearts (PSFDH)

The damage caused by shoot fly is visible in the form of typical deadheart symptom in which, the central leaf whorl is dried and the plant growth is stunted causing the losses in yield and productivity. The SFDH and PSFDH were recorded at 14 and 28 days after seedling emergence and the genotypes having lower incidence of SFDH were found to be resistant than the genotypes having more incidence of SFDH. In the rainy 2016 the only significant correlation was found between SFDH with PSFDH at 14 DAE and 28DAE while in post rainy the SFDH was found to be significantly correlated with the traits like PWSFE, TNE at both 14 and 28 DAE and negatively correlated with TD_{aba} (-0.450**) and TD_{ada} (-0.373*) which is an important criterion along with GS and LSP for selection of resistant genotypes (Gomashe *et al*, 2010). Similar results were found in the rainy and post rainy, 2017 with a significant positive correlation was found between PWSFE, TNE and negative correlation with TD_{aba} and TD_{ada} .

4.3.4 Plants with shoot fly eggs (PWSFE) and number of shoot fly eggs (TNE)

The number of shoot fly eggs and plants with shoot fly eggs directly correlates with the shoot fly deadhearts as the larvae from the eggs hatch out and attack the growing point of the plant. In rainy 2016, the PWSFE and TNW were directly correlated to SFDH at 28 DAE where as in post rainy, the correlation was significant with SFDH and PSFDH both at 14 & 28 DAE. The similar trend was followed in the 2017. The reason for the absence of the correlation with SFDH and PSFDH at 14 DAE in rainy season might be the heavy infestation of shoot fly during rains and irrespective of the number of eggs and plants with shoot fly eggs, the seedlings were suffering from deadhearts.

4.3.5 Trichome density (TD_{aba} and TD_{ada})

In most of the studies the trichome density at adaxial surface of leaf is found to be associated with the resistance to shoot fly as the trichomes poses hinderance to the shoot fly for laying eggs and also the movement of the larva on the underside of leaf thereby restricting

it to reach the growing point (Sharma, 1997; Dhillon *et al*, 2005). In present studies, the trichome density at both the surfaces were found to be correlated with less incidence of SFDH and shoot fly damage. Similar results were also found by the studies of Gomashe *et al*, 2010).

4.4 Combining ability studies

The combining ability studies are significant for understanding the nature and gene action for the traits involved in inheritance of the quantitative traits. Among various mating designs Line X Tester is most commonly used for the combining ability studies and gaining basic information regarding the potential of the parents used in a hybrid breeding program (Prakash *et al* 2010). For this specific combining ability (SCA) effects and general combining ability (GCA) effects are studied that signify, the non-fixable (generating heterotic hybrids) and fixable (generating superior genotypes) components of genetic variance (Mohammed 2009). In the present study, the parental lines were crossed in LXT fashion during rainy and post rainy season, 2016. The crosses among 10 CMS and 10 restorer lines yielded 100 hybrid combinations which were evaluated along with the parents to studying the specific combining ability (SCA) and general combining ability (GCA) effects of lines and testers.

4.4.1 Analysis of variance for lines and testers including parents

The variation based on the mean sum of squares (Table 4.3) was significantly different for all the traits except total number of eggs (TNE 1 & 2) and shoot fly deadhearts (SFDH 1& 2) in lines and testers respectively. Total number of plants (TNP), number of shoot fly deadhearts (SFDH), total number of plants with shoot fly eggs (TNPWE), days to flowering (DTF) and forage yield were significant at 5% level of significance and glossy score (GS), leaf sheath pigmentation (LSP), percent shoot fly deadhearts (SFDH 1 & 2 %), trichome density abaxial (TD_{aba}) and adaxial (TD_{ada}) were significant at 1 % level of significance for both lines and testers. For Line X Testers, all the traits were significantly different at 1% level of significance except GS and DTF (Table: 4.4 a).

4.4.2 General combining ability (GCA) effects of lines and testers

The positive combining ability for the traits like trichome density at both surfaces while negative combining ability for the traits like GS, SFDH, shoot fly eggs and DTF is considered good. Among the lines (Table 4.4) ICSA 487 showed significant and negative combining ability for the traits like GS (-0.3333**), SFDH (-3.715**), PSFDH (-11.7501**), TNE1 (-8.605**), PWSFE1 (-2.885**), SFDH2 (-3.165**), PSFDH (-3.8972**), TNE2 (-7.565**), PWSFE2 (-2.72**), and DTF (-3.065**), whereas a positive combining ability for the traits like TD_{aba} (7.1333**) and TD_{ada} (14.66**). Similarly, ICSA 84 recorded significant negative combining ability for traits like GS (-0.7333**), LSP (-0.5533**), PSFDH1 (-

9.2698**) and PSFDH2 (-11.9297**) while positive and significant combining ability for T_{aba} (11.2667**) and TD_{ada} (24.2933**). The other good combiners were ISCA 14037, ICSA 467, ICSA 458 and ICSA 14024 having significant general combining ability effects for the traits of importance for shoot fly resistance along with forage yield. Among the testers (Table 4.5) ICSV 700, ICSV 93046, ICSV 25337 and S-35 were found to be having significant negative combining ability for the traits like GS, LSP, SFDH, PSFDH and TNE while significant positive combining ability effects for the trichome density at both surfaces of leaf. The details are shown in table 4.3.

4.4.3 Specific combining ability (SCA) effects of lines

The SCA effects (Table 4.6) for GS ranged from -0.8667 (ICSV 14024 X ICSV 700) to 0.833 (ICSA 52 X S-35). Similarly, the SCA effects among the lines ranged from -0.88 (ICSA 14013 X ICSV 93046) to 1.62 (ICSA 487 X SSV 74) and the values for the SFDH ranged from -0.9085 (ICSA 467 X ICSV 25316) to 8.165 (ICSA 467 X ICSV 93046). Also, the SCA effects for the TD_{aba} ranged from -18.667 (ICSA 323 X ICSV 700) to 45.133 (ICSA 14024 X ICSV 700) and for TD_{ada} the value ranged from -35.4267 (ICSA 467 X ICSV 93046) to 45.74 (ICSA 14024 X ICSV 93046). The SCA effects of ICSA 14037 X SSV 74 (2.936) was found to be maximum for forage yield.

For any hybrid breeding program, selection of parents is the most critical step for the success of breeding program and studying the combining ability effects of the genotypes, forms a crucial platform to identify and select the parents with better specific and general combining abilities. Further these provides information regarding the heritability and components of variance based on which the choice of breeding program can be demarcated. The results in the present study revealed that the crosses involving the lines ICSA 458, ICSA 467, ICSA 487, ICSA 14024 and ICSA 14037 and testers ICSV 700, ICSV 93046, S-35, ICSV 25292 and ICSV 25337 had significant SCA and GCA effects for the traits important for shoot fly resistance. Thus, these lines and testers can be used in different combinations to achieve the desired objective of imparting resistance to shoot fly and with better yield. The mean sum of squares GCA and SCA for most of the traits were significant indicating the prevalence of both additive and dominance components. The heritability estimates (Table 4.7) revealed that low to moderate heritability for most of the traits with total number of eggs, plants with shoot fly eggs recorded minimum heritability estimates while for forage yield and days to flowering the estimates were highest. The same has been reported earlier for the traits important in shoot fly resistance (Mohammed *et al* 2015b).

Table 4.3: ANOVA for line x tester analysis including parents

Source	DF	Mean sum of squares							
		GS	LSP	TNP	SFDH1	SFDH1 (%)	TNE 1	TNPWE 1	DTF
Replications	2	1.452778*	1.002778**	180.075**	71.302083**	204.1235	1944.075**	85.852083**	45.01875*
Treatments	119	1.50803**	1.113702**	110.962185**	58.55667**	534.236587**	888.092437**	97.447006**	95.958561**
Parents	19	0.687719	0.325439**	108.165789**	65.913158**	363.781812**	1136.405921**	103.026316**	61.063816**
P. vs. C.	1	16.055556	12.33389	184.32	3.51125	2016.059	471.245	95.91125	888.3113
Crosses	99	1.518519	1.15165	110.757879**	57.700833**	551.982225**	844.646894**	96.391742**	94.65197*
Lines	9	5.392593**	3.260741**	216.703333*	120.4675*	1563.380323**	1264.041	146.434167*	270.0175*
Testers	9	5.362963**	2.616296**	236.22**	73.3675	409.7484	1310.140833*	203.934167*	280.284167*
Line X Testers	81	0.660905*	0.754568**	85.045926**	48.986019**	455.408415**	746.326019**	78.882315**	54.541111*
Error	238	0.475187	0.106419		8.999562	126.4245	126.0372	10.70923	3.84333

******, and ***** Significant at Fpr value 0.01% and 0.05% respectively

Table 4.3 contd...

		Mean sum of squares						
Source	DF	SFDH 2	SFDH2 (%)	TNE 2	TNPWE 2	Trichome Abaxial	Trichome Adaxial	Forage yield
Replications	2	95.408333**	284.971069**	1477.008333**	89.26875**	25.433333	23.725	1.268141
Treatments	119	78.389916**	247.436273**	618.456092**	96.38188**	461.306723**	2269.593277**	13.27622**
Parents	19	79.916447**	83.62776*	847.284868**	101.326974**	1.105263	982.091228**	10.379642**
P. vs. C.	1	59.405	773.02562	15.68	135.30125	1740.5	6094.08	55.77264
Crosses	99	78.288712**	273.565287**	580.628106**	95.039697**	536.707071**	2478.058451**	13.402872*
Lines	9	153.000833*	894.019843**	833.684167	140.336667*	1088.177778**	6401.146667**	58.168476**
Testers	9	93.63417	673.833199**	944.150833*	191.136667*	1182.859259**	11972.139259**	27.052997*
Line X Testers	81	68.282315**	160.151679**	512.119352**	79.329259**	403.63786**	987.261893**	6.912235**
Error	238	8.866317	45.74106	84.37388	9.697321	21.37451	90.111555	0.813854

**, and * Significant at Fpr value 0.01% and 0.05% respectively

Table 4.4: GCA effects due to lines

Lines	GS	LSP	TNP	SFDH 1	SFDH1 %	TNE1	TNPWE 1	SFDH 2	SFDH2 (%)	TNE 2	TNPWE 2	TD _{aba}	TD _{ada}	DTF	FY
ICSA 14013	-0.2	-0.0867	4.13**	2.735**	-0.3857	0.395	3.365**	3.685**	0.4681	-3.415**	2.43**	2.8333**	3.26	-2.39**	-2.3082**
ICSA 14024	0.4**	0.1467**	-0.07	0.185	1.4533	1.045	0.665	-0.415	-2.272*	2.385	0.73	2.1667**	-8.4733**	2.81**	1.028**
ICSA 14027	0.3667**	0.18**	1.63**	1.985**	4.2355*	11.295**	2.065**	2.485**	5.621**	6.935**	2.03**	-5.5667**	-11.2733**	0.01	1.6917**
ICSA 14037	0.2667*	0.28**	-2.12**	-0.465	7.9187**	1.045	-1.785**	-1.065*	5.746**	0.185	-1.42**	-6.0667**	-10.0733**	-1.89**	0.4269**
ICSA 323	-0.3667**	-0.3533**	-0.57	-1.415**	-7.8654**	-1.205	-0.735	-0.615	-0.6816	2.985*	-0.52	-1.3667	17.06**	3.11**	-0.5283**
ICSA 458	-0.1333	-0.2533**	-1.82**	-0.265	6.9191**	0.295	-0.785	-1.715**	-0.1454	0.185	-1.57**	-5.3333**	-10.0067**	-1.115**	0.8054**
ICSA 467	0.6333**	0.58**	0.18	1.735**	7.0315**	7.545**	0.815	1.435**	6.2913**	6.335**	0.88	-6.0667**	-20.3067**	4.96**	-2.2227**
ICSA 487	-0.3333**	-0.02	-2.97**	-3.715**	-11.7501**	-8.605**	-2.885**	-3.165**	-3.8972**	-7.565**	-2.72**	7.1333**	14.66**	-3.065**	0.0143
ICSA 52	0.1	0.08	-2.87**	-2.015**	1.7129	-10.755**	-3.035**	-2.465**	0.7996	-8.815**	-2.97**	1	0.86	-4.04**	1.4286**
ICSA 84	-0.7333**	-0.5533**	4.48**	1.235*	-9.2698**	-1.055	2.315**	1.835**	-11.9297**	0.785	3.13**	11.2667**	24.2933**	1.61**	-0.3357*
SE±	0.1259	0.0596	0.5739	0.5477	2.0528	2.0497	0.5975	0.5436	1.2348	1.677	0.5685	0.8441	1.7331	0.3579	0.1647

******, and ***** Significant at Fpr value 0.01% and 0.05% respectively

Table 4.5: GCA effects due to 10 testers

Testers	GS	LSP	TNP	SFDH1	SFDH1 (%)	TNE 1	TNPWE 1	SFDH 2	SFDH2 (%)	TNE 2	TNPWE 2	TD _{aba}	TD _{ada}	DTF	FY
Btx 623	0.4333**	0.2133**	-4.17**	-2.115**	7.4296**	-5.255**	-3.935**	-2.665**	7.2086**	-5.565**	-3.77**	-5.6667**	-15.5067**	-3.79**	-2.2264**
ICSV 25280	-0.3667**	-0.3867**	3.08**	1.585**	-1.1969	13.995**	3.065**	1.735**	-5.6809**	11.485**	3.23**	-5.9667**	-17.3733**	-3.74**	0.2611
ICSV 25292	0.1333	-0.0533	-2.17**	-1.765**	-2.5221	-7.255**	-2.135**	-1.915**	0.1709	-4.865**	-2.07**	-0.6667	-1.6067	2.035**	-0.4026**
ICSV 25303	0.1667	-0.0533	0.38	0.085	-0.1172	2.645	0.815	0.985*	3.4696**	1.685	0.28	-4.5667**	-11.9733**	-3.34**	-0.0863
ICSV 25316	0.4333**	0.3133**	-0.92	0.135	0.9048	-2.805	-1.085	-0.265	2.6624*	-3.365*	-0.92	2.9667**	-8.14**	2.785**	-0.1665
ICSV 25337	-0.2667*	-0.1533**	0.83	0.635	2.2352	2.495	0.515	0.585	-0.0794	0.235	0.38	1.0667	17.36**	-2.64**	0.0274
ICSV 700	-0.7**	-0.2867**	4.03**	2.535**	-2.0967	3.495*	3.565**	2.335**	-5.4738**	2.835*	3.53**	13.9667**	27.46**	0.16	1.0162**
ICSV 93046	-0.5**	-0.32**	3.38**	1.385**	-6.8618**	4.145*	3.065**	1.935**	-7.2651**	6.135**	2.88**	5.5333**	37.0933**	1.91**	1.2913**
S-35	0.2333*	0.28**	-2.42**	-1.415**	0.8413	-5.105**	-2.085**	-1.315**	3.8295**	-4.265**	-2.17**	-1.3333	-9.6067**	3.06**	0.4185**
SSV74	0.4333**	0.4467**	-2.02**	-1.065*	1.3838	-6.355**	-1.785**	-1.415**	1.1584	-4.315**	-1.37**	-5.3333**	-17.7067**	3.56**	-0.1327
SE±	0.1259	0.0596	0.5739	0.5477	2.0528	2.0497	0.5975	0.5436	1.2348	1.677	0.5685	0.8441	1.7331	0.3579	0.1647

**, and * Significant at Fpr value 0.01% and 0.05% respectively

Table 4.6: SCA effects due to 10 lines

Cross	GS	LSP	TNP	SFDH 1	SFDH1 %	TNE1	TNPWE1
ICSA 14013 X BTx 623	0.6	-0.08	-7.53**	-5.335**	1.7959	-13.045*	-6.315**
ICSA 14013 X ICSV 25280	0.7333*	1.1867**	-9.28**	-5.035**	7.8116	-18.795**	-7.815
ICSA 14013 X ICSV 25292	-0.1	-0.1467	-0.03	0.315*	0.6961	8.455	1.385
ICSA 14013 X ICSV 25303	-0.8*	-0.8133**	10.42**	3.965*	-12.7361	1.055	9.435**
ICSA 14013 X ICSV 25316	0.2667	0.4867**	2.72	4.415**	9.7713	34.505**	2.835
ICSA 14013 X ICSV 25337	-0.0333	-0.0467	1.97	-1.585	-16.9076**	-8.795	-1.265
ICSA 14013 X ICSV 700	-0.2667	0.0867	-3.73*	0.515	13.2523*	-10.295	-0.815
ICSA 14013 X ICSV 93046	-0.8*	-0.88**	1.92	-2.835	-15.3676**	-22.445**	-4.315*
ICSA 14013 X S-35	0.8*	0.52**	1.22	0.965	-0.7079	15.805**	3.335*
ICSA 14013 X SSV74	-0.4	-0.3133*	2.32	4.615**	12.392**	13.555*	3.535*
ICSA 14024 X BTx 623	0	0.02	4.17*	5.215**	10.1902	11.305	4.885**
ICSA 14024 X ICSV 25280	0.1333	-0.38	-3.08*	-1.985	-2.6372	-11.945*	-1.615
ICSA 14024 X ICSV 25292	0.6333*	0.62**	1.17	2.865	13.6631**	12.305*	-1.415
ICSA 14024 X ICSV 25303	0.2667*	-0.38*	-1.38	-1.485	-3.876	-8.095	-0.365
ICSA 14024 X ICSV 25316	0.6667*	0.2533	-3.08*	-0.535	19.4539	0.355	-3.965*
ICSA 14024 X ICSV 25337	0.0333	-0.28	3.67*	5.965**	12.0163*	16.055**	5.435**
ICSA 14024 X ICSV 700	-0.8667*	-0.1467	2.97	-2.435	-20.9884**	7.055	4.385*
ICSA 14024 X ICSV 93046	-0.7333*	-0.1133	-2.38	-3.285*	-8.2444	-12.095*	-1.615
ICSA 14024 X S-35	-0.1333	0.2867	0.92	1.015	1.4861	-4.845	-1.965
ICSA 14024 X SSV74	0	0.12	-2.98	-5.335**	-21.0636**	-10.095	-3.765*

Cross	GS	LSP	TNP	SFDH 1	SFDH1 %	TNE1	TNPWE1
ICSA 14027 X BTx 623	0.0333	0.32*	-3.53*	-3.585*	-4.8227	-21.445**	-2.015
ICSA 14027 X ICSV 25280	-0.1667	-0.08	-0.78	-2.285	-11.496*	15.305**	-0.515
ICSA 14027 X ICSV 25292	0.3333	-0.08	-5.53	-3.935*	4.4388	-15.945**	-4.815**
ICSA 14027 X ICSV 25303	-0.3667	0.2533	-1.08	-3.285*	-18.3102**	-1.845	-0.265
ICSA 14027 X ICSV 25316	0.3667	-0.78**	2.22	2.165	5.7935	-0.395	0.635
ICSA 14027 X ICSV 25337	-0.6	-0.3133*	6.47**	0.665	-20.7242**	25.805**	7.535**
ICSA 14027 X ICSV 700	0.5	0.4867**	-9.73**	-6.235**	9.9134	-38.695**	-12.015**
ICSA 14027 X ICSV 93046	0.3	-0.1467	4.42**	5.915**	10.1845	24.655**	5.985**
ICSA 14027 X S-35	-0.4333	0.2533	5.72**	6.715**	10.7741	-1.095	3.635
ICSA 14027 X SSV74	0.0333	0.0867	1.82	3.865*	14.2488*	13.655*	1.835
ICSA 14037 X BTx 623	-0.2	-0.4467**	-0.28	-0.135	5.8327	-1.695	-0.165
ICSA 14037 X ICSV 25280	-0.0667	-0.18	1.97	3.665*	5.6369	5.555	-0.665
ICSA 14037 X ICSV 25292	0.1	0.4867**	5.22**	4.515**	2.7888	11.805*	4.535**
ICSA 14037 X ICSV 25303	0.0667	0.4867**	2.67	1.165	-7.526	0.905	3.585*
ICSA 14037 X ICSV 25316	-0.2	-0.5467**	0.47	1.615	12.6506*	17.355**	1.985
ICSA 14037 X ICSV 25337	-0.1667	0.2533	-8.28**	-6.385**	0.5906	-21.445**	-8.115
ICSA 14037 X ICSV 700	0.2667	-0.28	2.02	0.215	-9.3036	9.055	2.835
ICSA 14037 X ICSV 93046	0.7333	0.0867	-4.83**	-2.635	5.4599	-4.595	-4.165
ICSA 14037 X S-35	-0.6667*	0.1533	1.47	-0.335	-8.2844	-6.345	0.485
ICSA 14037 X SSV74	0.1333	-0.0133	-0.43	-1.685	-7.8457	-10.595	-0.315*
ICSA 323 X BTx 623	0.1	0.52**	3.17*	5.815**	19.576**	8.555	3.285*

Cross	GS	LSP	TNP	SFDH 1	SFDH1 %	TNE1	TNPWE1
ICSA 323 X ICSV 25280	0.5667	0.4533**	-9.58**	-4.385**	18.6574**	-29.695	-8.215
ICSA 323 X ICSV 25292	0.0667	0.12	-2.33	-3.535*	-17.4894**	6.055	-0.015
ICSA 323 X ICSV 25303	0.3667	0.12	1.12	2.615	10.5596	3.655	-3.465*
ICSA 323 X ICSV 25316	-0.5667	-0.2467	2.42	0.565	-4.7949	-3.395	0.935
ICSA 323 X ICSV 25337	-0.2	0.5533**	2.17	0.565	-5.138	-1.195	1.835
ICSA 323 X ICSV 700	0.2333	-0.3133*	5.97**	5.165**	6.0692	16.305**	7.285**
ICSA 323 X ICSV 93046	0.0333	-0.28	0.62	-2.685	-16.7291**	6.155	2.285
ICSA 323 X S-35	-0.3667	-0.5467**	-2.08	-4.385**	-21.31	0.905	-2.565
ICSA 323 X SSV74	-0.2333	-0.38*	-1.48	0.265	10.5992	-7.345	-1.365
ICSA 458 X BTx 623	-0.8*	-0.2467	0.42	-1.335	-11.2056*	10.055	1.335
ICSA 458 X ICSV 25280	0.3333	0.02	1.17	0.965	-8.9502	-2.695	-0.165
ICSA 458 X ICSV 25292	-0.5	-0.6467**	-5.58**	-4.185**	7.7365	-19.445**	-5.965**
ICSA 458 X ICSV 25303	-0.2	0.02	-3.13	-2.535	-3.2795	-7.845	-3.915*
ICSA 458 X ICSV 25316	0.2	0.6533**	1.67	0.415	-1.2317	-7.395	1.985
ICSA 458 X ICSV 25337	0.2333	0.12	-2.08	-0.585	5.0447	-12.195	-2.615
ICSA 458 X ICSV 700	0	0.2533	4.72**	5.015**	3.5007	35.805**	4.835**
ICSA 458 X ICSV 93046	0.1333	0.2867	4.87**	3.165*	-1.61	1.655	4.835**
ICSA 458 X S-35	0.4	0.02	1.17	1.965	5.4754	5.905	1.985
ICSA 458 X SSV74	0.2	-0.48**	-3.23*	-2.885*	4.5198	-3.845	-2.315
ICSA 467 X BTx 623	-0.5667	-0.7467**	-5.08**	-5.335**	-2.6055	-8.695	-5.265**
ICSA 467 X ICSV 25280	0.5667	0.52**	-4.33*	-3.535**	-2.994	-13.945*	-4.265*

