Electronic Journal of Plant Breeding, 10 (3): 1133-1139(Sep 2019) DOI: 10.5958/0975-928X.2019.00144.3
ISSN 0975-928X

Research Article

Screening the genotypes of sorghum (*Sorghum bicolor* (L.) Moench) BC1 F_3 generation of the cross CO (S) 28 x IS18551 for shoot fly (*Atherigona soccata* (Rond.) resistance

M.L. Abinaya[1], N. Kumaravadivel[2], S. Varanavasiappan[3] and D. Kavithamani[4]
[1]M.Sc student, Department of Plant Biotechnology, Centre for Plant Molecular Biology, TNAU, Coimbatore-641003
[2]Professor and Head, Department of Plant Molecular Biology and Bioinformatics, CPMB&B, TNAU, Coimbatore-641003
[3]Assistant Professor, Department of Plant Biotechnology, Centre for Plant Molecular Biology, TNAU, Coimbatore-641003
[4] Assistant Professor, Department of Millets, Centre for Plant Breeding and Genetics, TNAU, Coimbatore-641003
E-Mail: mlabinaya@gmail.com

(Received: 13 Sep 2019; Revised: 21 Sep 2019; Accepted: 25 Sep 2019)

Abstract
Screening of sorghum genotypes of BC_1F_3 generation of the cross CO (S) 28 x IS18551 for shoot fly [*Atherigona soccata* (Rondani)] resistance was carried out during *kharif* 2018-19. The segregating population was subjected to Foreground selection using SSR markers flanking QTLs imparting shoot fly resistance which is in the linkage group SBI-05 and SBI-10. From the 100 plants, 29 plants with 4 QTLs, 35 plants with 3 QTLs, 24 plants with 2 QTLs and 9 plants with 1 QTL have been identified. Shoot fly component traits and phenotypic observations were recorded for the same 100 plants. Shoot fly infestation was found to be very less in the sorghum lines harboring 4 QTLs. Based on the genotypic data, 10 lines were selected and forwarded to next generation to study the agronomic performance. Grain yield per plant was comparatively higher in BC_1F_4 generation because of reduced infestation of shoot fly.

Key words
Sorghum shoot fly, screening, SSR Markers and resistant QTLs and shoot fly component traits

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] also known as jowar is an annual crop belonging to family Poaceae, subfamily Panicoidae, tribe Andropoganae and subtribe Sorghastrae (Price *et al.*, 2005). It is a diploid species (2n=20) with ten pairs of chromosomes. In India, it is cultivated in an area of about 4.01 million ha with a production of 3.70 million metric tonnes and productivity of 920 kg/ ha (USDA 2019). Tamil Nadu stands fourth after Maharashtra, Karnataka and Rajasthan in area (0.35 million ha) (Directorate of Economics and Statistics, 2018). The food and fuel production challenges of the approaching century would require production gains from the ancient crop breeding, genomic selection and editing with biotechnological approaches that develop plants with increased productivity and traits such as disease, pest and drought resistance, and canopies with high photosynthetic efficiency (Mullet *et al.*, 2014). In that context, it has a massive prospective for production of lingo – cellulosic ethanol and energy by means of biomass combustion.

Increased sorghum production has been observed over the past three decades, by exploiting the use of cytoplasmic male sterility. But sorghum cultivars yield potential is not achieved in farmer's field because of many biotic stresses (Aruna *et al.*, 2011). More than 150 species of insects attack the sorghum and complete several generation within the growing season causing a huge biomass loss (Guo *et al.*, 2011). In Asia, Africa and Europe, it is the most destructive pest preventing its production. In India, the grain yield loss due to shoot fly attack varies from 50% (Kishore and Jotwani, 1982) to 90% (Rao and Gowda, 1967). Increase in 1 % of dead heart symptom can cause a loss of about 143 kg grain yield/ ha (Dhaliwal *et al.*, 2004).

Sorghum Shootfly (*Atherigona soccata)* belongs to the order Diptera and family Muscidae. It completes its whole life cycle within 17-21 days. Female shoot fly lays single egg on lower surface of the leaves parallel to the mid rib under humid condition in 7 to 8 days old seedlings. Eggs are elongated, cigar shaped and white in color with dimension of 0.8 x 0.2 mm. In normal days,1to3 eggs/ tillers will be seen and it may vary with level of invasion (Padmaja *et al.*, 2010). After 2 days of egg hatchment, it crawls down to the central whorl and feeds on the growing point of young seedlings. Maggot stage takes about 7 – 12 days followed by pupal stage which occurs in base of dead shoot or sometimes in soil for about 7 days. From the pupae, adult emerges out resembling a small housefly. Male moth will be blackish. In female head and thorax will be pale gray and abdomen will be

yellow in color with paired brown patches. Adult will be active throughout the day.

Among the different stages of shoot fly, Maggot is the phase which causes a huge damage to crop. It causes a specific symptom called "dead heart" which is due to the death of central whorl - leaves inside which shoot apical meristem will present. As a result of loss of apical growth, axial tillers are produced which are rarely productive. Under heavy infestation, it will also be affected by shoot fly resulting in stunted growth, grain and forage yield loss.

To meet the future demand of both grain and fodder sorghum, genetic manipulation to impart shoot fly resistance in sorghum is very important. It would also decline the usage of