Cross	GS	LSP	TNP	SFDH 1	SFDH1 %	TNE1	TNPWE1
ICSA 467 X ICSV 25292	0.0667*	0.1867	2.92	3.315*	9.2343	2.305	3.935*
ICSA 467 X ICSV 25303	0.0333	0.1867	6.37**	6.965**	7.7475	14.405*	2.985
ICSA 467 X ICSV 25316	-0.2333	-0.18	-9.33**	-9.085**	-15.9555**	-29.145**	-7.615**
ICSA 467 X ICSV 25337	0.4667	0.2867	-0.58	-0.585	-1.349	-8.945	1.285
ICSA 467 X ICSV 700	0.2333	0.42*	-6.28**	-3.485*	6.9249	-9.445	-8.765**
ICSA 467 X ICSV 93046	0.3667	0.4533**	7.87**	8.165**	8.6761	37.905**	7.235**
ICSA 467 X S-35	-0.7*	-0.8133	6.67**	2.965*	-8.9501	16.655*	7.885**
ICSA 467 X SSV74	-0.2333	-0.3133*	1.77	0.615	-0.7287	-1.095	2.585
ICSA 487 X BTx 623	0.0667*	-0.48**	5.07**	0.115	-24.0981	7.455	3.435*
ICSA 487 X ICSV 25280	-0.8*	-0.5467**	11.82**	6.415**	-4.0076	27.705**	12.935**
ICSA 487 X ICSV 25292	0.0333	-0.2133	-3.43*	-3.235*	-15.9158	-8.545	-2.365
ICSA 487 X ICSV 25303	0	-0.2133	-7.98**	-4.085**	12.096*	-2.445	-6.315**
ICSA 487 X ICSV 25316	-0.2667	0.42*	-7.68**	-6.635**	-22.8149**	-18.995**	-6.415**
ICSA 487 X ICSV 25337	0.4333	-0.1133	-0.43	3.365*	25.9089**	3.205	-1.015
ICSA 487 X ICSV 700	-0.1333	0.02	2.37	2.465	6.7718	-1.795	0.435
ICSA 487 X ICSV 93046	0	0.0533	0.52	0.115	0.1104	-2.445	1.935
ICSA 487 X S-35	0.2667	-0.5467**	-4.18*	-1.085	21.8518**	-8.195	-2.915
ICSA 487 X SSV74	0.4	1.62**	3.92*	2.565	0.0976	4.055	0.285
ICSA 52 X BTx 623	-0.0333	-0.2467	1.47	1.415	1.5692	7.105	1.585
ICSA 52 X ICSV 25280	-0.5667	-0.6467**	-0.78	1.715	9.716	-7.645	0.085
ICSA 52 X ICSV 25292	-0.0667	0.02	0.47	1.065	0.8862	-2.395	-1.715

Cross	GS	LSP	TNP	SFDH 1	SFDH1 %	TNE1	TNPWE1
ICSA 52 X ICSV 25303	0.5667	0.02	-5.58**	-2.285	17.5056**	-10.295	-3.665*
ICSA 52 X ICSV 25316	0.3	0.6533**	4.72*	0.665	-13.8928*	2.155	2.735
ICSA 52 X ICSV 25337	-0.3333	-0.2133	-2.03	-0.335	5.9935	4.855	-0.865
ICSA 52 X ICSV 700	-0.2333	-0.08	7.27**	3.265*	-11.018*	9.355	7.085**
ICSA 52 X ICSV 93046	-0.7667*	-0.0467	-2.58	-1.085	3.0449	-3.295	-2.415
ICSA 52 X S-35	0.8333	0.6867**	-1.28	-1.785	-0.6588	-5.045	-2.265
ICSA 52 X SSV74	0.3	-0.1467	-1.68	-2.635	-13.1457*	5.205	-0.565
ICSA 84 X BTx 623	0.8*	1.3867**	2.12	3.165	3.7679	0.405	-0.765
ICSA 84 X ICSV 25280	-0.7333*	-0.3467*	12.87**	4.465	-11.7371*	36.155**	10.235**
ICSA 84 X ICSV 25292	-0.5667	-0.3467*	7.12**	2.815	-6.0384	5.405	6.435**
ICSA 84 X ICSV 25303	0.0667	0.32*	-1.43	-1.035	-2.181	10.505	1.985
ICSA 84 X ICSV 25316	-0.5333	-0.7133**	5.87**	6.415	11.0204*	4.955	6.885**
ICSA 84 X ICSV 25337	0.1667	-0.2467	-0.88	-1.085	-5.4352	2.655	-2.215
ICSA 84 X ICSV 700	0.2667	-0.4467**	-5.58**	-4.485	-5.1223	-17.345**	-5.265**
ICSA 84 X ICSV 93046	0.7333*	0.5867**	-10.43**	-4.835	14.4753*	-25.495**	-9.765**
ICSA 84 X S-35	0	-0.0133	-9.63**	-6.035	0.3239	-13.745*	-7.615**
ICSA 84 X SSV 74	-0.2	-0.18	-0.03	0.615	0.9264	-3.495	0.085
SE±	0.398	0.1883	1.8149	1.732	6.4916	6.4817	1.8894

**, and * Significant at Fpr value 0.01% and 0.05% respectively

Table 4.6 contd...

Cross	SFDH2	SFDH2%	TNE2	TNPWE2	TD _{aba}	TD _{ada}	DTF	Yield
ICSA 14013 X BT _x 623	-6.685**	2.6892	-11.035**	-5.53**	-3.2333	-8.3933	6.99**	1.6964**
ICSA 14013 X ICSV 25280	-6.585**	7.526*	-18.085**	-9.03**	-2.9333	-6.5267	-1.56	-0.6411
ICSA 14013 X ICSV 25292	1.065	4.2977	6.765	0.77	-8.2333**	-22.2933**	-3.335**	2.4166**
ICSA 14013 X ICSV 25303	7.665**	-9.1614	3.715	7.92**	9.6667**	22.4067**	10.04**	-0.3677
ICSA 14013 X ICSV 25316	4.415**	7.2355*	16.265**	4.12*	-11.8667**	-15.76**	8.415**	-0.7035
ICSA 14013 X ICSV 25337	-1.435	-15.4416**	-2.335	2.32	0.0333	10.4067*	0.84	-0.5324
ICSA 14013 X ICSV 700	-2.185	3.5247	-8.435	-2.83	17.1333**	31.9733**	-7.46**	-0.3082
ICSA 14013 X ICSV 93046	-2.285	-13.2955	-21.735**	-6.18**	10.5667**	8.6733	4.29**	-0.6433
ICSA 14013 X S-35	2.465	6.0684	15.665**	3.87*	-7.5667**	-14.2933**	-8.36**	-0.8925*
ICSA 14013 X SSV74	3.565*	6.557*	19.215**	4.57**	-3.5667	-6.1933	-9.86	-0.0243
ICSA 14024 X BT _x 623	4.415**	2.6648	7.665	4.17*	-2.5667	3.34	-6.21**	-2.6637**
ICSA 14024 X ICSV 25280	-2.485	-0.3343	-7.385	-2.83	-2.2667	5.2067	-2.76**	0.0988
ICSA 14024 X ICSV 25292	1.665	2.7135	9.965*	0.47	-7.5667**	-10.56	1.965*	-0.4176
ICSA 14024 X ICSV 25303	-0.735	3.3142	-0.585	-0.88	-3.6667	-0.1933	6.84**	0.2332
ICSA 14024 X ICSV 25316	-2.985*	-0.2744	0.465	-3.18*	-11.2**	-4.0267	3.715**	2.2383**
ICSA 14024 X ICSV 25337	5.165**	7.9555*	12.365*	4.52**	-9.3**	-28.86**	-2.36*	1.329**
ICSA 14024 X ICSV 700	0.415	-8.3852*	1.765	3.87*	45.1333**	-13.6267**	4.84**	-1.6734**
ICSA 14024 X ICSV 93046	-3.185	-5.1157	-14.535**	-1.48	1.2333	45.74**	1.59	0.7176
ICSA 14024 X S-35	1.565	5.1333	-2.135	0.07	-6.9**	-2.56	-8.56**	-0.4257

Cross	SFDH2	SFDH2%	TNE2	TNPWE2	TD _{aba}	TD _{ada}	DTF	Yield
ICSA 14024 X SSV74	-3.835*	-7.6715*	-7.585	-4.73**	-2.9	5.54	0.94	0.5636
ICSA 14027 X BTx 623	-3.985*	-2.4636	-13.885**	-3.13*	5.1667*	6.14	-3.91**	0.8086
ICSA 14027 X ICSV 25280	-0.885	-2.2993	13.565**	-0.13	5.4667*	8.0067	-2.46*	-2.7439**
ICSA 14027 X ICSV 25292	-4.735**	4.5741	-10.085*	-4.83**	0.1667	-5.4267	-1.235	-0.4583
ICSA 14027 X ICSV 25303	-1.135	-1.1921	-5.635	-0.18	4.0667	2.6067	-4.36**	0.301
ICSA 14027 X ICSV 25316	2.115	-0.161	-3.585	0.52	-3.4667	-1.2267	6.015**	1.9236**
ICSA 14027 X ICSV 25337	4.765**	-6.892*	18.815**	6.22**	-1.5667	17.2733**	1.94*	-3.0537**
ICSA 14027 X ICSV 700	-6.985**	10.2188**	-28.785**	-10.43**	-14.4667**	-8.4933	-2.86**	0.9989*
ICSA 14027 X ICSV 93046	4.915**	4.2114	16.915**	5.22**	-1.0333	-27.46**	-2.61*	0.3549
ICSA 14027 X S-35	4.665**	-3.8095	9.815*	5.27**	0.8333	0.24	4.74**	0.7101
ICSA 14027 X SSV74	1.265	-2.1868	2.865	1.47	4.8333*	8.34	4.74**	1.1589*
ICSA 14037 X BTx 623	-0.935	-2.5887	-2.635	-0.18	5.6667*	4.94	0.49	-1.1877*
ICSA 14037 X ICSV 25280	3.665*	7.6918*	2.315	0.32	5.9667*	6.8067	1.44	1.2309**
ICSA 14037 X ICSV 25292	4.815**	-1.1594	9.165*	4.62**	0.6667	-8.96	-5.835**	-0.865*
ICSA 14037 X ICSV 25303	0.415	-10.0218**	-1.885	3.27*	4.5667*	1.4067	-0.96	-0.1157
ICSA 14037 X ICSV 25316	-0.335	-5.2097	14.165**	0.97	-2.9667	-2.4267	-4.585**	-3.5426**
ICSA 14037 X ICSV 25337	-7.185**	4.6994	-12.435*	-7.33**	-1.0667	-27.9267**	1.34	-1.4454**
ICSA 14037 X ICSV 700	2.565	0.7143	10.465*	3.02*	-13.9667**	13.9733**	-0.46	1.2347**
ICSA 14037 X ICSV 93046	-3.035*	8.2221*	-3.335	-3.83*	-5.5333*	6.0067	-2.71**	2.2367**
ICSA 14037 X S-35	0.715	-2.6663	-2.935	0.22	1.3333	-0.96	5.64**	-0.4786
ICSA 14037 X SSV74	-0.685	0.3183	-12.885**	-1.08	5.3333*	7.14	5.64**	2.9326**

Cross	SFDH2	SFDH2%	TNE2	TNPWE2	TD _{aba}	TD _{ada}	DTF	Yield
ICSA 323 X BTx 623	3.615*	3.839	4.565	2.92	4.9667*	10.14*	6.49**	0.0436
ICSA 323 X ICSV 25280	-7.785**	1.4654	-25.985	-8.58**	1.2667	3.6733	1.94*	0.9601*
ICSA 323 X ICSV 25292	-3.635*	-10.319**	4.865	-1.28	18.9667**	-1.0933	-1.335	-1.8973**
ICSA 323 X ICSV 25303	0.965	-1.1615	3.315	-1.63	-0.1333	-1.7267	-1.46	2.0405**
ICSA 323 X ICSV 25316	1.215	-5.2656	-3.635	0.07	2	2.44	0.415	0.8486
ICSA 323 X ICSV 25337	1.365	-4.7804	-1.235	1.77	-0.7667	25.2733**	-0.66	-0.5882
ICSA 323 X ICSV 700	5.115**	-0.754	20.165**	7.12**	-18.6667**	-13.8267**	1.54	1.0959*
ICSA 323 X ICSV 93046	1.015	4.5353	7.865	1.77	-10.2333**	-30.7933**	-1.21	1.5469**
ICSA 323 X S-35	-1.235	7.2182*	-3.235	-1.18	-3.3667	1.24	-5.36**	-1.3539**
ICSA 323 X SSV74	-0.635	5.2226	-6.685	-0.98	5.9667*	4.6733	-0.36	-2.6961**
ICSA 458 X BTx 623	-0.785	-7.1251*	10.865*	0.97	4.9333*	4.8733	-3.285**	0.3529
ICSA 458 X ICSV 25280	2.315	5.5315	-5.185	1.97	6.2333*	10.74*	-0.335	1.0404*
ICSA 458 X ICSV 25292	-4.535**	3.7928	-16.335**	-5.23**	-0.0667	-1.6933	0.39	-1.3079**
ICSA 458 X ICSV 25303	-2.435	3.2719	-3.385	-2.08	3.8333	1.34	-4.235**	-1.6172**
ICSA 458 X ICSV 25316	2.815	7.849*	-0.335	2.62	2.6333	4.5067	0.39	1.1907*
ICSA 458 X ICSV 25337	-0.535	6.7921*	-13.935**	-2.68	-1.8	-26.9933**	0.065	1.1711*
ICSA 458 X ICSV 700	2.215	-9.5544	22.965**	4.17*	-14.7**	-10.4267*	0.265	0.6202
ICSA 458 X ICSV 93046	4.615**	2.9818	9.165*	6.32**	-6.2667*	9.6067*	1.015	0.1162
ICSA 458 X S-35	0.865	-2.2158	1.565	-3.13*	0.6	-1.0267	3.865**	0.5634
ICSA 458 X SSV74	-4.535**	-11.3237**	-5.385	-2.93	4.6*	9.0733	1.865	-2.1298**
ICSA 467 X BTx 623	-6.435**	-10.1181**	-12.785**	-4.98**	5.6667*	15.1733**	4.64**	0.2315

Cross	SFDH2	SFDH2%	TNE2	TNPWE2	TD _{aba}	TD _{ada}	DTF	Yield
ICSA 467 X ICSV 25280	-4.335**	-3.4344	-3.835	-3.98*	5.9667*	17.04**	6.59**	0.8775*
ICSA 467 X ICSV 25292	2.815	1.197	2.015	3.32*	0.6667	2.2733	-3.185**	2.9302**
ICSA 467 X ICSV 25303	5.915**	-1.6914	11.465*	3.97*	4.5667*	11.64*	0.19	0.6569
ICSA 467 X ICSV 25316	-9.335**	1.4123	-25.485**	-8.83**	-2.9667	7.8067	-5.435**	-0.1429
ICSA 467 X ICSV 25337	-0.185	1.507	-6.585	0.37	-1.0667	-17.6933**	3.99**	-0.2188
ICSA 467 X ICSV 700	-3.935*	9.5485**	-8.685	-7.28**	-13.9667**	-27.7933**	0.69	-1.4876**
ICSA 467 X ICSV 93046	9.965**	11.3398**	34.015**	8.87**	-5.5333*	-35.4267**	-1.56	-2.1972**
ICSA 467 X S-35	4.215**	-9.4446**	7.915	6.92**	1.3333	9.2733*	-2.71**	-1.323**
ICSA 467 X SSV74	1.315	-0.316	1.965	1.62	5.3333*	17.7067**	-3.21**	0.6733
ICSA 487 X BTx 623	5.165**	3.341	6.115	4.12*	-7.5333**	-7.46	-0.335	-0.405
ICSA 487 X ICSV 25280	8.265**	-4.1689	25.565**	12.62**	-7.2333**	-14.5933**	-0.385	0.013
ICSA 487 X ICSV 25292	-3.085*	-8.8244*	-8.085	-2.58	1.8	31.9733**	4.59**	0.0534
ICSA 487 X ICSV 25303	-6.985**	2.4603	-6.635	-6.93**	-8.6333**	-14.9933**	-2.285*	-1.6111**
ICSA 487 X ICSV 25316	-7.235**	-8.9547*	-16.085**	-7.23**	41.5**	0.84	-1.91*	-1.101*
ICSA 487 X ICSV 25337	0.415	3.5587	-0.685	-0.53	4.4	34.6733	-4.485**	1.6042**
ICSA 487 X ICSV 700	1.665	-2.717	0.215	0.32	-2.1667	2.5733	0.215	0.5883
ICSA 487 X ICSV 93046	0.065	-1.0907	-1.085	1.47	-4.4	10.2733*	0.465	-2.4777**
ICSA 487 X S-35	-3.685*	3.2909	-9.685*	-3.48*	-11.8667**	-25.6933**	5.815**	2.2565**
ICSA 487 X SSV74	5.415**	13.1048**	10.365*	2.22	-5.8667*	-17.5933**	-1.685	1.0793*
ICSA 52 X BTx 623	0.965	-2.8611	7.365	1.87	-1.4	0.6733	-2.86**	-0.1134
ICSA 52 X ICSV 25280	-3.435*	-19.2269**	-5.185	-0.63	-1.1	-2.7933	-0.91	-0.3699

Cross	SFDH2	SFDH2%	TNE2	TNPWE2	TD _{aba}	TD _{ada}	DTF	Yield
ICSA 52 X ICSV 25292	1.715	9.3954**	-7.335	-2.83	-6.4**	-16.2267**	6.315**	0.8808*
ICSA 52 X ICSV 25303	-5.185**	0.4323	-8.385	-4.18*	-2.5	4.4733	-2.31*	-1.6974**
ICSA 52 X ICSV 25316	5.565**	6.904*	6.665	4.02*	-8.7**	-4.0267	-6.935**	-1.0403*
ICSA 52 X ICSV 25337	-0.785	6.2291	6.065	-1.28	-8.1333**	2.4733	0.49	0.9519*
ICSA 52 X ICSV 700	5.965**	-2.1804	4.465	7.07**	5.3*	24.04**	1.19	-0.238
ICSA 52 X ICSV 93046	-2.135	-1.1706	-0.335	-1.78	30.4**	4.74	-1.06	-0.407
ICSA 52 X S-35	-0.885	5.7369	-3.935	-1.23	-5.7333*	-11.8933*	3.29**	2.2187**
ICSA 52 X SSV74	-1.785	-3.2587	0.615	-1.03	-1.7333	-1.46	2.79**	-0.1856
ICSA 84 X BTx 623	4.665**	12.6226**	3.765	-0.23	-11.6667**	-29.4267**	-2.01	1.2369**
ICSA 84 X ICSV 25280	11.265**	7.2493*	24.215**	10.27**	-11.3667**	-27.56**	-1.56	-0.4656
ICSA 84 X ICSV 25292	3.915*	-5.6678	9.065*	7.57**	0	32.0067**	1.665	-1.3349**
ICSA 84 X ICSV 25303	1.515	13.7494**	8.015	0.72	-11.7667**	-26.96**	-1.46	2.1773**
ICSA 84 X ICSV 25316	3.765*	-3.5352	11.565*	6.92**	-4.9667*	11.8733*	-0.085	0.329
ICSA 84 X ICSV 25337	-1.585	-3.6278	-0.035	-3.38*	19.2667**	11.3733*	-1.16	0.7821
ICSA 84 X ICSV 700	-4.835**	-0.4152	-14.135**	-5.03**	10.3667**	1.6067	2.04*	-0.8307
ICSA 84 X ICSV 93046	-9.935**	-10.6178**	-26.935**	-10.38**	-9.2**	8.64	1.79	0.7527
ICSA 84 X S-35	-8.685**	-9.3115**	-13.035**	-7.33**	31.3333**	45.6733**	1.64	-1.275**
ICSA 84 X SSV 74	-0.085	-0.446	-2.485	0.87	-12**	-27.2267**	-0.86	-1.3718**
SE $\pm$	1.7191	3.9047	5.3033	1.7979	2.6692	5.4806	1.1319	0.5208

**, and * Significant at Fpr value 0.01% and 0.05% respectively

Table 4.7: Estimation of genetic components

Genetic components	GS	LSP	TNP	SFDH1	SFDH1 (%)	TNE 1	TNPWE 1	SFDH 2
Cov. H.S. (Line)	0.1577	0.0835	4.3886	2.3827	36.9324	17.2572	2.2517	2.824
Cov. H.S. (Tester)	0.1567	0.0621	5.0391	0.8127	-1.522	18.7938	4.1684	0.8451
Cov. H.S. (Average)	0.0087	0.004	0.2619	0.0888	0.9836	1.0014	0.1783	0.1019
Cov. F.S.	1.0795	0.6872	55.5639	23.6696	224.2533	323.4279	43.5006	31.6787
Heritability	0.337311	0.347692	0.272833	0.254599	0.347974	0.142628	0.153403	0.244539

******, and ***** Significant at Fpr value 0.01% and 0.05% respectively

Table 4.7 contd...

Genetic components	SFDH2 (%)	TNE 2	TNPWE 2	Trichome Abaxial	Trichome Adaxial	DTF	Forage yield
Cov. H.S. (Line)	24.4623	10.7188	2.0336	22.818	180.4628	7.1825	1.7085
Cov. H.S. (Tester)	17.1227	14.401	3.7269	25.974	366.1626	7.5248	0.6714
Cov. H.S. (Average)	1.1551	0.6978	0.16	1.3553	15.184	0.4085	0.0661
Cov. F.S.	172.7105	223.8725	41.8522	285.3176	2067.9907	64.4938	9.7344
Heritability	0.365961	0.130049	0.146258	0.259053982	0.286559435	0.347391	0.489357

******, and ***** Significant at Fpr value 0.01% and 0.05% respectively

4.5 Biochemical components

The resistance against insect pest constitutes tolerance with minimal losses, anti-feeding or repelling and affecting the insect's biology thusly resisting the feeding and growth of insects. These traits are governed by wide array of complex regulatory networks which are involved in production of secondary metabolites (Charmarathi *et al* 2011). The herbivory and insect resistance are contributed to some extent by these secondary metabolites and have significant effects on insect behaviour and biology (Painter, 1958; Schoonhoven, 1968). The plants lacking such mechanisms are prone to substantial damage caused by insect pest in terms of yield and growth. In sorghum, the presence of certain nutritional and antinutritional compounds have been associated with the effects on developmental biology of insects and conferring resistance or susceptibility against shoot fly (Raina, 1985). Along with various micronutrients, the biochemical constituents like total amount of sugars, proteins, tannin and phenol content in sorghum has been associated with the resistance or susceptibility against shoot fly. In the present study, the lyophilized leaf powder was used to estimate the content of total soluble sugars, proteins, tannins and total phenols in the test genotypes. The leaf samples were collected during rainy and post rainy seasons in 2017. The analysis of variance (Appendix 9 and 10) revealed significant variation for these compounds in the test genotypes.

4.5.1 Total Soluble sugars (%)

TSS concentration in the sorghum genotypes varied for both the seasons with higher values during rainy season (Fig. 4.1). The value varied from 8.63 to 17.01 % with an average value of 14.99 % ($SE \pm 0.30$) during rainy season. ICSA 52 (17.01) recorded maximum TSS content, followed by ICSA 323 (16.99), ICSB 84 (16.86) and ICSV 25292 (16.72) while the SSV 74 recorded minimum TSS (8.63) followed by ICSV 25303 (12.15), ICSV 25316 (12.62), ICSB 458 (13.09) and ICSA 84 (13.54). In the post rainy season, the recorded value was relatively less than the rainy season and ranged from 4.05 to 14.51 % with a mean value of 6.53 % ($SE \pm 0.35$). The lowest value was recorded for ICSA 467, followed by ICSB 52, ICSB 467, ICSB 14024 and ICSB 487 and the maximum value was reported for ICSA 52.

4.5.2 Proteins

The concentration of protein (Fig. 4.2) in the test genotypes also varied for rainy and post rainy seasons (higher during rainy season) but the differences were not substantial as for TSS content. During rainy season the value ranged from 1.20 to 2.58 (mg equivalent of catechin/g of sample) with an average value of 1.82 ($SE \pm 0.06$). ICSB 467 recorded the minimum value of 1.20 followed by ICSB 323 (1.27), ICSB 14027 (1.32), ICSB 84 (1.37) and then ICSA 467 (1.44). Whereas ICSV 700 recorded highest amount of proteins (2.58) followed by ICSA 52 (2.45), ICSB 14037 (2.33) and then ICSB 52 (2.28). During post rainy season, the value ranged from 1.03 to 2.20 with a mean value of 1.59 ($SE \pm 0.05$). ICSB 467 reported the

minimum value followed by ICSB 14013, ICSV 25280, BTx 623, IS 18551 and the ICSB 14037 reported to be having highest value of 2.20 mg/g of sample.

4.5.3 Tannins

In the rainy season the tannin content (Fig. 4.3) varied from 18.64 to 56.62 with an average value of 38.48 (SE $\pm$ 1.64). BTx 623 (56.62) and ICSA 458 (56.62) recorded the maximum value followed by ICSV 25280 (51.22) and ICSV 25316 (48.61) while the ICSB 84 (18.64) recorded the minimum value followed by ICSA 323 (24.22), ICSB 323 (25.378), ICSA 14013 (27.35) and ICSB 14027 (27.53). Tannin content varied from 9.06 to 59.23 with an average of 27.83 (SE $\pm$ 2). ICSB 52 (59.23) recorded a maximum value of followed by ICSB 487 (47.91). ICSA 14037 (9.06) recorded the minimum value of followed by, Swarna (12.54), ICSB 84 (13.07), ICSV 25337 (16.72) and ICSV 25292 (16.90).

4.5.4 Total Phenols

The phenolic content (Fig. 4.4) of the test genotypes was not significantly different across seasons. During rainy season it ranged from 6.57 to 11.30 (mg/g sample) with an average value of 9.06 (SE $\pm$ 0.20). BTx 623 (11.30) recorded maximum value followed by ICSA 14037(11.23), ICSB 52 (10.50) and ICSB 458 (10.44) whereas ICSB 323 recorded lowest value (6.57) followed by ICSB 14027 (7.03), ICSV 25280 (7.68), ICSV 25337 (7.80) and ICSB 467 (7.85). In post rainy season, the amount of total phenols ranged from 7.62 to 11.68 of the samples with an average value of 10.73 (SE $\pm$ 0.17). ICSV 25316 (11.68) and ICSA 14037 (11.66) recorded maximum phenol content of followed by S-35 (11.54) whereas ICSB 467 recorded minimum phenol content (7.62) followed by ICSB 84 (8.18), ICSB 14013 (9.34), ICSA 467 (9.58) and ICSB 458 (9.86).

The higher content of total soluble sugars and tannins have been related with midge susceptibility in sorghum (Mohan *et al.*, 1997) and similar results were obtained in the present study for total soluble sugars, where the genotypes exhibiting moderate (ICSA 84, ICSB 52, ICSB 14024) and high (ICSA 467, and ICSB 487) resistance to shoot fly recorded lesser content of total soluble sugars (during post rainy season). While the tannin content was found to be high in most of the resistant genotypes with few exceptions which is in corroboration with the results of (Kamatar *et al* 2003 and Charmarthi *et al* 2010). Similarly, the higher protein content has been associated with shoot fly susceptibility (Kamatar *et al*, 2003; Singh *et al*, 2004). The resistant genotypes (ICSA/B 467, IS 18551, ICSB 487, ICSB 84, ICSB 14024 and ICSV 25292) showed lower protein content than the susceptible ones which exhibited higher protein concentration per gram samples. There are reports suggesting the role of phenolic compounds in imparting resistance against some insects in other cereals like aphids in wheat (Juan *et al* 2001), stemborer in maize (Kabre and Ghorpade, 1998) but in present studies, no such associations were found against shoot fly, where susceptible genotype (BTx 623) recorded maximum total phenols, which is in corroboration with Charmarthi *et al* (2011) and Riyazadin *et al* (2015).

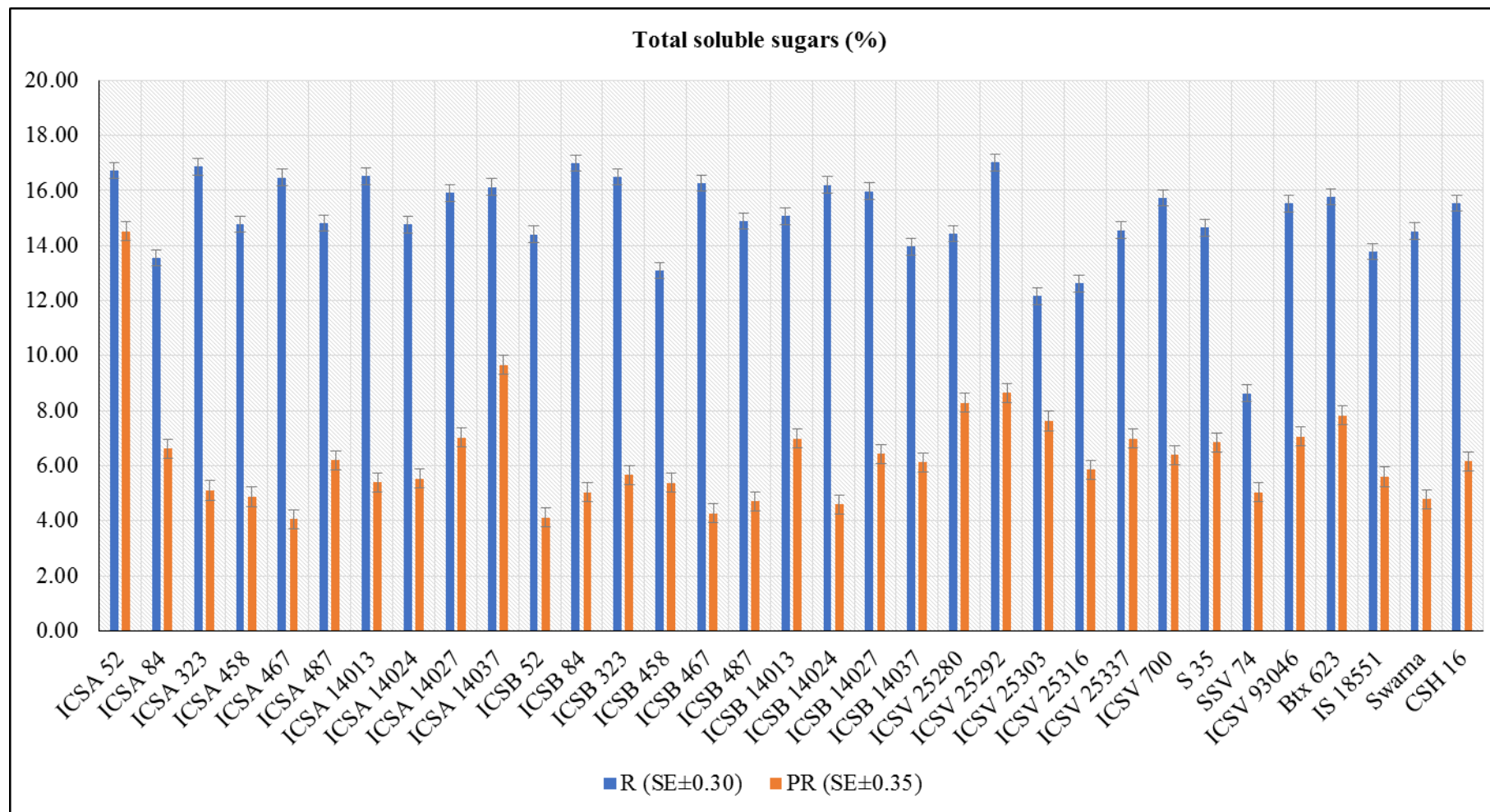


Fig. 4.1: TSS (%) in test genotypes during rainy and post rainy, 2017

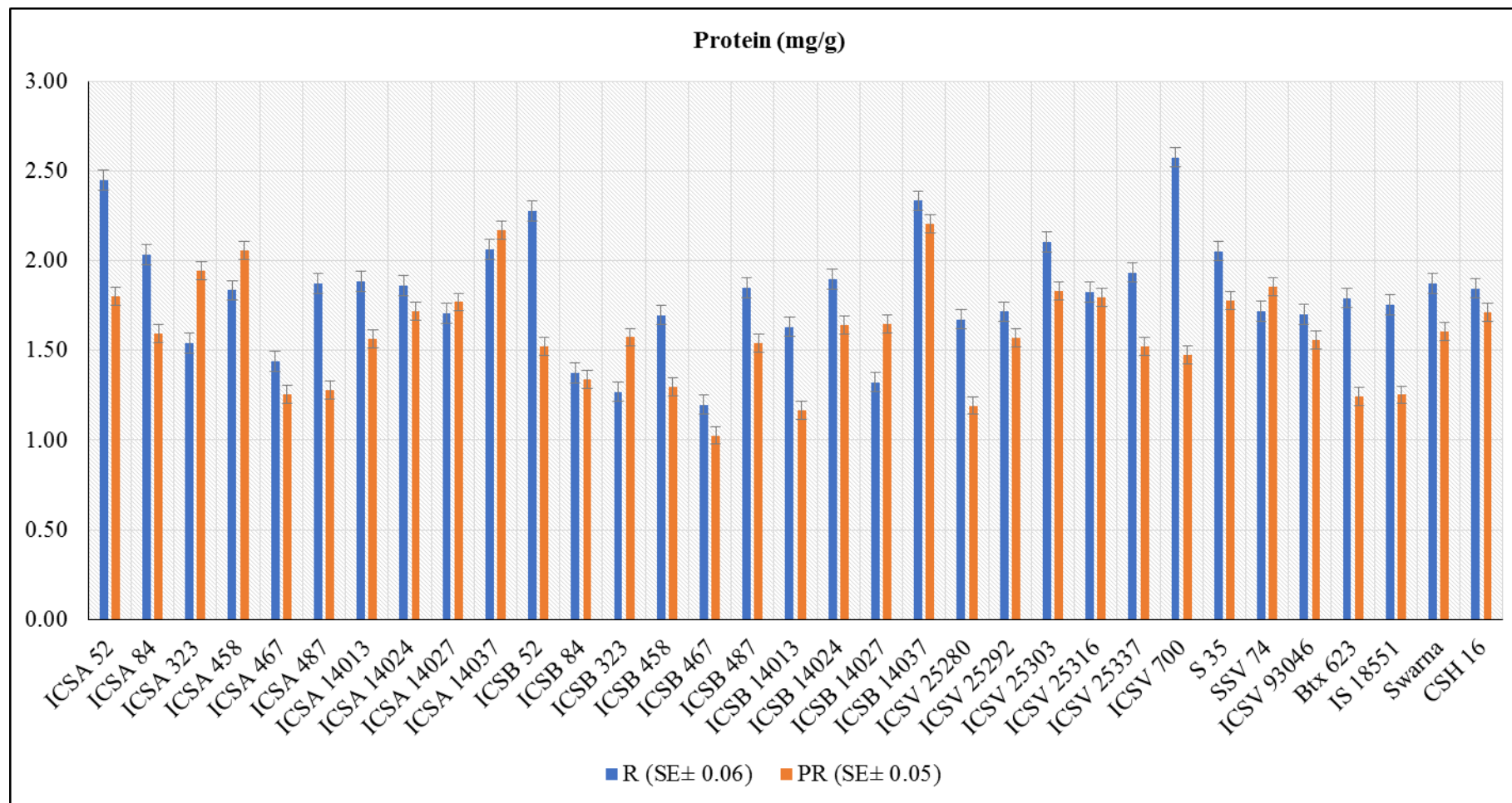


Fig. 4.2: Protein content in test genotypes during rainy and post rainy, 2017

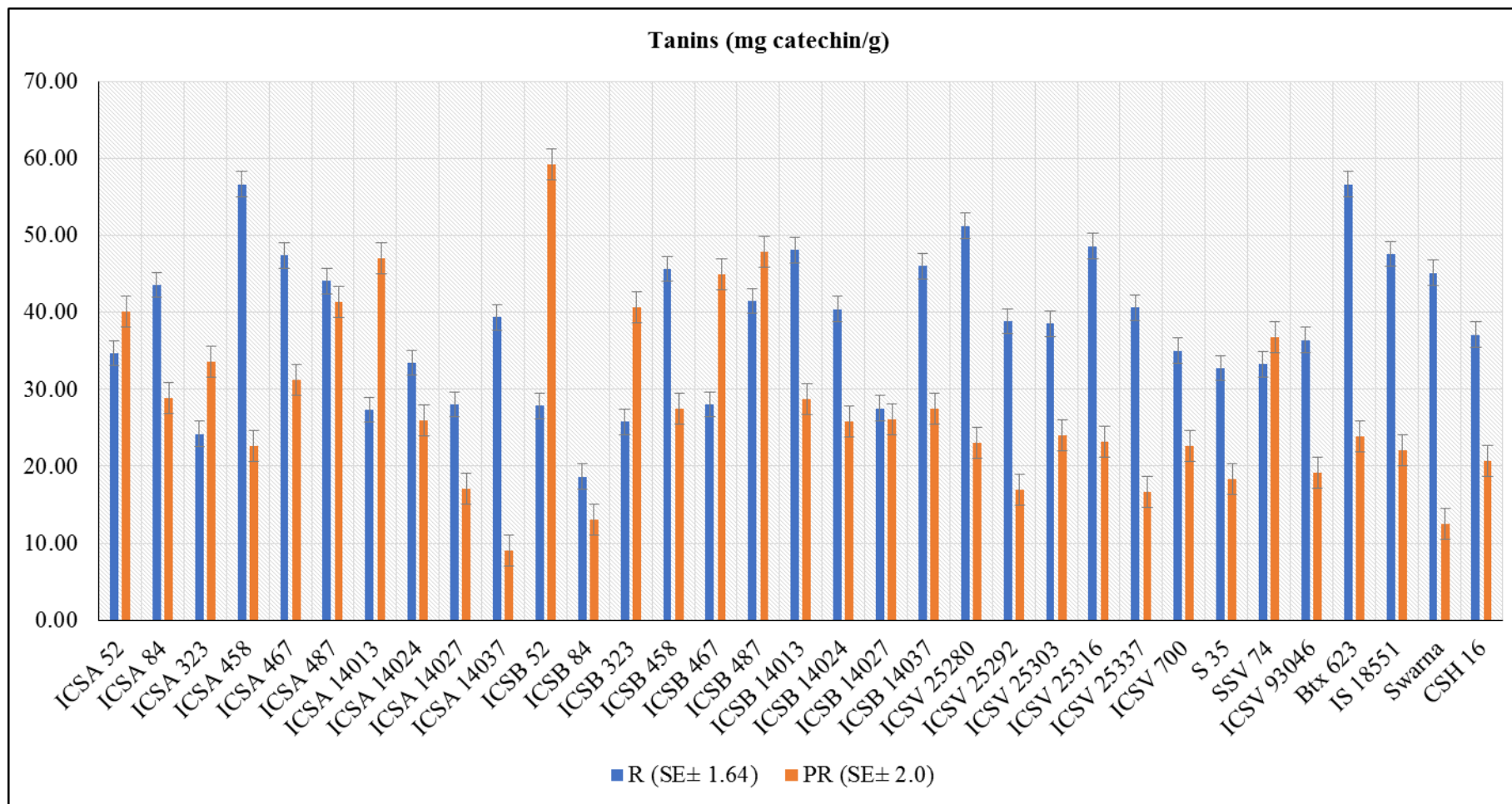


Fig. 4.3: Tannin content in test genotypes during rainy and post rainy, 2017

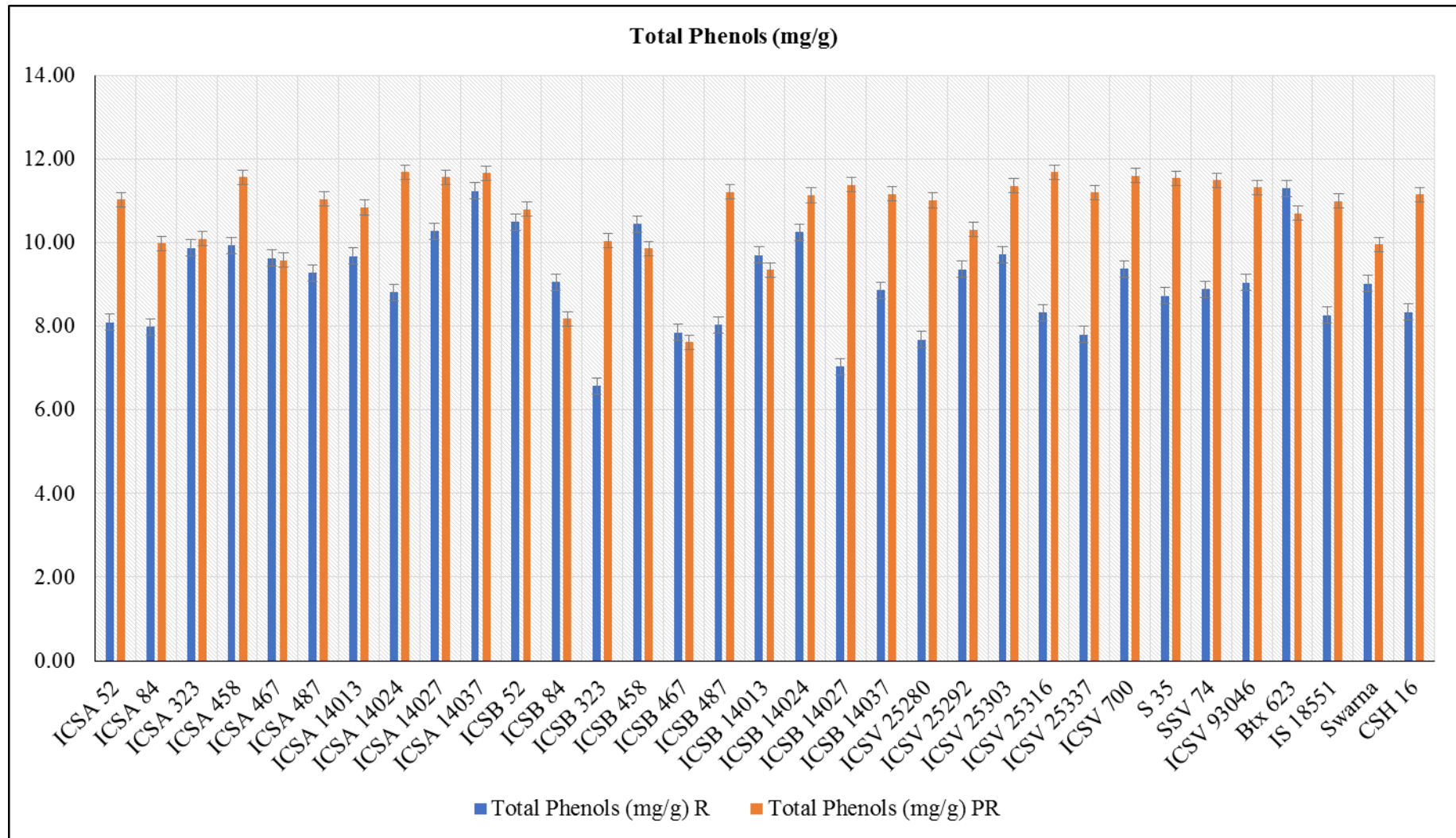


Fig. 4.4: Phenol content in test genotypes during rainy and post rainy, 2017

4.6 HPLC fingerprints

Resistance to different biotic and abiotic stress is the outcome of interaction of several factors like morphological, biochemical, environmental and molecular imparting resistance or susceptibility to a genotype. Resistance to sorghum shoot fly is also an outcome of interaction between various morphological and biochemical factors (Ogwaro 1978, Delobel 1982, Raina 1982). 3,4 Dihydroxy Benzoic acid is found in prominent concentrations during seedling stage in sorghum suggesting that it is a by-product of dhuririn hydrolysis. Low concentrations of some phenolics like Gentisic, Vanillic, Protocatechuic, γ -resorcylic acids (Woodhead and Bernays 1978) and their role in fungal diseases has been reported earlier (Hahn *et al*, 1983). In sorghum several phenolic compounds have been correlated with shoot fly resistance and susceptibility based on the presence or absence and even, the concentrations of these compounds (Pandey *et al* 2005).

The HPLC analysis of methanol extracts of leaf samples yielded chromatograms containing 25 different peaks in total with varying peak areas among genotypes (Fig 4.5). From 25 different peaks a total of 12 compounds were identified (matching with the reference set library) and quantified (Table 4.8-4.10). The phenolic constituents and their concentration varied in rainy and post rainy seasons. Many common peaks were observed in retention time 2.89 to 13.96 min. IS 18551 (resistant check) recorded 13 different peaks in total with four known compounds (3,7,4 Trihydroxy flavone, Coumaric acid, Gentisic acid and Protocatechuic acid) and Swarna (susceptible check) recorded 12 peaks in total with four known compounds (3,7,4 Trihydroxy flavone, Gentisic acid, Protocatechuic acid and Naringenin). The checks had 7 peaks in common with three known compounds in both with varying concentrations. They differed for Naringenin (in Swarna but absent in IS 18551) and coumaric acid (in IS 18551) while for other similar compounds there was variation in terms of their concentration. Swarna had higher concentration of Protocatechuic acid and 3,7,4-Trihydroxy flavone while IS 18551 had higher Gentisic acid content. During the rainy season when there was severe shoot fly attack, the absence of Protocatechuic acid in IS 18551 (suffered more deadhearts incidence) suggested its putative role in shoot fly resistance. Among the test entries, Gentisic acid was present in almost all genotypes during both the seasons with a few exceptions. Vanillic acid which has been associated with susceptibility of shoot fly (Pandey *et al*. 2005) was present in only one susceptible genotype (ICSA 323) during rainy season, when there was substantial shoot fly pressure while it was found in few genotypes (ICSA/B 14037, ICSB 458, ICSB 14037) which exhibited moderate to high

resistance to shoot fly in both the seasons. During rainy season, 3,4 Dihydroxy Benzoic acid was present only in A-lines (except ICSA- 487, 14024 and 14027) and absent in B and R-lines with few exceptions (ICSB 52 and ICSV-25280, 25292 respectively). Similarly, during post rainy season, it was found only in A-lines, except few an absent in B and R-lines. This compound has been associated with induction of oviposition and feeding of maggots in shoot fly (Alborn *et al.* 1992) but no such association could be made in the present study. Protocatechuic and Coumaric acid have been associated with resistance against shoot fly. Although, both of these compounds were found in most of the genotypes exhibiting susceptible and resistant reaction, but the concentration in the resistant was relatively more than susceptible genotypes. Along with Protocatechuic and coumaric acid, Naringenin was reported in most of the moderately to highly resistant genotypes (ICSB 84, ICSA- 467, 487, 14027 and ICSV-25292 700) during both the seasons in varying concentrations. The susceptible check (Swarna) also recorded the presence of Naringenin, during post rainy season only where as it was absent in resistant check (IS 18551). Contrasting results for Ferulic acid was found in present study where it was found in moderate to high resistance genotypes like ICSA/B 14037 and ICSB 14027) while three genotypes (ICSB 14013, ICSV 25303 and ICSV 25316) showing susceptibility to shoot fly also recorded this in relatively high concentrations. Certain compounds like Formononetin (ICSA 52, ICSA 14037) and Fisetin (ICSA 52) were present in few genotypes only suggesting their limited contribution towards resistance to shoot fly. Chlorogenic acid was also present in few genotypes with susceptible genotypes having relatively higher concentration then the resistant ones.

The biochemical pathways of the identified compounds should be studied to make stronger associations with the resistance against shoot fly. Also, there were a large number of peaks of unidentified compounds which could be associated with the resistance or susceptibility to shoot fly. Emphasis should be given to identify these compounds and understanding their putative role in shoot fly resistance or susceptibility.

Table 4.8: Flavonoids detected in male sterile (A) lines for rainy (R) and post rainy season (PR)

Flavonoids (µg/100µg)	Season	ICSA 52	ICSA 84	ICSA 323	ICSA 458	ICSA 467	ICSA 487	ICSA 14013	ICSA 14024	ICSA 14027	ICSA 14037
3,4 Dihydroxy Benzoic acid	R	0.30	1.39	0.36	0.23	0.42	-	0.91	-	-	0.53
	PR	0.25	0.31	0.31	0.04	-	0.23	-	0.27	0.52	0.53
Protocatechuic acid	R	-	-	-	-	-	-	0.90	0.71	0.63	0.67
	PR	-	-	-	-	-	-	0.90	0.74	0.36	0.54
Chlorogenic acid	R	4.70		3.16	2.47	2.84	2.49	-	-	-	-
	PR	4.96	3.92	5.07	3.00	1.39	2.72	-	-	-	-
Fisetin	R	1.03	-	-	-		-	-	-	-	-
	PR	5.04	-	-	-		-	-	-	-	-
3,7,4 Trihydroxy flavone	R	-	-	-	-	0.07	-	-	-	2.09	4.70
	PR	1.41	-	0.08	-	-	-	10.74	-	1.53	0.05
Gentisic acid	R	10.59	15.15	8.42	10.27	6.63	9.42	7.72	3.90	8.29	12.95
	PR	17.30	11.81	6.57	10.27	4.61	9.42	5.53	3.67	9.69	12.95
Vanillic acid	R	-	-	1.14	-	-	-	-	-	-	-
	PR	-	-	-	1.16	-	-	-	-	-	0.91
Coumaric acid	R	0.82	-	-	2.48	3.51	2.42	0.26	-	0.21	1.12
	PR	1.99	-	1.40	2.60				-	2.43	1.12
Ferulic acid	R	-	-	-	-	-	-	-	-		0.84
	PR	-	-	-	-	-	-	-	-	0.28	0.67
Formononetin	R	1.45	-	-	-	-	-	-	-	-	10.95
	PR	1.45	-	-	-	-	-	-	-	0.44	-
Naringenin	R	-	-	-	-	0.50	0.49	-	-	1.07	-
	PR	-	-	-	0.43	-	0.15	0.05	-	-	-

Table 4.9: Flavonoids detected in maintainer (B) lines for rainy (R) and post rainy season (PR)

Flavonoids (µg/100µg)	Replication	ICSB 52	ICSB 84	ICSB 323	ICSB 458	ICSB 467	ICSB 487	ICSB 14013	ICSB 14024	ICSB 14027	ICSB 14037
3,4 Dihydroxy Benzoic acid	R	0.29	-	-	-	-	-	-			
	PR	-	-	-	-	-	-	-	0.55	0.47	32.29
Naringin	R	-	-	-	-	-	-	-	-	-	
	PR	-	-	-	-	-	-	-	-	-	21.61
Gallic acid	R	-	-	-	-	-	-	-	-	-	
	PR	-	-	-	-	-	-	-	-	-	0.29
Protocatechuic acid	R	0.95	1.00	0.87	0.03	0.73	0.52	0.69	1.05	1.15	1.00
	PR	1.01	1.02	0.49	0.68	0.59	0.97	0.72	0.71	0.63	-
3,7,4 Trihydroxy flavone	R	-	-	5.14	0.23	0.78	1.61	10.83	2.97	2.87	6.59
	PR	0.02	5.63	1.72	7.92	5.57	0.38	2.38	1.53	1.46	5.77
Gentisic acid	R	9.40	6.58	2.88	0.60	6.45	5.75	6.62	5.48	6.04	5.58
	PR	8.84	6.21	-	5.37	5.30	10.20	10.05	16.93	12.72	20.65
Vanillic acid	R	-	-	-	-	-	-	-	-	-	-
	PR	-	-	-	-	-	-	-	-	0.04	0.79
Coumaric acid	R	1.08	2.69	-	0.35	-	-	0.27	-	1.86	-
	PR	1.40	1.67	-	1.13	-	2.90	0.15	1.99	-	-
Ferulic acid	R	-	-	-	-	-	-	0.33	-	0.42	0.53
	PR	-	-	-	-	-	0.86	0.66	0.62	0.97	0.70
Naringenin	R	-	0.90	-	-	-	-	-	-	-	-
	PR	-		-	-	-	1.21	-	0.91	1.17	2.93

Table 4.10: Flavonoids detected in restorer (R) lines for rainy (R) and post rainy season (PR)

Flavonoids (µg/100µg)	Season	ICSV 25280	ICSV 25292	ICSV 25303	ICSV 25316	ICSV 25337	ICSV 700	S 35	SSV 74	ICSV 93046	Btx 623	IS 18551	Swarna
3,4 Dihydroxy Benzoic acid	R	0.60	0.62	-	-	-	-	-	-	-	-	-	-
	PR	-	-	-	-	0.40	-	-	-	-	-	-	-
Protocatechuic acid	R	-	-	0.72	0.61	0.80	1.25	0.88	0.53	0.63	0.58	-	0.61
	PR	-	0.16	1.12	0.90	1.65	0.66	-	0.74	1.04	1.01	0.70	0.93
3,7,4 Trihydroxy flavone	R	1.70	2.17	4.01	2.58	2.03	3.53	5.33	1.31	3.27	2.36	1.31	2.59
	PR	-	1.00	2.55	3.24	5.08	7.23	-	3.45	6.41	10.02	4.35	10.42
Gentisic acid	R	-	-	4.40	5.48	5.00	10.77	5.04	3.48	3.28	-	6.03	2.47
	PR	7.95	1.28	4.43	8.73	10.79	7.07	-	5.67	5.20	-	8.54	5.66
Coumaric acid	R	-	-	0.21	1.17	1.36	2.19	-	0.86	1.00	-	1.67	-
	PR	-	-	1.15	1.67	-	0.91	-	0.99	-	-	1.30	-
Ferulic acid	R	-	-	-	-	-	-	-	-	-	-	-	-
	PR	-		1.22	0.88	-	-	-	-	-	-	-	-
Naringenin	R	-	0.50	-	-	-	1.66	-	-	-	-	-	0.76
	PR	-	0.50	-	-	-	1.66	-	-	-	-	-	0.76

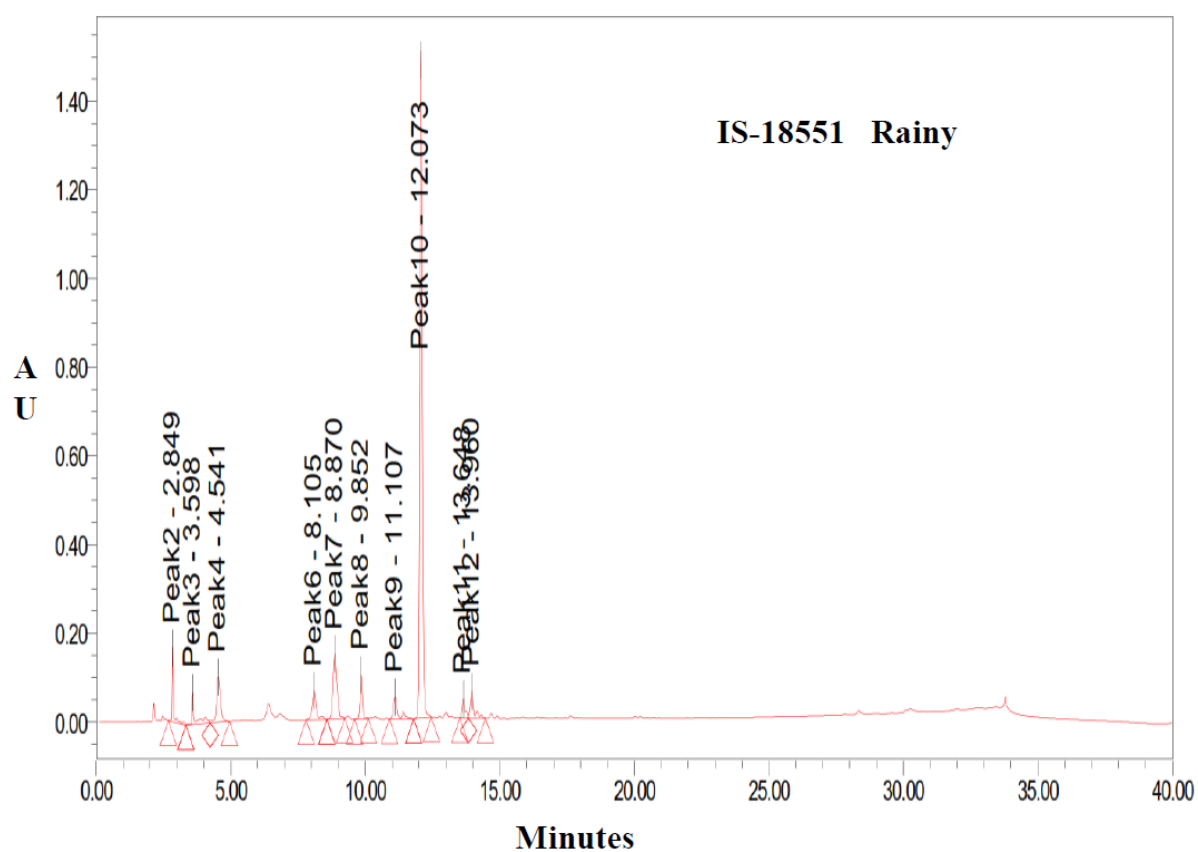
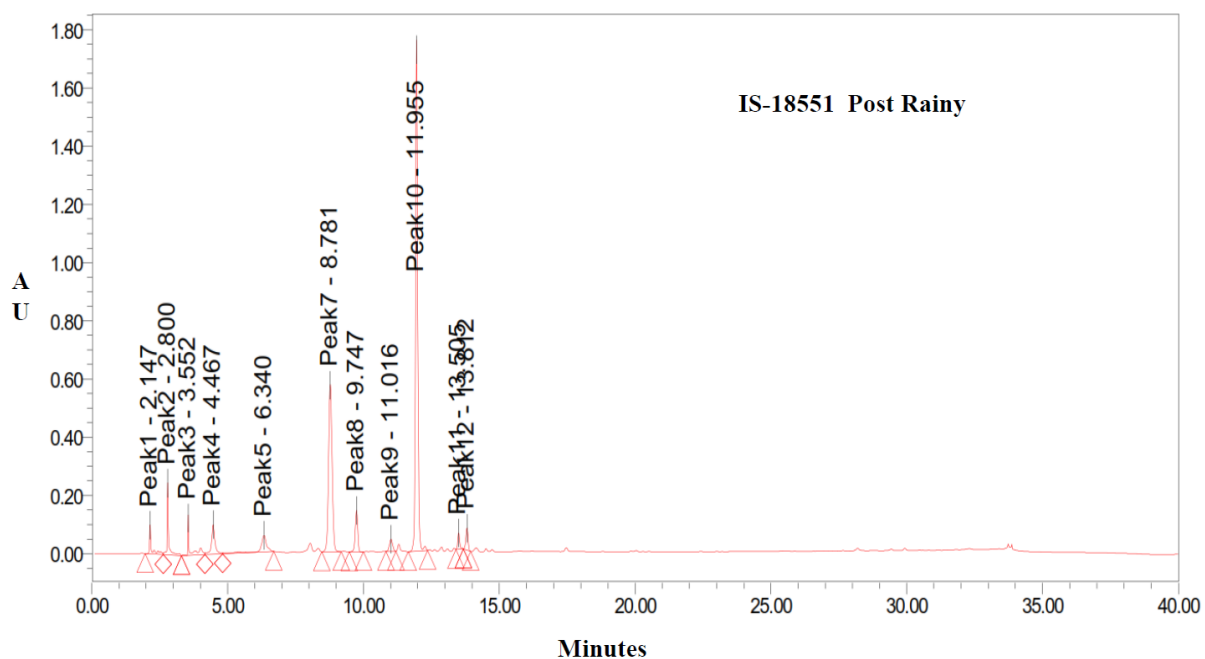


Fig. 4.5: HPLC chromatographic profile of phenolic compounds of five genotypes

Fig. 4.5 contd....

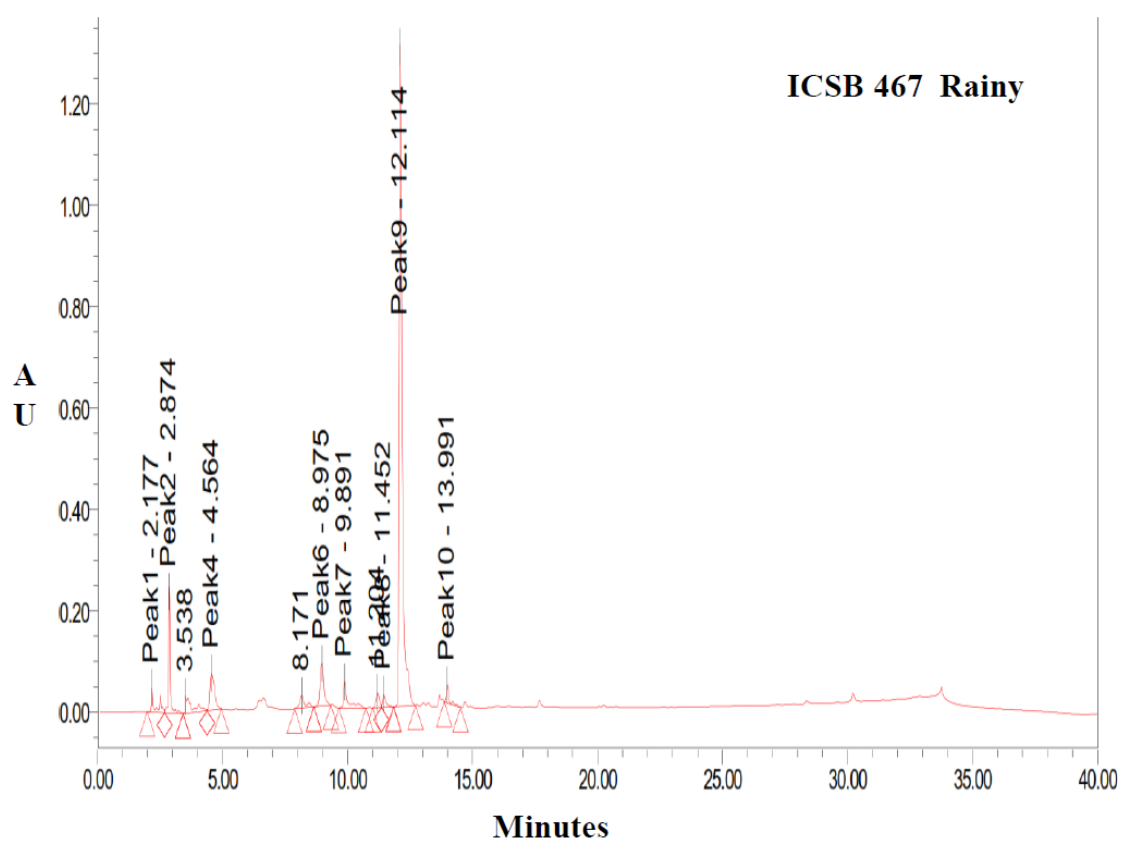
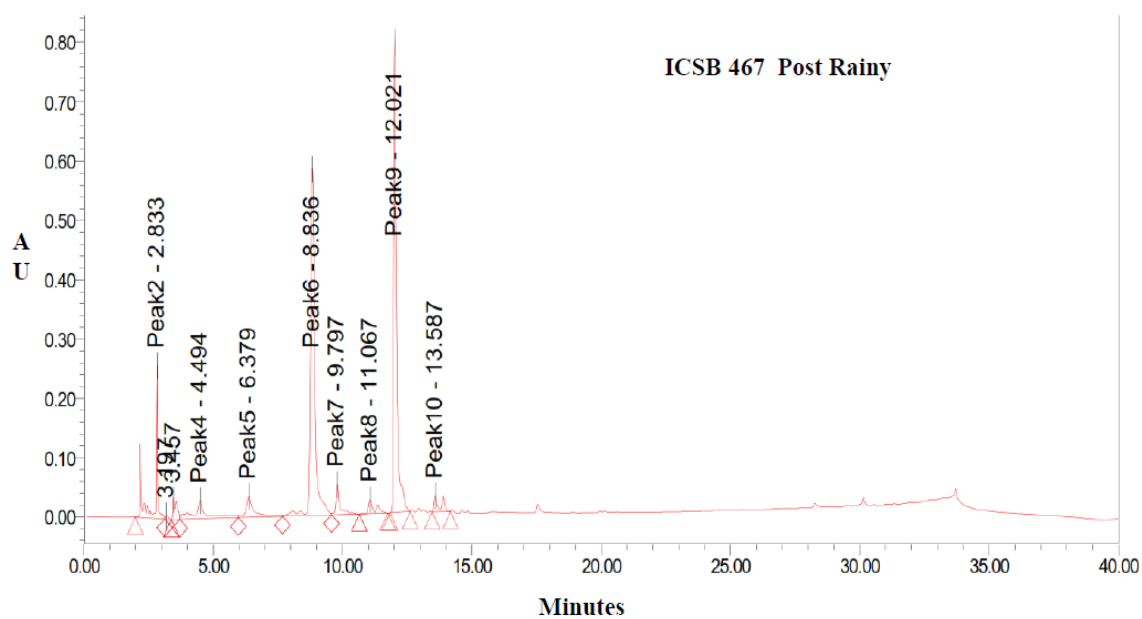


Fig. 4.5 contd....

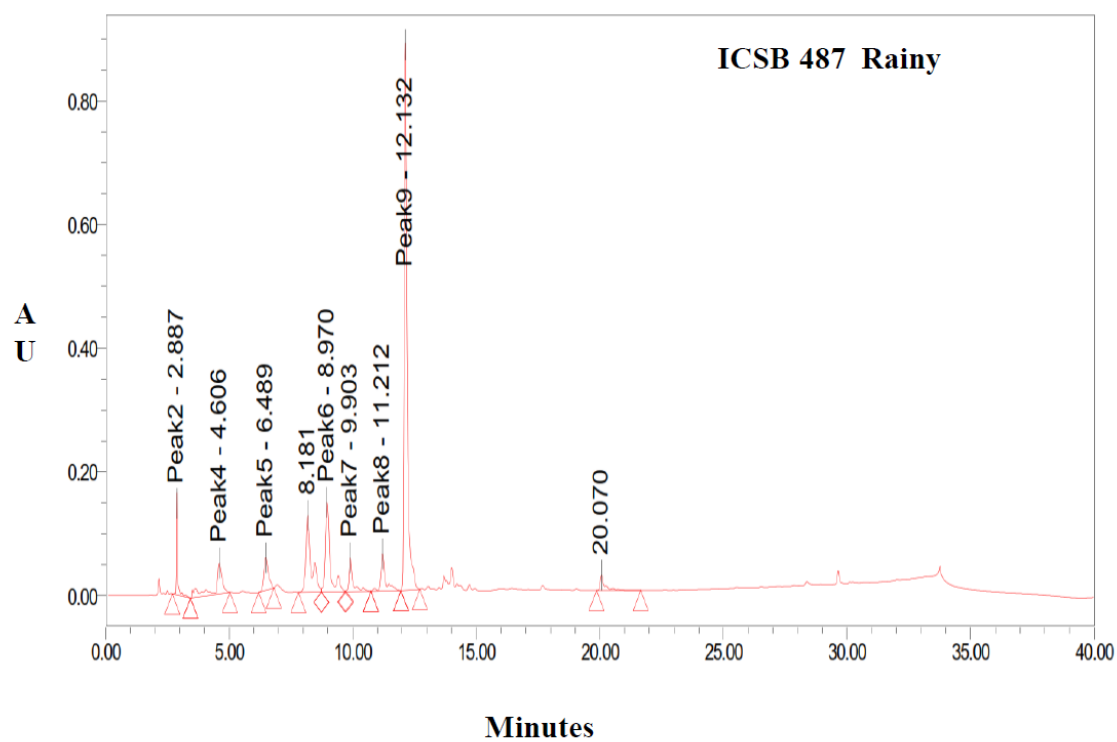
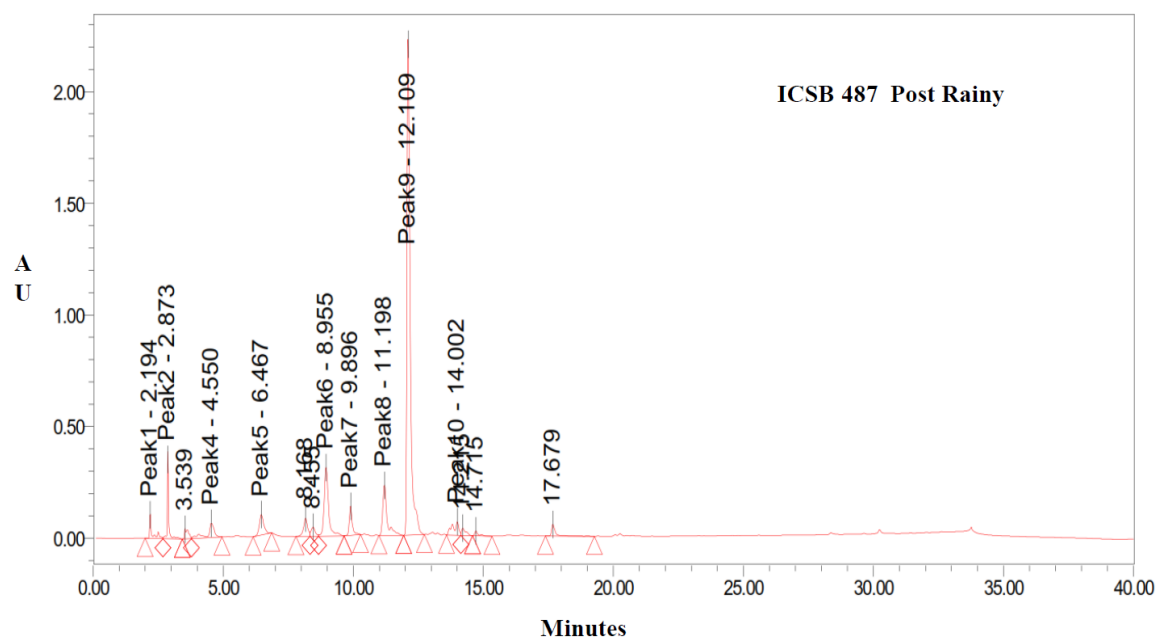


Fig. 4.5 contd....

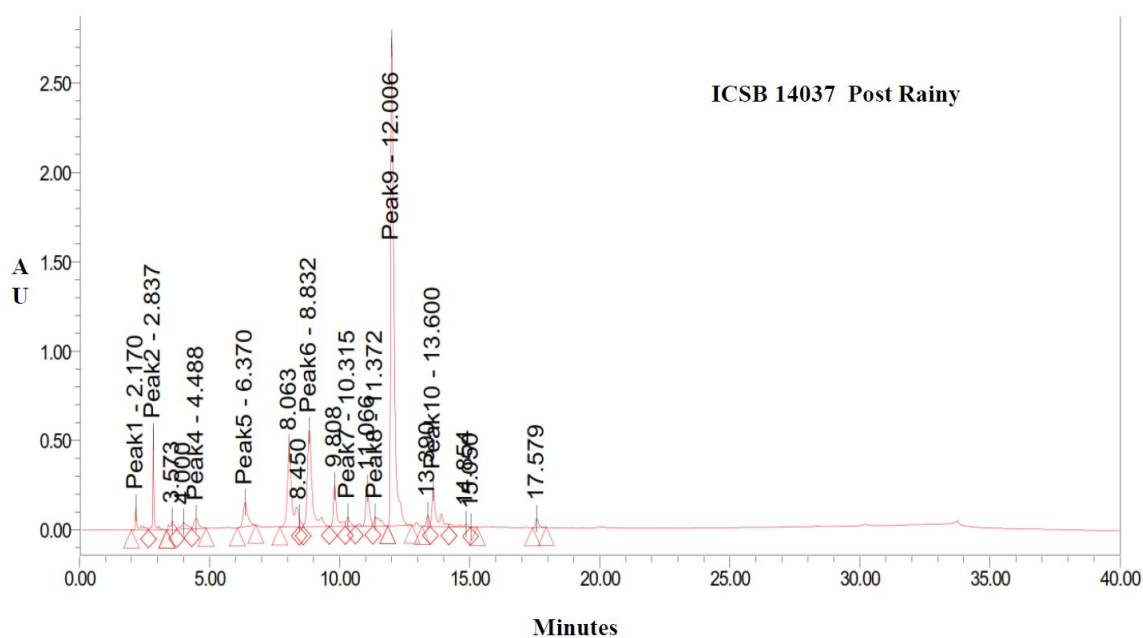
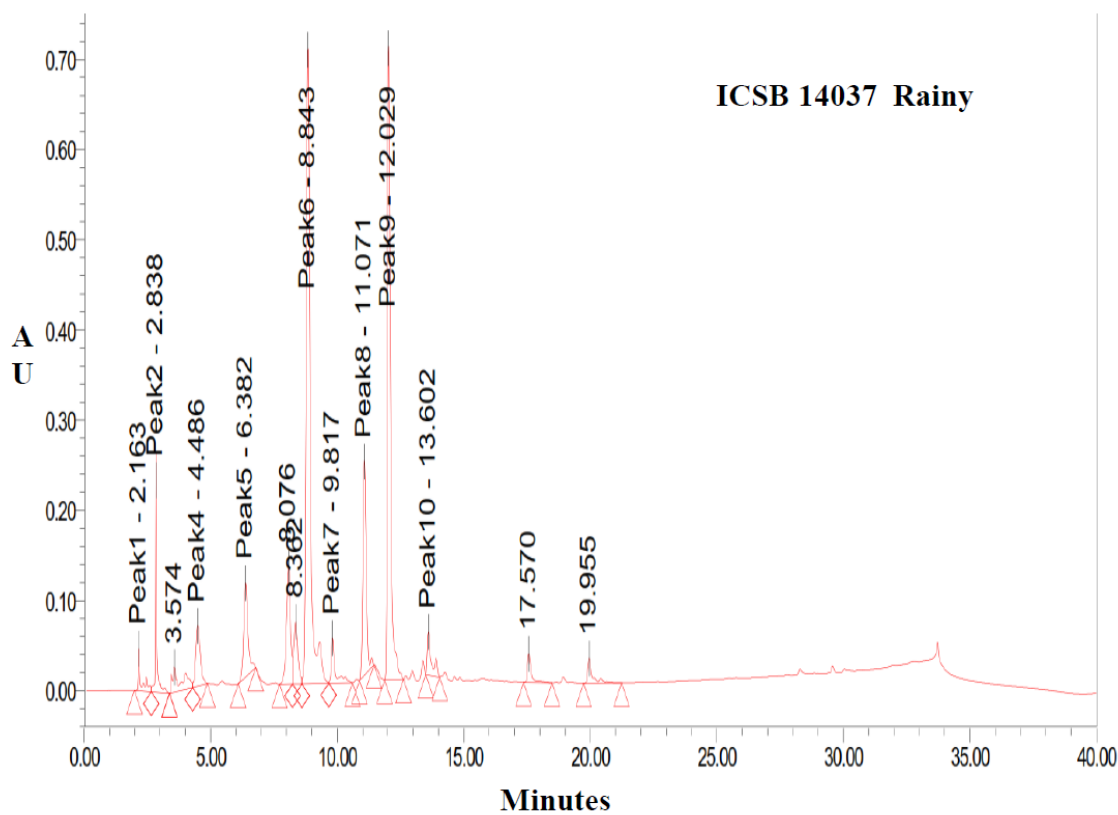
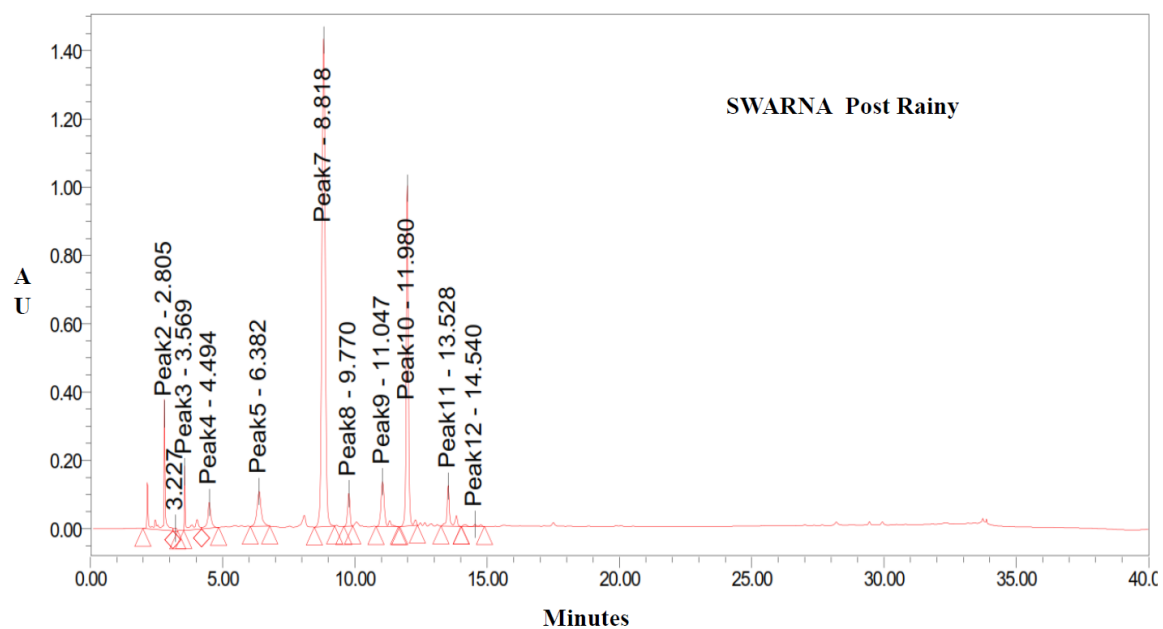
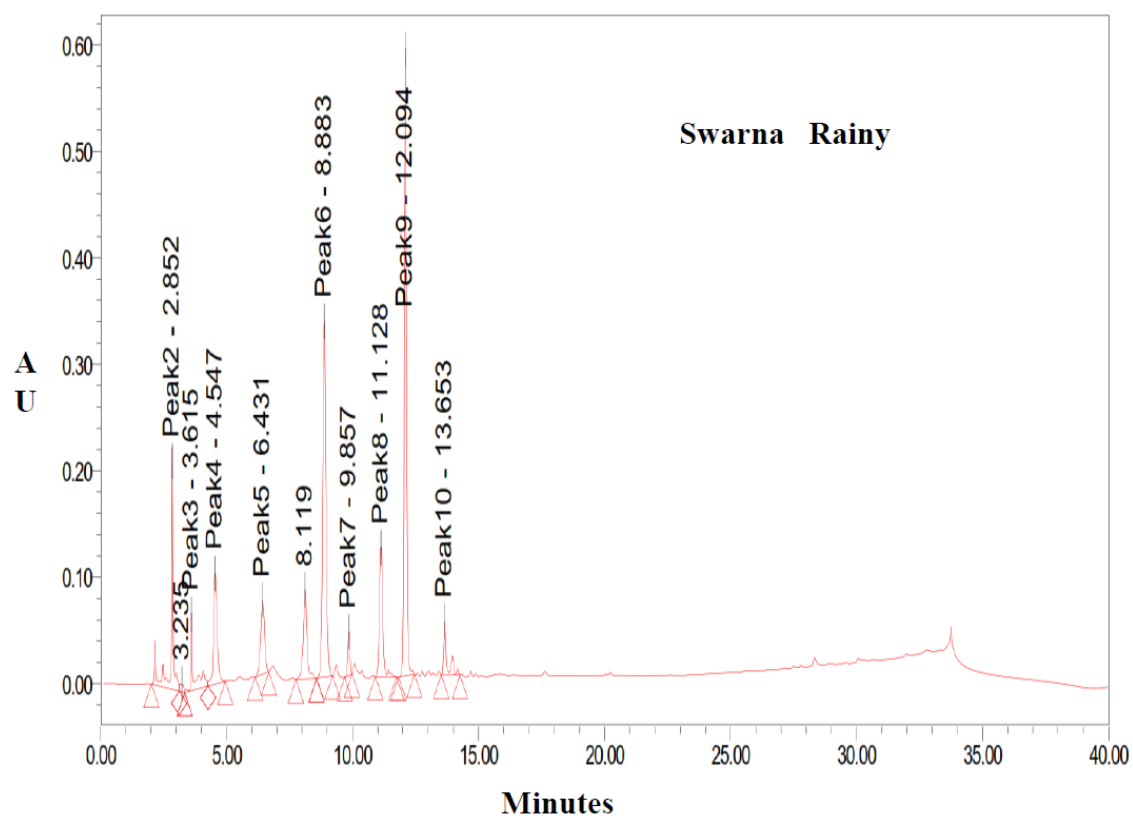


Fig. 4.5 contd....



4.7 Diversity and association studies

4.7.1 Genetic Diversity analysis of Sorghum Germplasm Set and parental lines

The SNP data of 53 sorghum germplasm lines was obtained from centre of excellence in genomics lab, ICRISAT. A total of 60787 SNPs covering 10 chromosomes, on the basis of minor allele frequency (MAF) >1% were obtained. Chromosome wise SNP distribution (Table 4.11) showed that the number of SNPs on the chromosome is variable with chromosome 1 and 2 having maximum number of SNPs while the chromosome 7 has the minimum. The SNP density plot (Fig. 4.6) revealed that there were some regions in all chromosomes where no SNP has been called whereas some regions have more than 324 SNPs per Mb.

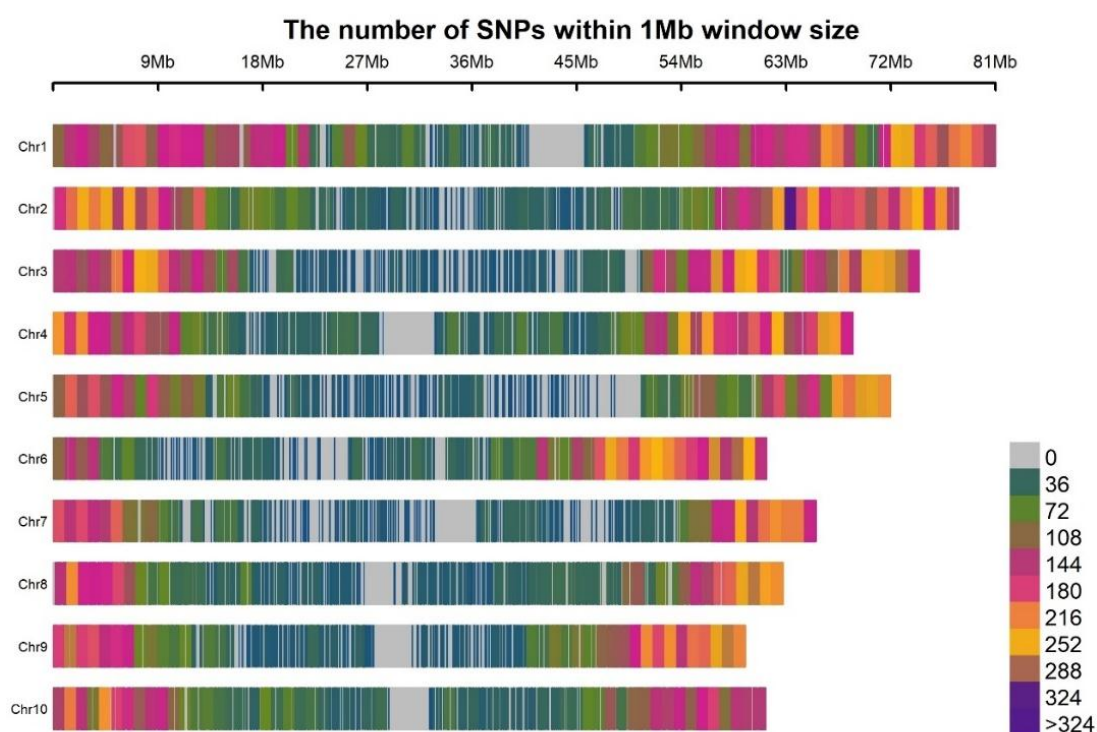


Fig. 4.6: SNP density plot

Table 4.11: Chromosome wise SNP distribution

Chromo- some	01	02	SB- 03	SB- 04	SB- 05	SB- 06	SB- 07	SB- 08	SB- 09	SB-10
No. of SNPs	8785	8355	7432	6497	5149	5382	4179	4666	5172	5170

The SNP data was used to analyse diversity within germplasm lines based on dissimilarity matrix using DARwin version 6.0. Based on diversity analysis, the germplasm

lines were grouped into three major clusters (Fig. 4.7). Based on the field evaluation of these lines, the genotypes (IS 5480, IS 4995, IS 5470, IS 2122, IS 2146, IS 5604, IS 18368, IS 2205, IS 2312, IS 12308, IS 21879, IS 8671, IS 18551, IS 5622, IS 5566, IS 2123, and IS 5490) which exhibited resistance to shoot fly were grouped into one cluster (shown in red), the genotypes which were moderately resistant to shoot fly (IS 1051, IS 7005, IS 20643, IS 21879, IS 10712, IS 13100, IS 18698, IS 18662 and IS 1104) fall into the second cluster (shown in green) and the genotypes which were found to be susceptible and showed very low level of resistance (IS 17610, IS 19512, IS 8151, IS 14334, IS 21443, IS 21444 and IS 19955) against shoot fly were grouped into separate cluster (shown in blue). The clustering of these lines based on the resistance against shoot fly depicts that there is good amount of variability present in the material and these can be utilized in breeding program for transferring resistance to shoot fly, thereby widening the genetic base of the cultivated material.

Similarly, out of the 20 test genotypes (maintainers and restorers in the parental lines) the SNP data of 10 genotypes was obtained from centre of excellence in genomics (ICRISAT) and diversity was studied using DARwin 6.0. Likewise, the germplasm lines, these 10 genotypes were also grouped into 3 clusters (Fig. 4.8) and each cluster consisted of resistant (R), moderately resistant (MR) and susceptible lines (S) based on the morphological evaluation of these genotypes. The first cluster consisted of SSV 74 (S), ICSB 467 (R) and ICSB 14024 (MR), the second cluster included the genotypes, ICSB 14027 (MR) ICSV 700 (R), IS 18551 (R) while the third cluster consisted of ICSB 323 (S), Swarna (S), and ICSB 487 (R). The three resistant lines showed diversity at molecular level and fall into three different clusters. These lines may contain different alleles for resistance which can be utilized in breeding programme.

4.7.2 Genome wide association study (GWAS) for the traits governing the shoot fly resistance

The phenotypic data for the 9 traits (Table 4.2) for shoot fly resistance and SNP genotyping data was used to find association of the SNP markers to various traits. GWAS was carried out using GLM (Generalized linear model), MLM (Mixed linear model) and FarmCPU (Fixed and random model Circulating Probability Unification) approaches using MVP (A memory-efficient, Visualization-enhanced and parallel-accelerated) tool (<https://github.com/XiaoleiLiuBio/MVP>). Population structure was analysed using principle component analysis (PCA). The first three PC were sufficient enough to explain maximum diversity within population structure. The Fig. 4.9 shows the distribution of germplasm lines in three distinct clusters based on first two principle components (PC). For GWAS, first three PCs were used as a covariate.

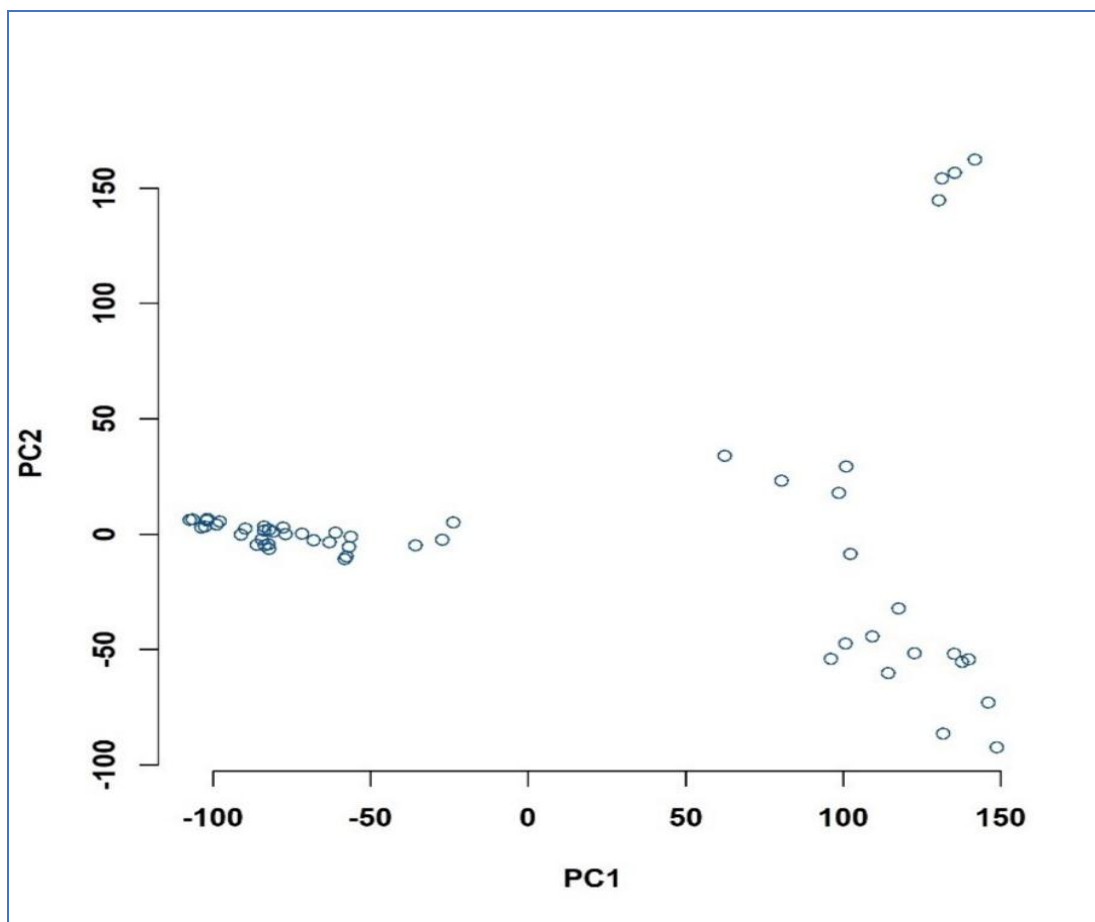


Fig. 4.9: Population structure based on two principle components

Significant association of SNPs were found on different chromosomes for the glossy score, leaf sheath pigmentation, percent plants with shoot fly eggs, percent tillers with shoot fly deadhearts and plant vigour score (Table 4.12, Plate 1). While for percent shoot fly deadhearts, shoot fly eggs per plant and number of tillers, no significant association was found.

4.7.2.1 Glossy score

Four SNPs were associated above the Bonferroni significance threshold for GS. These SNPs were S3_56295016 located on chromosome 3 with probability value (p) of 3.73×10^{-12} , S10_52451215 $p=8.36 \times 10^{-7}$ while two SNPs S9_53301253 and S9_1114230 were located on chromosome 9 with $p=1.16 \times 10^{-11}$ and 5.635×10^{-9} respectively.

4.7.2.2 Leaf sheath pigmentation

A large number of SNPs showed significant association for LSP on all chromosomes indicating the complex nature of the trait.

4.7.2.3 Percent plants with shoot fly eggs

Only a single SNP located on chromosome 2 position S2_97765436 with $p=0.71 \times 10^{-7}$ was associated with PPSFE.

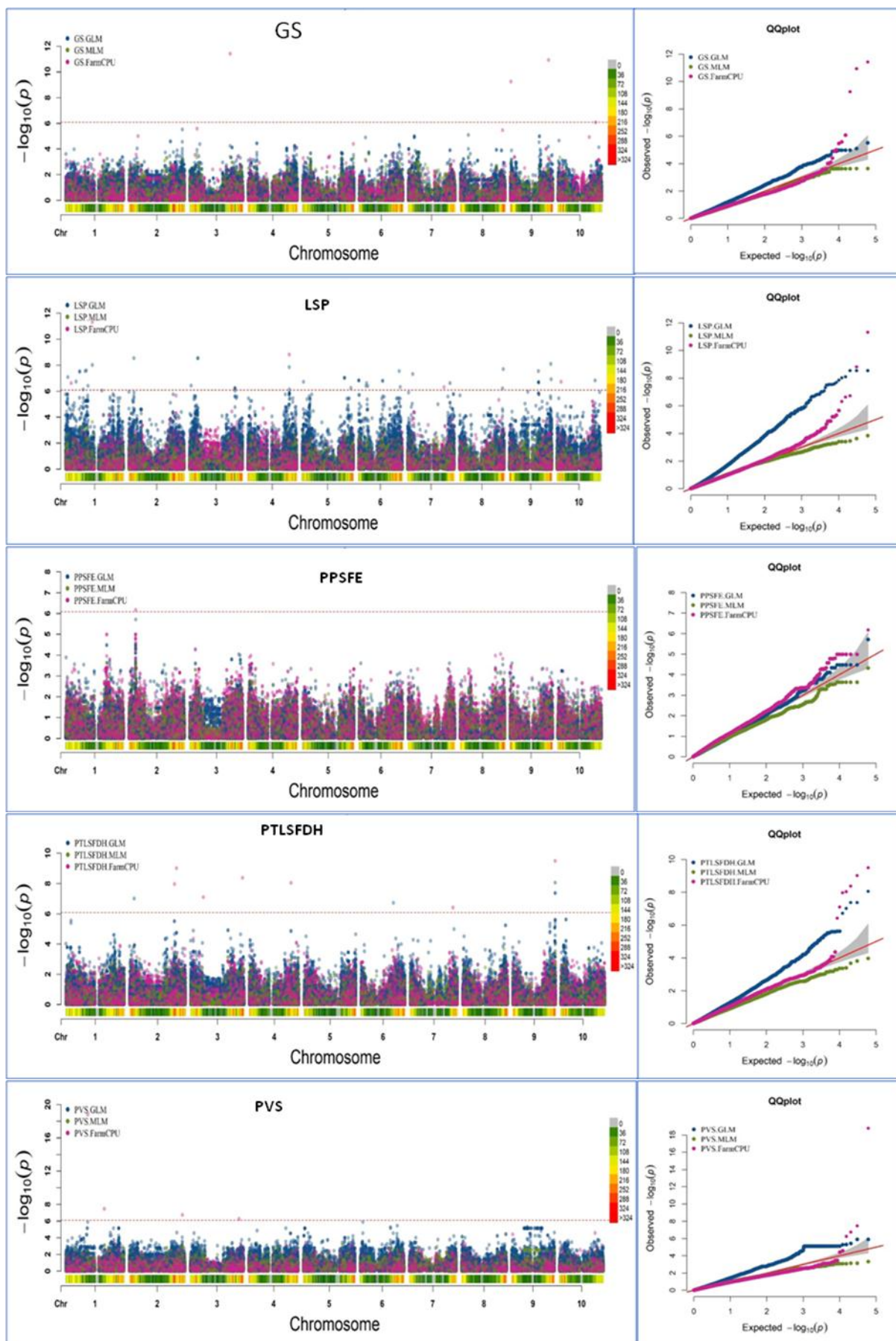
4.7.2.4 Percent tillers with shoot fly deadhearts

Like LSP, a large number of SNPs (Table 4.6) were associated on chromosomes 2, 3, 4, 6, 7 and 9 again indicating the complex nature of this trait.

4.7.2.5 Plant Vigour Score

SNPs on chromosomes 1, 2, 3, and 6 were associated with PVS. The SNPs S1_30327735 on chromosome 1 with $p=1.66 \times 10^{-19}$ and S1_53491750 $p=3.49 \times 10^{-08}$, on chromosome 2, S2_73710457 with $p=1.87 \times 10^{-07}$, on chromosome 3, S3_67683816 with $p=5.74 \times 10^{-07}$, and on chromosome 6 at position S6_3956013 with $p=1.28 \times 10^{-06}$.

So, in present studies multiple SNPs were associated with different chromosomes which are in corroboration with earlier studies (Satish *et al*, 2009; Kiranmayee *et al* 2016). This is also important as finding pleiotropic QTLs is always beneficial in simultaneous improvement of multiple traits at the same time (Apotikar *et al*, 2011), but further validation of the associated SNPs found in the present study is required for transferring the QTLs into adapted cultivars. To exploit the full potential of these lines, traits like trichome density should also be studied and the SNPs associated should be defined and validated.



Manhattan plot
Plate 1: Manhattan plots for different traits

Q-Q Plot

Table 4.12: SNPs with significant association with particular traits above significance threshold

	SNP*	Chromosome	Position	p value	Approach
GS	S3_56295016	3	56295016	3.74E-12	FCPU ¹
	S9_53301253	9	53301253	1.17E-11	FCPU
	S9_1114230	9	1114230	5.64E-10	FCPU
	S10_52451215	10	52451215	8.37E-07	FCPU
LSP	S1_37262961	1	37262961	4.79E-12	FCPU
	S1_3175287	1	3175287	8.28E-08	GLM ²
	S2_7325676	2	7325676	2.92E-09	GLM
	S3_11819911	3	11819911	2.92E-09	GLM
	S3_11819869	3	11819869	2.92E-09	GLM
	S4_57943809	4	57943809	1.57E-09	FCPU
	S8_61201912	8	61201912	2.05E-08	GLM
	S9_57950962	9	57950962	8.12E-09	GLM
	S10_5951065	10	5951065	1.93E-07	FCPU
	S10_53185764	10	53185764	1.50E-07	GLM
PPSFE	S2_9776543	2	9776543	6.71E-07	FCPU
PTLSFDH	S9_58728329	9	58728329	3.22E-10	FCPU
	S9_58728450	9	58728450	4.28E-08	GLM
	S2_6684745	2	6684745	9.82E-08	GLM
	S2_65028947	2	65028947	1.82E-05	GLM
	S3_71866629	3	71866629	4.23E-09	FCPU
	S4_57558556	4	57558556	9.30E-09	FCPU
	S2_62395211	2	62395211	1.09E-08	FCPU
	S3_17589266	3	17589266	8.02E-08	FCPU
	S7_59143038	7	59143038	3.86E-07	FCPU
	S3_3720432	3	3720432	4.46E-05	FCPU
PVS	S6_44897947	6	44897947	1.91E-07	GLM
	S1_30327735	1	30327735	1.67E-19	FCPU
	S1_53491750	1	53491750	3.49E-08	FCPU
	S2_73710457	2	73710457	1.87E-07	FCPU
	S3_67683816	3	67683816	5.75E-07	FCPU
	S6_3956013	6	3956013	1.28E-06	GLM

*Significantly associated SNP at Bonferroni test threshold =0.05, ¹FarmCPU approach, ²Generalized linear approach

CHAPTER V

SUMMARY

Despite of the high yield potential of sorghum (up to 10 tons ha⁻¹), this could not be realized and the average sorghum grain yields on farmers' field in India hardly exceeds 500–800 kg ha⁻¹ (Sharma 1985). This has been attributed to several biotic and abiotic stresses, of which insect pests are the most important constraint worldwide. Insect pests causes economic losses valued over US\$1 billion annually in the semi-arid tropics (Sharma *et al.* 2005). Sorghum, particularly rainy season crop is prone to various biotic constraints including shoot fly, stem borer, midge, grain mold, striga (Sharma 1985; Thakur *et al.* 2006). It is damaged by over 150 insect species, of which sorghum shoot fly, *Atherigona soccata* (Rondani) is a major pest in Asia, Africa and the Mediterranean Europe (Sharma, 1993). Sorghum shoot fly damages the plant at the seedling stage (5 to 30 days after seedling emergence) producing typical dead-heart symptoms, which is formed as a result of drying up of the central leaf when maggot cuts the growing point. The complete life cycle is completed in 15 to 18 days under favourable temperature (20-30°C) and relative humidity (>60%), (Srivastava, 1985). Sorghum shoot fly causes substantial damage in the late-sown crops affecting the grain and fodder yields. Minimization of the losses can be achieved by following appropriate cultural practices including appropriate planting time and chemical control of the insect pest (Sharma 1985), but their efficacy is dependent on the economic, edaphic and climatic factors like costly chemicals beyond the reach of resource poor farmers and highly erratic rainfall in semi-arid tropics (SAT) shortening the sowing window (Sharma 1993).

To reduce the extent of losses due to shoot fly damage in grain and stover sorghums, cultural practices and host plant resistance (HPR) can be used to keep the infestation below economic threshold levels. HPR being a highly complex trait is the final outcome of interaction among the multiple component traits imparting resistance to the genotype (Dhillon 2004). Several genotypes with resistance to shoot fly have been identified (Jotwani 1978; Taneja & Leuschner 1985; Sharma *et al.* 2003). With the introduction of improved sorghum varieties and hybrids having narrow genetic base and susceptibility to shoot fly, along with the inappropriate cultural practices like mono-cropping and ratooning has imparted the sorghum shoot fly, a status of a principal pest in the Indian subcontinent (Singh and Rana 1986).

Where ever feasible, combining genetic and management (cultural) methods are more effective in overcoming the constraints in breeding for shoot fly resistance. To broaden the genetic base for shoot fly resistance, there is a need for gaining and understanding different mechanisms of resistance to this insect in a wide array of shoot fly resistant/susceptible

genotypes. Though sorghum germplasm is rich in variability for shoot fly resistance, the use of such resources is limited. This is primarily because of the linkage drag comprising of several undesirable traits in terms of low yield (grain and fodder), poor nutritional quality, cross incompatibility etc., limiting their use in a breeding program. Also, the traits conferring resistance to shoot fly are complex and are controlled by several genes with a high G X E interaction (Mohammed *et al* 2015a,b). Identification of the genomic regions containing quantitative trait loci (QTLs) for resistance to shoot fly, and the linked markers will be useful in sorghum improvement through marker-assisted selection (MAS). Simultaneous identification of QTLs and phenotyping can be used to improve the efficiency of introgressing shoot fly resistant genes into the locally adapted cultivars. So, the current study was undertaken to address the following objectives: (1) Assess morphological, biochemical, and genetic diversity among the shoot fly-resistant forage/sweet sorghums. (2) Study the inheritance of shoot fly resistance in forage/sweet sorghums. (3) Study the inheritance of QTLs linked to shoot fly resistance and component traits in F₁ hybrids of shoot fly resistant sweet sorghums and locally adapted cultivars.

The test genotypes exhibited significant variation for all the traits except shoot fly deadhearts 28DAE (during rainy season) in 2016, while in 2017, significant variation was found for most of the traits except shoot fly deadhearts (14DAE), number of plants with shoot fly eggs (14DAE) and total number of shoot fly eggs (rainy season) and trichome density at adaxial and abaxial surface (post rainy) in 2017. From the inheritance studies it was concluded that, for GS, the GCA effects varied from -0.733 (ICSB 84) to 0.633 (ICSB 467) and the lines ICSB 323 (-0.3667) and ICSB 487 (-0.3333) had significant negative GCA for GS. Similarly, for LSP the GCA for lines varied from -0.5533 (ICSB 84) to ICSB 467 (0.58) and the genotypes ICSB 487 and ICSB 458 showed significant negative GCA for LSP. The line ICSB 487 also performed well in terms of SFDH with significant negative GCA (-3.715) followed by ICSB 323 (-1.415) and ICSB 14037 (-0.465). The GCA effects for TD_{ada} and TD_{aba} were found to be maximum for ICSB 84 (24.2933 & 11.267) followed by ICSB 323 (TD_{ada} 17.06) and ICSB 487 (14.66 & 7.133) respectively. The line ICSB 487 was also found to be having significant positive GCA effects in terms of forage yield whereas ICSB 84 was found to be having negative GCA for yield. ICSB 14037 also performed better in terms of forage yield and was found to be having significant positive GCA of 0.4269. The moderately resistant line ICSB 14027 was having maximum GCA for forage yield. Among the testers, the GCA for LSP varied from -0.3867 (ICSV 25280) to 0.4467 (SSV 74) and again ICSV 93046 (-0.32), ICSV 700 (-0.2867) and ICSV 25337 (-0.1533) were found to be having significant negative GCA for LSP. Also, for PSFDH, ICSV 93046 (-6.8618), ICSV 700 (-2.0967) and ICSV 25292 (-2.5221) were having significant negative GCA effects. while

for the PSFDH ICSV 93046 (-6.8618), ICSV 25292 (-2.5221) and ICSV 700 (-2.0967) were having significant negative GCA effects. These testers also had significant positive GCA effects for TD and forage yield which is good criterion for the selection of shoot fly resistance and yield perspective. The heritability for the important traits for shoot fly resistance varied from 0.1426 to 0.3659 and it was found to be maximum for PSFDH 1 (0.3479) and PSFDH 2 (0.3659) while among the agronomic traits of importance, it was maximum for forage yield (0.489) followed by DTF (0.3473). It was observed that some of genotypes exhibiting resistance to shoot fly were having significantly negative combining ability for forage yield. Although that is not a desirable trait, but instead of using such lines or testers directly in breeding programs, they can be utilized as a diverse source for transferring resistance to cultivated lines or further the selection can be made for the favourable traits in the segregating generations. Recently, diversifying the parents for hybrid breeding programs for increasing the grain and forage yield along with the resistance components have gained importance and plays a major role in improving sorghum breeding programs. From these inheritance studies, the lines, ICSB 487, ICSB 14037, ICSB 467, ICSB 84, ICSB 458 and testers, ICSV 93046, ICSV 700, S-35, ICSV 25337 were found to perform better individually or in combinations for various traits and can be used in various breeding programs as hybrid parents. Also, the hybrid combinations ICSB 487 X ICSV 25337, ICSB 14037 X ICSV 93046, ICSB 487 X ICSV 700, ICSB 458 X ICSV 700 were out performing than the check variety for forage yield and exhibited shoot fly resistance and these can be evaluated further on a larger scale in various trials and programs for resistance and stability over multiple locations.

The estimation of biochemical components, revealed that the genotypes exhibiting moderate (ICSA 84, ICSB 52, ICSB 14024) and high (ICSA 467, and ICSB 487) resistance to shoot fly recorded lesser content of total soluble sugars, proteins and higher content of tannins while there was no significant association found for phenol content. From the HPLC analysis of methanol extracts of leaf samples a total of 25 different peaks with 12 known compounds were recorded.

The SNP data was used to analyse diversity within germplasm and parental lines based on dissimilarity matrix using DARwin version 6.0. Based on diversity analysis, the germplasm and parental lines were grouped into three major clusters consisting of resistant, moderately resistant and susceptible genotypes. The phenotypic data for the 9 traits for shoot fly resistance and SNP genotyping data was used to find association of the SNP markers to various traits. GWAS was carried using MVP (A memory-efficient, Visualization-enhanced and parallel-accelerated) tool Population structure was analysed using principle component analysis (PCA). The first three PC were sufficient enough to explain maximum diversity within population structure. The Fig. shows the distribution of germplasm lines in three

distinct clusters based on first two principle components (PC). For GWAS, first three PCs were used as a covariate. Significant association of SNPs were found on different chromosomes for the glossy score, leaf sheath pigmentation, percent plants with shoot fly eggs, percent tillers with shoot fly deadhearts and plant vigour score. While for percent shoot fly deadhearts, shoot fly eggs per plant and number of tillers, no significant association was found.

Future work

- ✓ Combining parents with diverse mechanisms of resistance including morphological and biochemical traits should be selected and involved in breeding programs which should be validated for adaptability in the different agroclimatic zones of Punjab.
- ✓ Focus on multiple insect resistance should be given so as to identify sorghum genotypes exhibiting resistance to shoot fly for resistance against other insect pests such as stem borer etc.
- ✓ The unknown compounds from the HPLC fingerprints should be explored further so as to understand their chemical nature and mechanism of action if any in imparting resistance or susceptibility to sorghum shoot fly. This would enable us to have better insights in studying the mechanism of resistance against insect pests along with their inheritance.
- ✓ The SNPs association with the traits important for shoot fly resistance should be thoroughly probed to identify strong associations, stable under varying environments.

REFERENCES

- Agrama H, Widle G, Reese J, Campbell L and Tuinstra M (2002) Genetic mapping of QTLs associated with greenbug resistance and tolerance in *Sorghum bicolor*. *Theor Appl Genet* **104**: 1373-78.
- Agrawal BL and CV Abraham (1985) Breeding sorghum for resistance to shoot fly and midge. pp 371-83 *In Pro Int Sorghum Ento Workshop*, Texas A&M Univ College Station, Texas, USA, 15-21 July 1984, ICRISAT, Patancheru 502324, Andhra Pradesh, India.
- Agrawal BL and House LR (1982) Breeding for pest resistance in sorghum. pp 435-46. In: House LR *et al.* (ed.) *Sorghum in Eighties, Proc Int Symp Sorghum*, 2-7 November 1981, International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, Andhra Pradesh, India.
- Akabari V R and Parmar H P (2014) Heterosis response and combining ability for green fodder yield and its quality traits in forage sorghum. *J Prog Agric* **5**: 9-14.
- Alborn H, Stenhagen G and Leuschner K (1992) Biochemical selection of sorghum crop varieties resistant to sorghum shoot fly (*Atherigona soccata*) and stem borer (*Chilo partellus*): role of allelochemicals. In *Allelopathy* (pp.101-117). Springer, Dordrecht.
- Anandan A, Huliraj H and Veerabadhiran P (2009) Analysis of resistance mechanism to *Atherigona soccata* in crosses of sorghum. *Pl Br* **128**: 443-50.
- Anonymous (2017) Sugarcane. Package of Practices for Crops of Punjab (Kharif 2017) Punjab Agricultural University, Ludhiana, Punjab, India.
- Apotikar D B, Venkateswarlu D, Ghorade R B, Wadaskar R, Patil J V and Kulwal P L (2011) Mapping of shoot fly tolerance loci in sorghum using SSR markers. *J Genetics* **90**: 59-66.
- Aruna C and Padmaja PG (2009) Evaluation of genetic potential of shoot fly resistant sources in sorghum (*Sorghum bicolor* L. Moench). *The J Agric Sci* **147**: 71-80.
- Aruna C, Bhagwat VR, Madhusudhana R, Sharma V, Hussain T, Ghorade RB and Seetharama N (2011) Identification and validation of genomic regions that affect shoot fly resistance in sorghum [*Sorghum bicolor* (L.) Moench]. *Theor Appl Genet* **122**: 1617-30.
- Baker RJ (1978) Issues in Diallel analysis. *Crop Sci* **18**: 533-36.
- Balakotaiah K, Rana BS, Tripathi DP and Rao N P (1975) Genetic analysis of some exotic x Indian crosses in sorghum. X. Inheritance of resistance to sorghum shoot fly. *Indian J Genet Pl Br* **35**: 344-49.
- Biradar SG and Borikar ST (1985) Genetic analysis of shoot fly resistance in relation to growth stages in Sorghum. *Zeitschrift fur Pflanzenzuchtung= J Pl Br pp* 173-78.

- Biradar SG, Borikar ST and Chundurwar RD (1986) Trichome density in some progeny of sorghum. *Sorghum News l* **29**: 107.
- Borikar S T and Chopde P R 1981(b) Shoot fly resistance in Sorghum. *Indian J Genet Pl Br* **41**:191- 99.
- Borikar S T and Chopde P R (1982) Genetics of resistance to sorghutn shoot fly. *Zeitschrift fur Pflanzenzuchtung* **20**: 220-24.
- Borikar ST and Chopde P R (1980) Inheritance of shoot fly resistance under three levels of infestation in sorghum. *Maydica* **25**: 175-83.
- Bray H C and Thorpe W V (1954) Analysis of phenolic compounds of interest in metabolism. *Meth Biochem Analysis* **1**: 27-52.
- Chamarthi S K, Sharma H C, Deshpande S P, Hash C T, Rajaram V, Ramu P and Narasu L M (2012a) Genomic diversity among sorghum genotypes with resistance to sorghum shoot fly, *Atherigona soccata*. *Pl Biochem Biotech* **21**: 242-51.
- Chamarthi S K, Vijay M P, Sharma H C and Narasu L M (2012b) Constitutive and inducible resistance to *Atherigona soccata* (Diptera: Muscidae) in *Sorghum bicolor* *J Econ Ent* **105**: 1069-76.
- Dabholkar A R, Lal G S, Mishra R C and Barche N B (1989) Combining ability analysis of resistance of sorghum to shoot fly. *Indian J Genet Pl Br* **49**: 325-30.
- Delobel A G (1982) Effects of sorghum density on oviposition and survival of the sorghum shoot fly, *Atherigona soccata*. *Entomologia Experimentalis et Applicata* **31**:170-74.
- Devi N L, Shobha S, Tang X, Shaur S A, Dogan H and Alavi S (2013) Development of protein-rich sorghum-based expanded snacks using extrusion technology. *Int J Food Prop* **16**: 263-76.
- Dhillon M K (2004) *Effects of cytoplasmic male-sterility on expression of resistance to Sorghum shoot fly*. Ph.D. Thesis, CCS Haryana Agricultural University, Hissar, India.
- Dhillon M K, Sharma H C, Reddy B V S, Ram S, Naresh J S and Kaizhu (2005) Relative susceptibility of different male-sterile cytoplasm in sorghum to shoot fly, *Atherigona soccata*. *Euphytica* **144**: 275-83.
- Dhillon M K, Sharma H C, Reddy B V S, Singh R and Naresh J S (2006) Inheritance of resistance to sorghum shoot fly, *Atherigona soccata*. *Crop Sci* **46**:1377-83.
- Dhillon M K, Sharma H C, Singh R and Naresh J S (2005b) Mechanisms of resistance to shoot fly, *Atherigona soccata* in sorghum. *Euphytica* **144**: 301-12.
- Doggett H, Starks K J and Eberhart S A (1970) Breeding for resistance to the sorghum shoot fly. *Crop Sci.* **10**: 528-31.
- Duvick D N (1999) Commercial strategies for exploitation of heterosis: The Genetics and Exploitation of Heterosis in Crops. Wisconsin, USA. pp 19-29.

- Elbadawi A A, Nour A M and Pathak R S (1997) Combining ability of resistance to the sorghum shoot fly, *Atherigona soccata* and stem borer *Chilo partellus* in grain sorghum, *Sorghum bicolor*. *Int J Tropical Insec Sci* **17**: 329-33.
- Elshire R J, Glaubitz J C, Sun Q, Poland J A, Kawamoto K, Buckler, E S and Mitchell S E (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLOS one* **6(5)**: e19379.
- FAO (2013) Area, Production and Productivity of Sorghum in India and world. URL: <http://www.fao.org>.
- FAO (2016) Area, Production and Productivity of Sorghum in India and world. URL: <http://www.fao.org>.
- Ganapathy K N, Gomashe S S, Rakshit S, Prabhakar B, Ambekar S S, Ghorade R B, Biradar B D, Saxena U and Patil J V (2012) Genetic diversity revealed utility of SSR markers in classifying parental lines and elite genotypes of sorghum (*Sorghum bicolor* (L.) Moench). *Aust J Crop Sci* **6**: 1486-93.
- Ganesamurthy K, Punitha D and Elangovan M (2010) Genetic diversity among the land races of sorghum collected in Tamil Nadu. *Electronic J Pl Br* **1**: 1375-79.
- Gibson P T and Maiti R K (1983) Trichomes in segregating generations of sorghum matings. I. Inheritance of presence and density. *Crop Sci* **23**: 73-75.
- Gomashe S, Misal M B, Ganapathy K N and Rakshit S (2010) Correlation studies for shoot fly resistance traits in sorghum (*Sorghum bicolor* (L.) Moench). *Electronic J Plant Breed* **1(4)**: 899-902.
- Hahn D D, Faubion J M and Rooney L W (1983) Sorghum phenolic acids: their high-performance liquid chromatography separation and their relation to fungal resistance. *Cereal Chem* **60**: 255.
- Hallali M S, Gowda B T S, Kulkarni K A and Goud J V (1982) Inheritance of resistance to shoot fly (*Atherigona soccata* Rond.) in sorghum (*Sorghum bicolor* (L.) Moench). *Sabrao J* **14**: 165-70.
- Hedge J E and Hofreiter B T (1962) Anthrone Determination for carbohydrate. In: Whistley R L, Be Miller J N (Eds.) *Carbohydrate Chemistry*. Academic Press: New York, NY, USA.
- Jotwani M G (1978) Investigations on Insect Pests of Sorghum and Millets with special reference to Host Plant Resistance. Final Technical Report (1972-1975). *Res Bull Div Entomol IARI*, New Delhi, India, pp114.
- 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. pp 159.

- Juan LS, Wu Y, Liu AZ, Li SG and Han SP (2001) Studies on biochemical mechanism of *Acta Phytophysiol. Sin* **28**: 331-34.
- Juerg B, Thompson W, Rooney W and Bean B (2009) Management of biomass and sweet sorghum in the southwest US. *Abst. 2009 Int Annual Meetings ASA-CSSA-SSSA*, 1-5 Nov Pittsburgh, PA, USA, pp 1-9.
- Kabre G B, 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 F₁ hybrids. *J Insect Sci* **12**: 37-40.
- Kamatar M Y, Salimath P M, Kumar R L R and Rao T S (2003) Heterosis for biochemical traits governing resistance to shoot fly in sorghum [*Sorghum bicolor* (L.) Moench.]. *Indian J Genet* **63**: 124-27.
- Kenga R, Alabi S O and Gupta S C (2004) Combining ability studies in tropical sorghum (*Sorghum bicolor* (L.) Moench) *Field Crops Res* **88**: 251-60.
- Kiranmayee K U, Kishor P ., Hash C T and Deshpande S P (2016) Evaluation of QTLs for Shoot Fly (*Atherigona soccata*) Resistance Component Traits of Seedling Leaf Blade Glossiness and Trichome Density on Sorghum (*Sorghum bicolor*) Chromosome SBI-10L. *Tropical Plant Biol* **9(1)**: 12-28.
- Liu X, Huang M, Fan B, Buckler E S and Zhang Z (2016) Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. *PLoS Genet* **12(2)**: e1005767.
- Lowry O H, Rosebrough N J, Farr A L and Randall R J (1951) Protein measurement with Folin phenol reagent. *J Biol Chem* **193**: 265-75.
- Maiti R K and Bidinger F R (1979) Simple approaches to the identification of shoot fly tolerance in sorghum. *Indian J Plant Prot* **7**: 135-40.
- Maiti R K and Gibson P T (1983) Trichomes in segregating generations of sorghum matings. II. Association with shoot fly resistance. *Crop Sci* **23**:76-79.
- Maiti R K, Rao K E, Raju P S and House L R (1984) The glossy trait in sorghum: its characteristics and significance in crop improvement. *Field Crops Res* **9**: 279-89.
- Makanda I, Tongoona P, Derera J, Sibiya J and Fato P (2010) Combining ability and cultivar superiority of sorghum germplasm for grain yield across tropical low-and mid-altitude environments. *Field Crops Res* **116**: 75-85.
- Mohamed M I and Talib N H (2008) Heterosis and combining ability for quality traits in sorghum. *Aust J Basic Appl Sci* **2**: 99-104.
- Mohammed Maarouf I (2007) Potential of locally developed forage sorghum hybrids in the Sudan. *Sci Res Essay* **2**: 330-37.

- Mohammed Maarouf I (2009) Line x tester analysis across locations and years in Sudanese X exotic lines of forage sorghum. *J Pl Br Crop Sci* **1**: 311-19.
- Mohammed MI, Gamal EK, Ghada HA and Mohammed IE (2008) Improvement of the traditional forage sorghum cultivar 'Abu Sab' in'. *Sudan J Agric Res* **11**: 25-33.
- Mohammed R, Are AK, Bhavanasi R, Munghate RS, Kavi Kishor PB and Sharma HC (2015b) Quantitative genetic analysis of agronomic and morphological traits in sorghum, *Sorghum bicolor*. *Front. Plant Sci.* **6**:945.
- Mohammed R, Kavi Kishor P B, Kumar A A, Reddy B V S, Munghate R S and Sharma H C (2015a) Mechanisms and diversity of resistance to sorghum shoot fly, “*Atherigona soccata*. *Plant Breeding* **134**: 423-36.
- Mohammed R, Munghate R S, Are A K, Polavarapu KK B, Reddy B V, and Sharma HC (2016) Components of resistance to sorghum shoot fly, *Atherigona soccata*. *Euphytica* **207(2)**: 419- 438.
- Nagaraj N, Reese J C, Tuinstra M R, Smith C M, Amand P S, Kirkham M B and Wilde G E (2005) Molecular mapping of sorghum genes expressing tolerance to damage by greenbug (Homoptera: Aphididae). *J Eco Ent* **98**: 595-602.
- Nimbalkar V S and Bapat D R (1992) Inheritance of shoot fly resistance in sorghum. *J Maharashtra Agric Univ* **17**: 93-96.
- Nwanze K F (1997) Screening for resistance to sorghum shoot fly, pp 35-37. In: Sharma H C, Singh F and Nwanze K F (Eds.) *Plant Resistance to Insects in Sorghum*. Patancheru 502324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.
- Ogwaro K (1978) Ovipositional behaviour and host-plant preference of the sorghum shoot fly, *Atherigona soccata* (Diptera; Anthomyiidae). *Entomologia Experimentalis et Applicata* **23**: 189-99.
- 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 Agri Res Quatr* **17**: 215-18.
- Painter R H (1958) Resistance of plants to insects. *Ann Rev Entomol* **3**: 267-90.
- Pandey P K, R Singh and P K Shrotria (2005). Reversed phase HPLC separation of phenolic acids in sorghum and their relation to shoot fly (*Atherigona soccata* Rond.) resistance. *Indian J Entomol* **67**: 170-74.
- Prabhakar and Raut M S (2010). Exploitation of heterosis using diverse parental lines in Rabi Sorghum. *Electronic J of Plant Breed* **1(4)**: 680-684
- Prakash R, Ganesamurthy K, Nirmalakumari A and Nagarajan P (2010) Combining ability for fodder yield and its components in Sorghum (*Sorghum bicolor* L). *Elec J Pl Br* **2**: 124-28.

- Raina A K (1982) Daily rhythms in the sorghum shootfly, *Atherigona soccata*: Oviposition, egg hatch and adult eclosion. *Physiol Entomol* **7**: 65-70.
- 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, USA. Ed. by Kumble V, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, pp. 131-36.
- Ramu P, Billot C, Rami J F, Senthilvel S, Upadhyaya H D, Reddy L Ananda and Hash C T (2013) Assessment of genetic diversity in the sorghum reference set using EST-SSR markers. *Theor Appl Genet* **126**: 2051-64.
- Rana B S, Jotwani M G and Rao NGP (1980) Inheritance of host plant resistance to sorghum shoot fly. In: *Insect science and its application*, ICIPE, 4-8 May. 1980. Nairobi, Kenya.
- Rao N G P, Rana B S, Balakotaiah K, Tripathi D P and Fayed M F S (1974) Genetic analysis of some exotic X Indian crosses in sorghum. VIII. F1 analysis of ovipositional non-preference underlying resistance to sorghum shoot fly. *Indian J Genet Pl Br* **34**: 122-27.
- Rao P S, Rao S S, Seetharama N, Umakanth A V, Reddy P S, Reddy B V S and Gowda C L L (2009) Sweet Sorghum for biofuel and strategies for its improvement. *Info Bull No* 77, ICRISAT, Patancheru, AP, India, pp 80: ISBN: 978-92-9066-518-2.
- Reddy B V S, Ashok Kumar A, Sharma H C, Srinivasa Rao P, Blümmel M, Ravinder Reddy R, Deshpande S, DattaMazumdar S and Dinakaran E. (2012). *Sorghum improvement* (1980-2010): Status and way forward. *Journal of SAT Agricultural Research* 10.
- Reddy B V S, Kumar A A and Reddy P S (2010) Physiological and morphological basis of shoot fly resistance. *Recent Advances in Sorghum Improvement Research at ICRISAT* **506**: 499-506.
- Reddy B V S, Ramesh S, Reddy P S and Kumar A A (2009) Genetic enhancement for drought tolerance in sorghum. *Pl Br Rev* **31**: 189-222.
- Reddy B V S, Rangel A F, Ramaiah B and Ortiz R (2005) A research and network strategy for sustainable sorghum production systems for Latin America, pp.139-148, In: M.C.S. Ross W M, Gorz H J, Haskins F A and Kofoi K D (1979) "Combining Ability in Forage Sorghum Hybrids" *Maydica* **24**: 83-93.
- Reddy Belum V S, Rattunde H F W and Stenhouse J W (1997) Breeding sorghums for insect resistance. In: Sharma H C, Singh F and Nwanze K F (Eds.) *Plant Resistance to*

- Insects in Sorghum*. Patancheru 502324, Andhra Pradesh, India: ICRISAT, pp 115-26.
- Robert E B (1971) Method for estimation of tannin in grain sorghum. *Agron. J* **63**: 511.
- Ross W M, Gorz H J, Haskins F A and Kofoed K D (1979) Combining ability in forage sorghum hybrids. *Maydica* **24**: 83-93.
- Satish K, Srinivas G, Madhusudhana R, Padmaja P G, Reddy R N, Mohan S M and Seetharama (2009) Identification of quantitative trait loci for resistance to shoot fly in sorghum [*Sorghum bicolor* (L.) Moench]. *Theor Appl Genet* **119**: 1425-39.
- Schoonhoven L M (1968) Chemosensory basis of host plant selection. *Ann Rev Entomol* **13**: 115-36.
- Sharm H C, Abraham C V, Vidyasagar P and Stenhouse J W (1996) Gene action for resistance in sorghum to midge, *Contarinia sorghicola*. *Crop Sci* **36**: 259-65.
- Sharma G C, Jotwani M G, Rana B S and Rao N G P (1977) Resistance to the sorghum shoot-fly, *Atherigona soccata* (Rondani) and its genetic analysis. *J Ent Res* **1**: 1-12.
- Sharma H C (1985) Future strategies for pest control in sorghum in India. *Trop. Pest Manag.* **31**: 167-185.
- Sharma H C (1993) Host plant resistance to insects in sorghum and its role in integrated pest management. *Crop Protec* **12**: 11-34.
- Sharma H C (2001) Cytoplasmic male-sterility and source of pollen influence the expression of resistance to sorghum midge, *Stenodiplosis sorghicola*. *Euphytica* **122**: 391-95.
- Sharma H C (2006). Integrated pest management research at ICRISAT: Present status and future priorities. International Crops Research Institute for the Semi-Arid Tropics, Patancheru 502324, AP, India: pp 48.
- Sharma H C, Bhagwat V R and Padmaja P G (2008). Techniques to screen sorghums for resistance to insect pests. pp 31-49 in Sorghum improvement in the new millennium (Belum VS Reddy, Ramesh S, Ashok Kumar A and Gowda CLL, eds.). Patancheru 502324, Andhra Pradesh, India: ICRISAT.
- Sharma H C, Dhillon M K, Naresh J S, Singh R, Pampapathy G, and Reddy B V S (2004) Influence of cytoplasmic male-sterility on the expression of resistance to insects in sorghum. In: Fisher T, Turner N, Angus J, McIntyre L, Robertson M, Borrell A and Llyod D(eds.), Fourth International Crop Science Congress, 25 September-1 October 2004, Brisbane, Australia, Queensland, Brisbane, Australia ([http://www.cropscience.org.au](http://www.cropsscience.org.au)).
- Sharma H C, Nwanze K F and Subramanian V (1997) Mechanisms of resistance to insects and their usefulness in sorghum improvement. *Plant Resistance to Insects in Sorghum*. pp. 81-100.

- Sharma H C, Reddy B V, Dhillon M K, Venkateswaran K, Singh B U, Pampapathy G and Sharma K K (2005) Host plant resistance to insects in sorghum: present status and need for future research. *Int Sorghum Millets Newsletter* **46**: 36-43.
- Sharma H C, Taneja S L, Kameswara N R and Rao P K E (2003) Evaluation of sorghum germplasm for resistance to insect pests. *Info Bull No 63* pp 177 ICRISAT, Patancheru 502324, Andhra Pradesh, India.
- 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, Andhra Pradesh, India.
- Singh B U and B S Rana (1986). Resistance in sorghum to the shoot fly, *Atherigona soccata* Rondani. *Int J Trop Insect Sci* **7**: 577-87.
- 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**: 279-89.
- Singh F (1997) Inheritance of resistance to insect pests in sorghum. Plant resistance to insects in sorghum. Patancheru 502 324, Andhra Pradesh, India. *International Crops Research Institute for the Semi-Arid Tropics* **216**: 101-11.
- Singhania D L (1980) Heterosis and combining ability studies in grain sorghum. *Indian J Genet Pl Br* **40**: 463-71.
- Sivakumar C, Sharma HC, Lakshmi Narasu M and Pampapathy G (2008) Mechanisms and diversity of resistance to shoot fly, *Atherigona soccata* in *Sorghum bicolor*. *Indian J Plant Prot* **36**: 249-56.
- Soto P E (1974) Ovipositional preference and antibiosis in relation to resistance to sorghum shoot fly. *J Eco Ent* **67**: 265-67.
- Srivastava K P (1985) Sorghum insect pests in India. In: Proceedings of the International Sorghum Entomology Workshop, 15-21 July 1984, pp 65-72, Texas A & M University and ICRISAT.
- Stephens J C and Holland R F (1954) Cytoplasmic male sterility for sorghum seed production. *Agron J* **46**: 20-23.
- Sukhani T R and Jotwani M G (1980) Ovipositional preference and damage of sorghum shoot fly on different stages of tillers of ratoon crop. *Indian Journal of Entomology* **42**: 488-93.
- Taneja S L and Leuschner K (1985) Resistance screening and mechanism of resistance in sorghum to shoot fly. Proc. of the Int. Sorghum Ento. Workshop, 15-21 July 1984, Texas A & M University, College Station, TX, USA, pp 115-129.

- Tao Y Z, Hardy A, Drenth J, Henzell R G, Franzmann B A, Jordan D R and McIntyre C L (2003) Identifications of two different mechanisms for sorghum midge resistance through QTL mapping. *Theor Appl Genet* **107**: 116-22.
- Tarumoto I (1980) Inheritance of glossiness of leaf blades in sorghum, *Sorghum bicolor* (L.) Moench. *Japanese J Br* **30**: 237-40.
- Tarumoto I (1983) Multiple alleles on glossy locus in *Sorghum bicolor* (L.) Moench. *Abst. of contributed papers of the Fifteenth Int. Congress of Genet.* 12-21 December 1983, New Delhi, **2**: 643.
- Thakur R P, Reddy B V S, Indira S, Rao V P, Navi S S, Yang X B and Ramesh S (2006). Sorghum grain mold. *Information Bulletin No. 72*. Patancheru 502324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 32 p.
- Vavilov N I (1951) The origin, variation, immunity and breeding of cultivated plants. *Soil Sci* **72**: 482.
- Woodhead S and Bernays E A (1978) The chemical basis of resistance of *Sorghum bicolor* to attack by *Locusta migratoria*. *Entomologia experimentalis et applicate* **24**: 123-44.
- Wu Y and Huang Y (2008) Molecular mapping of QTLs for resistance to the greenbug *Schizaphis graminum* (Rondani) in *Sorghum bicolor* (Moench). *Theor Appl Genet* **117**: 117-24.

APPENDIX I

Evaluation of sorghum genotypes for resistance to shoot fly (Rainy 2016)

Genotype	Glossy score	Leaf sheath pigmentation	Total no. of plants	Seedling height (cm)	No. of shoot fly dead hearts	Shoot fly deadhearts (%)	No. of plants with SF eggs	Total no. of SF eggs
Btx 623	5.0	3.5	25.5	5.5	11.0	46.7	18.5	32.0
ICSA 14013	3.5	3.0	21.0	4.5	18.5	88.2	18.0	45.5
ICSA 14024	4.5	3.5	19.0	4.5	8.5	44.6	16.0	22.5
ICSA 14027	3.5	2.0	20.5	6.8	6.5	31.7	14.5	43.5
ICSA 14037	3.0	2.0	27.0	8.0	10.0	37.1	25.0	45.5
ICSA 323	4.5	4.0	27.0	4.0	17.0	63.3	22.5	63.0
ICSA 458	3.5	2.0	36.0	6.8	9.0	25.0	31.0	59.0
ICSA 467	2.5	2.0	36.0	4.5	15.0	40.9	28.0	34.0
ICSA 487	2.0	1.0	30.5	5.0	10.5	34.4	21.0	48.5
ICSA 52	4.5	3.5	26.0	4.8	21.5	82.7	21.5	42.5
ICSA 84	4.0	3.0	36.5	5.3	19.0	52.0	33.5	50.5
ICSB 14013	4.5	3.5	27.0	7.0	26.5	97.8	24.0	27.5
ICSB 14024	3.5	2.5	27.5	6.8	17.0	62.0	24.5	62.0
ICSB 14027	3.0	2.5	29.5	7.3	18.5	62.0	23.0	36.5
ICSB 14037	3.5	2.0	26.5	4.5	9.0	34.7	17.5	31.5
ICSB 323	5.0	3.5	18.0	4.9	9.5	66.0	16.5	51.5
ICSB 458	3.0	3.0	26.5	7.3	3.5	13.7	20.0	56.0
ICSB 467	3.0	2.0	20.5	5.8	7.5	36.2	17.0	34.0
ICSB 487	2.0	1.0	35.0	4.0	6.0	16.7	27.0	55.0
ICSB 52	5.0	4.0	21.5	5.3	15.0	69.1	19.5	47.0
ICSB 84	4.0	3.0	31.5	5.0	22.5	69.7	25.0	51.5
ICSV 25280	2.5	1.5	22.5	8.3	8.0	36.0	21.0	82.0
ICSV 25292	5.0	3.5	19.0	7.5	13.5	68.8	13.0	28.0
ICSV 25303	4.0	2.5	23.0	5.8	19.0	79.5	19.5	45.5
ICSV 25316	3.0	2.5	17.0	8.6	13.5	79.5	14.5	20.5
ICSV 25337	4.0	2.0	27.5	6.0	21.5	81.4	26.5	63.5
ICSV 700	2.0	1.0	27.0	4.5	8.0	29.2	22.0	50.5
ICSV 93046	2.5	1.5	28.5	6.0	7.0	25.0	22.5	36.0
IS 18551	1.0	2.0	29.0	12.3	8.5	29.5	21.5	40.5
S 35	2.5	2.5	26.0	4.5	7.0	26.8	19.5	29.0
SSV 74	4.5	3.5	26.5	7.3	26.0	97.8	25.5	61.5
Swarna	4.5	3.5	14.0	11.8	10.5	75.7	11.0	23.5
Fp	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	0.007	<0.001
Vr	9.63**	6.77**	2.97*	113.27**	5.04**	9.29**	2.48*	4.1**
Mean	3.52	2.58	25.89	6.23	13.25	53.20	21.25	44.40
SE±	0.34	0.34	3.28	0.19	2.77	8.03	3.22	7.07
LSD (P 0.05)	0.98	0.98	9.47	0.54	8.00	23.15	9.29	20.40
CV (%)	13.60	18.60	17.90	4.30	29.60	21.30	21.40	22.60

**** and * Significant at Fpr value 0.01% and 0.05% respectively**

Appendix1 contd...

Genotype	No. of shoot fly dead hearts 1	Shoot fly deadhearts (%) 1	No. of plants with SF eggs 1	Total no. of SF eggs 1	Trichome abaxial	Trichome adaxial	Plant height (cm)
Btx 623	25.5	100.0	23.0	40.0	0.0	4.0	95.0
ICSA 14013	21.0	100.0	21.0	52.5	0.0	0.0	100.0
ICSA 14024	18.0	95.5	18.5	27.5	0.0	0.0	110.0
ICSA 14027	20.0	97.6	19.0	43.5	12.0	0.0	155.0
ICSA 14037	27.0	100.0	22.0	37.5	0.0	12.0	137.5
ICSA 323	27.0	100.0	26.5	53.0	0.0	0.0	110.0
ICSA 458	36.0	100.0	33.5	51.0	24.0	0.0	100.0
ICSA 467	31.0	84.7	26.5	30.5	14.0	5.5	135.0
ICSA 487	26.5	87.5	19.0	32.5	12.5	0.0	165.0
ICSA 52	26.0	100.0	25.0	42.5	0.0	0.0	130.0
ICSA 84	33.0	89.6	33.0	45.0	0.0	0.0	110.0
ICSB 14013	27.0	100.0	26.5	33.0	0.0	24.5	90.0
ICSB 14024	27.5	100.0	27.5	65.5	0.0	0.0	112.5
ICSB 14027	27.5	92.3	21.0	35.0	46.0	52.0	175.0
ICSB 14037	26.5	100.0	19.0	26.5	0.0	69.5	165.0
ICSB 323	18.0	100.0	18.0	40.0	0.0	0.0	125.0
ICSB 458	25.5	96.2	23.0	55.5	0.0	7.5	110.0
ICSB 467	19.0	92.1	19.0	35.0	32.0	50.5	135.0
ICSB 487	29.0	78.6	23.5	40.5	26.5	67.0	132.5
ICSB 52	21.5	100.0	21.5	54.0	9.5	48.0	112.5
ICSB 84	30.0	93.8	29.5	55.0	0.0	0.0	120.0
ICSV 25280	21.5	95.2	22.5	87.0	13.5	56.0	207.5
ICSV 25292	18.0	96.0	14.0	26.5	0.0	0.5	245.0
ICSV 25303	23.0	100.0	21.5	44.0	0.0	0.0	155.0
ICSV 25316	17.0	100.0	17.0	27.5	39.5	60.0	230.0
ICSV 25337	27.5	100.0	27.5	53.0	18.0	40.5	150.0
ICSV 700	26.0	95.8	19.0	38.0	8.0	72.0	295.0
ICSV 93046	27.0	92.1	22.0	27.5	0.0	52.5	275.0
IS 18551	25.5	86.3	22.0	31.0	18.0	66.0	255.0
S 35	24.5	93.2	24.0	35.0	0.0	0.0	207.5
SSV 74	26.5	100.0	26.5	59.5	0.0	0.0	225.0
Swarna	14.0	100.0	14.0	34.5	0.0	15.0	125.0
Fp	0.051	0.352	0.035	<0.001	<0.001	<0.001	<0.001
Vr	1.81*	1.15	1.94*	5.03**	10.44**	20.38**	10.81**
Mean	24.78	95.80	22.67	42.50	8.55	22.00	156.00
SE±	3.64	5.18	3.37	5.99	3.98	6.06	17.20
LSD (P 0.05)	10.48	14.95	9.72	17.29	11.47	17.47	49.70
CV (%)	20.70	7.70	21.00	20.00	65.80	39.00	15.60

******, and ***** Significant at Fpr value 0.01% and 0.05% respectively

APPENDIX 2

Evaluation of sorghum genotypes for resistance to shoot fly (Post rainy 2016)

Genotype	Glossy score	Leaf sheath pigmentation	Total no. of plants	Seedling height (cm)	No. of shoot fly dead hearts	Shoot fly dead hearts (%)	No. of plants with SF eggs	Total no. of SF eggs
Btx 623	4.0	4.0	20.5	8.3	10.5	51.7	13.5	16.0
ICSA 14013	4.0	3.0	25.5	7.0	11.0	43.2	15.5	19.0
ICSA 14024	4.0	3.5	25.5	8.3	12.0	46.4	15.5	15.5
ICSA 14027	2.5	3.0	28.5	9.5	10.5	35.8	18.5	16.5
ICSA 14037	2.0	2.5	32.0	9.3	5.0	15.7	12.5	15.5
ICSA 323	4.5	4.5	26.5	5.4	11.5	43.6	22.5	23.0
ICSA 458	4.0	3.0	31.5	4.9	4.0	12.5	5.5	5.5
ICSA 467	2.5	2.5	25.0	6.8	2.5	10.6	6.0	7.0
ICSA 487	1.5	1.0	21.5	5.9	3.0	15.9	5.0	6.5
ICSA 52	4.0	4.5	28.0	6.1	18.5	66.5	24.5	29.0
ICSA 84	3.5	3.5	31.5	5.7	17.0	53.8	21.5	24.5
ICSB 14013	4.5	4.5	30.0	6.8	13.0	44.8	18.0	22.0
ICSB 14024	4.0	3.5	33.0	8.4	17.0	51.5	21.5	25.5
ICSB 14027	3.0	3.0	28.5	8.0	6.0	21.5	13.0	17.5
ICSB 14037	2.5	2.5	28.5	9.0	3.5	12.2	12.5	15.5
ICSB 323	5.0	4.5	27.0	5.1	9.5	35.2	22.5	30.0
ICSB 458	3.5	3.0	20.0	4.9	3.0	14.8	3.5	5.0
ICSB 467	3.0	2.5	13.5	8.0	2.0	15.9	3.0	4.5
ICSB 487	2.0	2.0	25.0	7.3	2.5	9.8	6.0	9.5
ICSB 52	4.5	4.5	31.0	5.3	13.0	43.9	20.5	22.5
ICSB 84	4.0	3.5	29.0	5.8	9.5	33.0	17.5	17.0
ICSV 25280	3.5	2.0	24.5	7.0	10.0	40.0	11.0	14.5
ICSV 25292	4.0	3.0	25.5	11.0	14.0	55.3	20.5	23.5
ICSV 25303	4.0	3.0	21.5	9.3	10.5	39.0	13.0	17.5
ICSV 25316	3.0	3.5	14.5	6.4	8.0	54.3	9.0	12.0
ICSV 25337	3.0	3.0	28.0	8.5	6.0	21.4	14.0	16.5
ICSV 700	2.0	1.0	31.0	10.0	8.5	27.4	11.5	12.5
ICSV 93046	1.5	1.5	24.5	7.3	4.5	18.5	7.0	8.0
IS 18551	1.0	2.0	28.5	11.0	1.5	5.2	3.5	4.5
S 35	2.5	2.5	17.0	7.8	7.0	41.2	13.5	15.5
SSV 74	4.5	3.5	21.5	11.5	16.0	75.4	20.5	24.0
Swarna	5.0	3.5	29.5	9.0	11.5	38.5	22.0	27.5
Fp	<0.001	<0.001	0.010	<0.001	<0.001	<0.001	<0.001	<0.001
Vr	11.26**	4.07**	2.35**	43.01**	3.66**	4.11**	6.1**	6.57**
Mean	3.33	3.03	25.86	7.62	8.81	34.20	13.88	16.34
SE±	0.32	0.48	3.25	0.28	2.54	8.95	2.65	2.91
LSD (P 0.05)	0.92	1.71	9.38	0.82	7.33	25.80	7.65	8.39
CV (%)	13.60	22.20	17.80	5.30	40.80	37.00	27.00	25.20

******, and ***** Significant at Fpr value 0.01% and 0.05% respectively

Appendix 2 cont.

Genotype	No. of shoot fly dead hearts 1	Shoot fly deadhearts (%) 1	No. of plants with SF eggs 1	Total no. of SF eggs 1	Trichome abaxial	Trichome adaxial	Plant height (cm)
Btx 623	14.5	70.7	15.0	16.5	0.0	1.0	113.9
ICSA 14013	17.5	68.4	20.0	24.0	0.0	0.0	106.7
ICSA 14024	14.0	53.1	17.0	20.0	0.0	0.0	100.2
ICSA 14027	20.0	69.7	20.5	24.0	15.5	0.0	138.1
ICSA 14037	16.5	52.0	22.5	26.0	0.0	6.5	142.6
ICSA 323	21.5	81.3	22.0	29.5	0.0	0.0	89.2
ICSA 458	14.0	42.6	9.5	9.5	28.5	0.0	88.1
ICSA 467	9.0	37.6	13.0	13.0	9.5	2.0	109.0
ICSA 487	8.0	33.1	6.5	6.5	13.0	0.0	117.1
ICSA 52	23.5	83.7	25.5	32.5	0.0	0.0	124.5
ICSA 84	22.0	69.9	25.0	32.0	0.0	0.0	127.8
ICSB 14013	24.5	82.3	25.0	28.0	0.0	35.0	108.6
ICSB 14024	23.0	69.7	18.0	25.5	0.0	0.0	114.1
ICSB 14027	15.5	54.8	20.0	21.5	59.5	58.0	160.1
ICSB 14037	16.5	57.8	18.0	17.5	0.0	67.0	144.8
ICSB 323	22.0	81.5	22.5	29.5	0.0	0.0	115.1
ICSB 458	8.0	35.7	11.0	7.5	0.0	7.5	90.0
ICSB 467	4.0	27.7	7.5	7.5	42.0	52.5	120.8
ICSB 487	12.5	49.5	11.5	13.0	25.0	46.5	123.9
ICSB 52	21.0	66.8	24.5	24.5	14.0	67.0	134.7
ICSB 84	16.0	55.7	13.5	21.5	0.0	0.0	134.4
ICSV 25280	11.5	46.5	15.5	21.0	19.5	69.0	187.1
ICSV 25292	21.5	85.0	21.0	24.5	0.0	1.5	196.6
ICSV 25303	17.0	72.7	19.0	22.5	0.0	0.0	167.5
ICSV 25316	12.5	86.8	13.0	15.0	32.0	55.5	219.7
ICSV 25337	13.5	48.2	21.0	22.0	19.0	51.5	205.4
ICSV 700	15.0	48.4	14.5	16.0	13.0	79.5	214.3
ICSV 93046	9.0	35.7	11.0	11.0	0.0	59.5	199.2
IS 18551	5.5	19.3	6.5	6.5	19.5	62.5	211.7
S 35	14.5	85.3	12.0	15.5	0.0	0.0	139.2
SSV 74	17.5	82.2	21.0	25.5	0.0	0.0	173.4
Swarna	21.0	70.8	28.5	43.5	0.0	10.5	126.5
Fp	0.005	<0.001	0.003	<0.001	<0.001	<0.001	<0.001
Vr	2.56*	4.49**	2.71*	4.71**	18.79**	13.12**	812.88**
Mean	15.69	60.10	17.22	20.39	9.69	22.90	142.01
SE±	3.41	9.05	3.63	3.99	3.40	8.03	1.39
LSD (P 0.05)	9.84	26.10	10.47	11.51	9.82	23.15	4.00
CV (%)	30.70	21.30	29.80	27.70	49.70	49.60	1.40

**** and * Significant at Fpr value 0.01% and 0.05% respectively**

APPENDIX 3

Evaluation of sorghum genotypes for resistance to shoot fly (Rainy 2017)

Genotype	Glossy score	Leaf sheath pigmentation	Total no. of plants	Seedling height (cm)	No. of shoot fly dead hearts	Shoot fly deadhearts (%)	No. of plants with SF eggs	Total no. of SF eggs
BTx 623	3.5	3.0	13.5	4.3	13.0	96.9	10.5	33.0
ICSA 14013	4.0	3.0	22.0	4.8	18.5	82.5	20.0	39.5
ICSA 14024	3.0	2.5	22.0	4.3	19.0	86.4	13.5	24.5
ICSA 14027	3.0	2.0	26.0	7.3	21.0	81.5	17.0	31.5
ICSA 14037	2.0	1.5	28.5	8.8	17.0	60.1	18.0	33.5
ICSA 323	4.0	3.0	6.0	4.0	3.5	68.8	5.5	22.5
ICSA 458	2.0	2.0	25.5	6.3	14.5	55.5	20.0	39.0
ICSA 467	2.0	2.0	16.0	4.3	8.5	53.6	10.0	13.5
ICSA 487	1.0	1.5	27.0	5.3	15.0	52.5	13.0	25.0
ICSA 52	3.5	2.5	9.5	4.8	7.5	77.8	9.0	21.5
ICSA 84	3.5	2.5	17.0	5.2	10.5	69.4	14.0	27.5
ICSB 14013	4.0	2.5	15.0	6.8	13.0	81.8	14.0	22.5
ICSB 14024	3.5	2.5	6.5	7.3	6.0	94.4	6.5	14.0
ICSB 14027	3.5	2.0	20.5	6.8	15.5	76.6	16.0	28.0
ICSB 14037	2.0	2.0	33.0	4.5	18.0	53.7	20.5	38.5
ICSB 323	3.5	3.0	19.0	4.7	11.0	58.3	18.0	58.0
ICSB 458	2.0	2.0	19.5	6.8	9.0	45.2	15.0	30.0
ICSB 467	2.0	2.0	19.0	5.5	8.0	45.2	11.0	17.0
ICSB 487	1.5	1.5	22.0	4.3	10.5	47.1	10.5	16.0
ICSB 52	3.5	3.0	11.5	5.2	8.0	69.7	9.5	27.5
ICSB 84	3.0	3.0	26.0	4.9	20.5	75.2	17.5	36.5
ICSV 25280	2.0	1.5	13.5	8.0	8.5	62.1	11.0	26.0
ICSV 25292	4.5	3.0	13.5	7.0	11.0	78.7	11.5	34.0
ICSV 25303	3.5	2.0	15.0	5.0	13.0	84.1	12.0	29.5
ICSV 25316	4.0	3.0	15.0	8.7	14.0	92.5	11.0	33.0
ICSV 25337	3.5	2.0	17.0	6.0	11.5	74.4	13.5	34.5
ICSV 700	1.5	1.5	33.5	4.3	24.0	71.3	22.5	38.0
ICSV 93046	1.5	2.0	20.5	5.8	10.0	46.9	14.0	27.0
IS 18551	2.0	2.0	19.5	12.4	6.5	37.8	14.5	23.0
S-35	4.0	3.0	19.0	4.5	17.5	92.2	15.0	39.5
SSV74	3.5	2.5	12.0	7.3	10.0	81.8	10.0	29.0
Swarna	4.0	2.5	11.5	10.8	10.5	83.3	9.5	35.0
Fp	<0.001	0.002	0.002	<0.001	0.261	0.308	0.235	0.126
Vr	6.71**	2.99*	2.85*	33.31**	1.26	1.20	1.30	1.52
Mean	3.00	2.00	18.59	6.09	13.00	69.90	13.55	29.60
SE±	0.40	0.30	4.07	0.34	4.40	15.06	3.62	7.35
LSD (P 0.05)	1.10	0.90	11.73	0.99	12.60	43.45	10.45	21.19
CV (%)	18.10	18.90	30.90	8.00	48.80	30.50	37.80	35.10

******, and ***** Significant at Fpr value 0.01% and 0.05% respectively

Appendix 3 cont

Genotype	No. of shoot fly dead hearts 1	Shoot fly deadhearts (%) 1	No. of plants with SF eggs 1	Total no. of SF eggs 1	Trichome abaxial	Trichome adaxial	Plant height (cm)
BTx 623	13.5	100.0	12.5	36.5	0.0	2.5	95.0
ICSA 14013	22.0	100.0	22.0	46.5	0.0	0.0	103.7
ICSA 14024	21.5	97.7	18.5	32.5	0.0	0.0	110.6
ICSA 14027	25.0	96.7	21.5	43.5	12.0	0.0	158.1
ICSA 14037	25.5	89.5	19.5	34.5	0.0	6.0	138.5
ICSA 323	6.0	100.0	6.0	27.0	0.0	0.0	115.8
ICSA 458	19.5	76.0	23.5	47.0	24.0	0.0	103.5
ICSA 467	12.5	78.2	14.5	21.0	14.0	2.5	145.5
ICSA 487	17.5	65.6	11.0	25.0	12.5	0.0	160.0
ICSA 52	9.0	94.4	9.5	25.5	0.0	0.0	137.9
ICSA 84	15.5	93.5	17.0	35.0	0.0	0.0	103.5
ICSB 14013	15.0	100.0	15.0	27.5	0.0	15.0	91.2
ICSB 14024	6.0	94.4	6.5	16.5	0.0	0.0	108.1
ICSB 14027	20.0	97.7	19.0	29.0	46.0	55.0	176.0
ICSB 14037	30.5	92.5	18.5	34.0	0.0	55.0	163.8
ICSB 323	18.0	95.7	19.0	33.5	0.0	0.0	125.1
ICSB 458	15.5	79.8	18.5	40.5	0.0	10.5	105.2
ICSB 467	16.0	84.5	14.0	24.0	32.0	41.0	136.5
ICSB 487	15.5	71.5	14.5	25.5	26.5	69.5	134.5
ICSB 52	11.5	100.0	11.5	34.0	9.5	44.0	118.9
ICSB 84	26.0	100.0	25.5	44.5	0.0	0.0	117.2
ICSV 25280	13.0	96.2	13.5	32.0	13.5	73.5	203.5
ICSV 25292	13.0	96.9	13.0	36.5	0.0	7.5	228.0
ICSV 25303	14.5	97.6	14.0	36.5	0.0	0.0	153.6
ICSV 25316	15.0	100.0	14.0	35.0	39.5	60.5	215.5
ICSV 25337	16.0	96.0	12.5	35.0	18.0	51.5	175.0
ICSV 700	27.5	82.2	26.0	45.0	8.0	67.0	285.0
ICSV 93046	16.0	79.9	18.0	39.0	0.0	43.0	265.2
IS 18551	14.0	73.2	15.5	27.0	18.0	66.0	268.0
S-35	17.5	92.2	17.0	40.0	0.0	0.0	215.5
SSV74	11.5	95.5	12.0	36.5	0.0	0.0	224.0
Swarna	11.5	100.0	11.5	43.5	0.0	9.5	126.9
Fp	0.002	<0.001	0.055	0.447	<0.001	<0.001	<0.001
Vr	2.95*	7.29**	1.79*	1.05	10.44**	27.36**	15.81**
Mean	17.00	91.20	15.77	34.00	8.55	21.23	157.00
SE±	3.40	3.69	3.64	7.52	3.98	5.21	13.70
LSD (P 0.05)	9.80	10.64	10.51	21.70	11.47	15.02	39.50
CV (%)	29.00	5.70	32.70	31.30	65.80	34.70	12.40

******, and ***** Significant at Fpr value 0.01% and 0.05% respectively

APPENDIX 4

Evaluation of sorghum genotypes for resistance to shoot fly (Post rainy 2017)

Genotype	Glossy score	Leaf sheath pigmentation	Total no. of plants	Seedling height (cm)	No. of shoot fly dead hearts	Shoot fly deadhearts (%)	No. of plants with SF eggs	Total no. of SF eggs
Btx 623	4.00	4.50	21.00	8.90	7.75	42.43	9.75	13.00
ICSA 14013	4.00	4.00	21.50	7.25	12.00	56.03	14.75	19.50
ICSA 14024	4.00	3.50	25.00	8.75	15.50	61.67	17.25	18.25
ICSA 14027	3.00	3.50	22.00	9.75	9.25	41.87	16.75	19.25
ICSA 14037	2.00	3.00	25.50	9.50	9.50	36.34	13.75	15.25
ICSA 323	4.50	4.50	21.50	6.15	13.25	63.72	19.50	24.00
ICSA 458	3.50	3.50	21.50	6.15	2.50	12.61	3.75	3.75
ICSA 467	2.50	3.00	23.50	6.50	6.25	24.02	8.00	9.00
ICSA 487	1.50	1.50	26.75	6.15	5.00	17.91	8.00	8.75
ICSA 52	4.50	5.00	26.00	6.10	15.25	58.04	21.25	26.00
ICSA 84	4.50	4.00	31.50	6.25	16.50	52.58	23.25	24.75
ICSB 14013	4.50	3.50	31.50	7.75	15.00	47.42	21.50	24.50
ICSB 14024	4.00	4.50	24.00	7.25	13.00	52.86	19.25	24.75
ICSB 14027	3.00	3.50	24.00	8.25	4.00	16.35	14.00	19.25
ICSB 14037	2.00	3.00	29.25	9.50	4.25	14.47	12.75	14.75
ICSB 323	4.50	4.50	28.50	5.75	9.75	34.26	21.25	26.00
ICSB 458	4.00	4.50	22.00	5.15	4.50	20.34	6.25	7.00
ICSB 467	3.00	3.50	10.25	7.90	2.50	21.96	3.50	4.25
ICSB 487	1.00	2.00	23.00	7.25	4.75	21.12	9.50	11.25
ICSB 52	4.00	4.50	25.50	7.15	14.50	57.00	19.25	22.75
ICSB 84	4.50	4.50	19.00	6.50	7.25	37.65	11.25	13.50
ICSV 25280	3.00	4.50	22.00	8.00	10.00	46.32	10.00	12.75
ICSV 25292	4.50	3.50	19.25	11.30	10.50	54.37	16.25	19.75
ICSV 25303	3.50	4.00	18.00	8.50	11.75	66.88	13.50	15.75
ICSV 25316	3.50	4.00	15.25	8.25	9.00	58.84	9.00	13.00
ICSV 25337	3.00	3.00	23.50	8.40	6.50	27.72	15.00	17.25
ICSV 700	1.00	1.50	22.00	10.00	4.75	18.68	7.25	8.25
ICSV 93046	2.00	1.50	17.50	7.15	4.25	24.34	5.50	6.50
IS 18551	1.00	2.00	22.00	11.40	1.25	6.09	4.25	4.75
S 35	3.00	3.00	16.00	8.00	6.00	37.25	10.25	12.25
SSV 74	5.00	4.50	13.00	11.15	9.50	65.00	12.00	15.50
Swarna	5.00	4.50	25.00	8.75	11.75	47.12	18.50	22.25
Fp	<0.001	<0.001	0.072	<0.001	0.001	<0.001	<0.001	<0.001
Vr	15.38**	8.9**	1.7*	22.39**	3.05**	4.78**	3.91**	6.42**
Mean	3.34	3.56	22.38	7.96	8.67	39.00	13.00	15.55
SE±	0.30	0.33	3.69	0.35	2.47	8.30	2.89	2.69
LSD (P 0.05)	0.88	0.96	10.65	1.00	7.13	23.80	8.34	7.77
CV (%)	12.90	13.30	23.30	6.20	40.30	30.00	31.40	24.50

******, and ***** Significant at Fpr value 0.01% and 0.05% respectively

Appendix 4 cont

Genotype	No. of shoot fly dead hearts 1	Shoot fly deadhearts (%) 1	No. of plants with SF eggs 1	Total no. of SF eggs 1	Trichome abaxial	Trichome adaxial	Plant height (cm)
Btx 623	11.75	62.62	11.50	13.75	0.00	17.00	105.00
ICSA 14013	19.25	89.80	19.00	22.00	5.42	0.00	111.50
ICSA 14024	18.00	71.67	19.00	25.50	0.00	1.83	99.00
ICSA 14027	17.50	79.19	19.25	24.00	10.42	4.67	142.50
ICSA 14037	18.25	71.41	21.25	23.00	7.00	17.17	153.50
ICSA 323	19.50	92.59	21.00	31.25	0.00	14.17	97.50
ICSA 458	10.50	51.43	7.75	8.75	10.00	26.75	87.50
ICSA 467	12.50	53.14	16.50	17.50	20.00	57.92	99.00
ICSA 487	9.00	34.23	8.75	8.25	12.50	35.75	117.50
ICSA 52	20.25	77.38	22.25	30.25	0.00	0.00	114.00
ICSA 84	23.00	73.03	25.50	32.50	0.00	18.17	122.50
ICSB 14013	25.25	80.23	25.50	24.00	0.00	16.67	112.50
ICSB 14024	18.00	73.57	19.00	23.25	16.42	20.08	122.50
ICSB 14027	13.75	57.09	17.50	21.75	22.50	35.58	169.50
ICSB 14037	16.25	55.61	20.50	20.75	0.00	36.33	144.00
ICSB 323	23.50	82.41	24.25	29.25	0.00	0.00	112.50
ICSB 458	10.00	47.36	12.50	9.25	0.67	4.25	86.50
ICSB 467	5.00	43.92	5.75	6.25	22.00	24.00	119.50
ICSB 487	10.75	46.50	12.25	11.00	40.00	30.50	115.50
ICSB 52	22.00	86.38	23.75	23.75	9.08	44.58	137.00
ICSB 84	11.50	58.33	11.25	16.25	0.00	17.67	136.50
ICSV 25280	17.25	76.26	13.25	16.00	17.08	33.08	183.50
ICSV 25292	14.25	69.08	16.50	27.25	0.75	1.58	189.00
ICSV 25303	15.00	83.13	16.50	17.75	0.00	0.00	159.00
ICSV 25316	13.25	86.85	14.00	22.00	20.50	45.75	227.50
ICSV 25337	15.25	65.08	19.50	20.00	7.50	32.08	188.50
ICSV 700	8.50	33.66	9.25	10.00	33.50	54.83	227.50
ICSV 93046	8.00	45.56	9.50	12.00	0.00	42.92	203.50
IS 18551	5.75	26.82	6.25	6.25	16.00	36.67	219.00
S 35	9.75	59.31	11.50	13.75	0.00	0.00	142.50
SSV 74	10.75	77.08	12.50	15.75	14.33	0.00	166.50
Swarna	21.00	84.13	23.50	35.75	1.67	5.83	134.00
Fp	0.004	<0.001	<0.001	<0.001	0.501	0.560	<0.001
Vr	2.64*	3.38**	4.93**	4.79**	1.00	1.27	351.48**
Mean	14.82	65.50	16.13	19.34	9.00	21.00	142.06
SE±	3.30	9.68	2.62	3.68	10.70	15.70	2.17
LSD (P 0.05)	9.51	27.93	7.56	10.61	30.90	45.40	6.27
CV (%)	31.40	20.90	23.00	26.90	168.90	105.40	2.20

******, and ***** Significant at Fpr value 0.01% and 0.05% respectively

APPENDIX 5

Pearson correlation coefficient for rainy season (2016)

	GS	LSP	TNP	SH	NSFDH	PSFDH	PWSFE	SFE	SFDH 1	PSFDH1	PWSFE 1	SFE 1	Taba	Tada	PH
GS	1.000														
LSP	0.844**	1.000													
TNP	-0.394*	-0.391*	1.000												
SH	-0.165	0.012	-0.286	1.000											
NSFDH	0.475**	0.461**	0.139	-0.090	1.000										
PSFDH	0.647**	0.625**	-0.348	0.072	0.861**	1.000									
PWSFE	-0.219	-0.252	0.904**	-0.237	0.332	-0.116	1.000								
SFE	-0.103	-0.204	0.344	-0.118	0.099	-0.079	0.507**	1.000							
SFDH 1	-0.255	-0.288	0.964**	-0.292	0.239	-0.243	0.921**	0.399*	1.000						
PSFDH1	0.659**	0.535**	-0.536**	0.087	0.307	0.541**	-0.321	0.020	-0.296	1.000					
PWSFE 1	0.019	0.024	0.768**	-0.246	0.429*	0.024	0.889**	0.499**	0.850**	-0.070	1.000				
SFE 1	0.111	0.073	0.081	-0.005	0.208	0.129	0.298	0.860**	0.172	0.246	0.448*	1.000			
Taba	-0.436*	-0.428*	0.119	0.190	-0.141	-0.165	0.079	-0.044	0.031	-0.351*	-0.075	-0.120	1.000		
Tada	-0.506**	-0.501**	0.008	0.197	-0.286	-0.268	-0.042	-0.043	-0.057	-0.277	-0.264	-0.149	0.544**	1.000	
PH	-0.487**	-0.439*	-0.091	0.278	-0.225	-0.189	-0.167	-0.077	-0.148	-0.204	-0.360*	-0.224	0.177	0.494**	1.000

**, and * Significant at Fpr value 0.01% and 0.05% respectively

APPENDIX 6

Pearson correlation coefficient for post-rainy season (2016)

	GS	LSP	TNP	SH	SFDH	PSFDH	PWSFE	TNE	SFDH1	PSFDH1	PWSFE	TNE1	Tabax	Tadax	PH
GS	1.000														
LSP	0.828**	1.000													
TNP	0.117	0.155	1.000												
SH	-0.252	-0.314	0.009	1.000											
SFDH	0.677**	0.635**	0.288	0.047	1.000										
PSFDH	0.643**	0.609**	-0.061	0.084	0.919**	1.000									
PWSFE	0.689**	0.707**	0.427*	0.030	0.882**	0.759**	1.000								
TNE	0.707**	0.699**	0.383*	0.030	0.852**	0.741**	0.971**	1.000							
SFDH1	0.656**	0.686**	0.541**	-0.040	0.817**	0.651**	0.923**	0.913**	1.000						
PSFDH1	0.631**	0.678**	0.008	0.009	0.752**	0.818**	0.798**	0.811**	0.818**	1.000					
PWSFE	0.626**	0.656**	0.487**	0.054	0.725**	0.572**	0.868**	0.887**	0.869**	0.684**	1.000				
TNE1	0.661**	0.629**	0.462**	0.048	0.764**	0.615**	0.906**	0.924**	0.864**	0.699**	0.945**	1.000			
Taba	-0.308	-0.261	-0.178	-0.027	-0.450**	-0.386*	-0.472**	-0.437*	-0.434*	-0.383*	-0.356*	-0.395*	1.000		
Tada	-0.420*	-0.378*	0.008	0.148	-0.373*	-0.364*	-0.374*	-0.328	-0.350*	-0.381*	-0.207	-0.306	0.529**	1.000	
PH	-0.413*	-0.412*	-0.093	0.555**	-0.077	0.019	-0.135	-0.107	-0.209	-0.062	-0.101	-0.123	0.236	0.596**	1.000

** and * Significant at Fpr value 0.01% and 0.05% respectively

APPENDIX 7

Pearson correlation coefficient for rainy season (2017)

	GS	LSP	TNP	SH	NSFDH	PSFDH	PWSFE	SFE	SFDH 1	PSFDH1	PWSFE 1	SFE 1	Taba	Tada	PH
GS	1.000														
LSP	0.813**	1.000													
TNP	-0.607**	-0.491**	1.000												
SH	0.032	-0.158	-0.137	1.000											
NSFDH	-0.102	-0.105	0.779**	-0.211	1.000										
PSFDH	0.773**	0.572**	-0.372*	-0.078	0.268	1.000									
PWSFE	-0.302	-0.237	0.868**	-0.081	0.770**	-0.202	1.000								
TNE	0.225	0.301	0.370*	-0.051	0.495**	0.151	0.644**	1.000							
SFDH 1	-0.354*	-0.288	0.937**	-0.156	0.879**	-0.138	0.895**	0.495**	1.000						
PSFDH1	0.835**	0.656**	-0.422*	-0.032	0.088	0.759**	-0.161	0.276	-0.087	1.000					
PWSFE 1	-0.299	-0.178	0.808**	-0.110	0.774**	-0.165	0.911**	0.526**	0.856**	-0.128	1.000				
SFE 1	0.116	0.162	0.401*	0.016	0.594**	0.189	0.604**	0.712**	0.510**	0.209	0.664**	1.000			
Taba	-0.219	-0.313	0.125	0.203	-0.020	-0.220	0.003	-0.214	0.044	-0.262	0.058	-0.215	1.000		
Tada	-0.375*	-0.428*	0.206	0.238	-0.041	-0.363*	0.098	-0.128	0.141	-0.217	0.066	-0.121	0.586**	1.000	
PH	-0.231	-0.266	0.193	0.304	0.108	-0.149	0.147	0.080	0.111	-0.254	0.111	0.113	0.197	0.533**	1.000

**, and * Significant at Fpr value 0.01% and 0.05% respectively

APPENDIX 8

Pearson correlation coefficient for post-rainy season (2017)

	GS	LSP	TNP	SH	SFDH	PSFDH	PWSFE	TNE	SFDH1	PSFDH1	PWSFE	TNE1	Taba	Tada	PH
GS	1.000														
LSP	0.868**	1.000													
TNP	0.022	0.000	1.000												
SH	-0.215	-0.244	-0.266	1.000											
SFDH	0.670**	0.585**	0.381	-0.131	1.000										
PSFDH	0.708**	0.646**	-0.075	0.040	0.873**	1.000									
PWSFE	0.610**	0.515**	0.580**	-0.115	0.863**	0.642**	1.000								
TNE	0.638**	0.572**	0.508**	-0.105	0.842**	0.667**	0.985**	1.000							
SFDH1	0.567**	0.540**	0.669**	-0.215	0.841**	0.581**	0.917**	0.897**	1.000						
PSFDH1	0.728**	0.732**	0.126	-0.080	0.816**	0.857**	0.749**	0.788**	0.802**	1.000					
PWSFE	0.509**	0.465**	0.668**	-0.153	0.777**	0.516**	0.925**	0.900**	0.953**	0.741**	1.000				
TNE1	0.627**	0.539**	0.470**	-0.068	0.799**	0.637**	0.908**	0.907**	0.873**	0.792**	0.903**	1.000			
Taba	-0.589**	-0.419*	-0.228	0.171	-0.433*	-0.378*	-0.435**	-0.394*	-0.432*	-0.412*	-0.424*	-0.449*	1.000		
Tada	-0.635**	-0.534**	0.054	-0.012	-0.450**	-0.523**	-0.450**	-0.450**	-0.340	-0.485**	-0.304	-0.388*	0.583**	1.000	
PH	-0.389*	-0.384*	-0.312	0.626**	-0.259	-0.093	-0.252	-0.216	-0.308	-0.175	-0.266	-0.161	0.305	0.396*	1.000

**, and * Significant at Fpr value 0.01% and 0.05% respectively

APPENDIX 9

Evaluation of biochemical components in sorghum lines (rainy season)

S No.	Genotypes	Total soluble sugars (%)	Proteins (mg/g)	Tannins (mg catechin/g)	Total Phenols (mg/g)
1	ICSA 52	16.72	2.45	34.67	8.09
2	ICSA 84	13.54	2.03	43.55	7.98
3	ICSA 323	16.86	1.54	24.22	9.87
4	ICSA 458	14.77	1.83	56.62	9.94
5	ICSA 467	16.46	1.44	47.39	9.63
6	ICSA 487	14.81	1.87	44.08	9.27
7	ICSA 14013	16.52	1.88	27.35	9.68
8	ICSA 14024	14.76	1.86	33.45	8.81
9	ICSA 14027	15.90	1.71	28.05	10.27
10	ICSA 14037	16.12	2.06	39.37	11.23
11	ICSB 52	14.40	2.28	27.87	10.50
12	ICSB 84	16.99	1.37	18.64	9.06
13	ICSB 323	16.49	1.27	25.78	6.57
14	ICSB 458	13.09	1.70	45.64	10.44
15	ICSB 467	16.27	1.20	28.05	7.85
16	ICSB 487	14.89	1.85	41.46	8.04
17	ICSB 14013	15.06	1.63	48.08	9.70
18	ICSB 14024	16.19	1.90	40.42	10.25
19	ICSB 14027	15.97	1.32	27.53	7.03
20	ICSB 14037	13.95	2.33	45.99	8.86
21	ICSV 25280	14.43	1.67	51.22	7.68
22	ICSV 25292	17.01	1.72	38.85	9.36
23	ICSV 25303	12.15	2.10	38.50	9.71
24	ICSV 25316	12.62	1.83	48.61	8.33
25	ICSV 25337	14.55	1.93	40.59	7.80
26	ICSV 700	15.72	2.58	35.02	9.37
27	S 35	14.64	2.05	32.75	8.72
28	SSV 74	8.63	1.72	33.28	8.88
29	ICSV 93046	15.52	1.70	36.41	9.05
30	Btx 623	15.77	1.79	56.62	11.30
31	IS 18551 (R)	13.78	1.75	47.56	8.26
32	Swarna (S)	14.52	1.87	45.12	9.02
33	CSH 16	15.53	1.84	37.11	8.34
Min		8.63	1.20	18.64	6.57
Max		17.01	2.58	56.62	11.30
Mean		14.99	1.82	38.48	9.06
SE		0.30	0.06	1.64	0.20
SD		1.70	0.32	9.44	1.12
CV (%)		11.37	17.36	24.54	12.37

APPENDIX 10

Evaluation of sorghum lines for biochemical components (post rainy)

S No.	Genotypes	Total soluble sugars (%)	Proteins (mg/g)	Tannins (mg catechin/g)	Total Phenols (mg/g)
1	ICSA 52	14.51	1.80	40.07	11.03
2	ICSA 84	6.62	1.60	28.92	9.98
3	ICSA 323	5.10	1.94	33.62	10.09
4	ICSA 458	4.87	2.06	22.65	11.56
5	ICSA 467	4.05	1.26	31.18	9.58
6	ICSA 487	6.20	1.28	41.29	11.04
7	ICSA 14013	5.40	1.57	47.04	10.84
8	ICSA 14024	5.53	1.72	25.96	11.68
9	ICSA 14027	7.02	1.77	17.07	11.56
10	ICSA 14037	9.66	2.17	9.06	11.66
11	ICSB 52	4.11	1.52	59.23	10.79
12	ICSB 84	5.03	1.34	13.07	8.18
13	ICSB 323	5.65	1.57	40.59	10.05
14	ICSB 458	5.38	1.30	27.53	9.86
15	ICSB 467	4.26	1.03	44.95	7.62
16	ICSB 487	4.70	1.54	47.91	11.22
17	ICSB 14013	6.99	1.17	28.75	9.34
18	ICSB 14024	4.58	1.64	25.78	11.13
19	ICSB 14027	6.43	1.65	26.13	11.39
20	ICSB 14037	6.12	2.20	27.53	11.16
21	ICSV 25280	8.29	1.19	23.00	11.01
22	ICSV 25292	8.65	1.57	16.90	10.31
23	ICSV 25303	7.62	1.83	24.04	11.36
24	ICSV 25316	5.85	1.80	23.17	11.68
25	ICSV 25337	6.99	1.52	16.72	11.20
26	ICSV 700	6.38	1.47	22.65	11.60
27	S 35	6.85	1.78	18.29	11.54
28	SSV 74	5.03	1.86	36.76	11.49
29	ICSV 93046	7.06	1.56	19.16	11.31
30	Btx 623	7.82	1.24	23.87	10.69
31	IS 18551 (R)	5.59	1.25	22.13	10.99
32	Swarna (S)	4.78	1.61	12.54	9.96
33	CSH 16	6.15	1.71	20.73	11.15
Min		4.05	1.03	9.06	7.62
Max		14.51	2.20	59.23	11.68
Mean		6.34	1.59	27.83	10.73
SE		0.35	0.05	2.00	0.17
SD		1.99	0.29	11.49	0.98
CV (%)		31.37	18.07	41.28	9.16

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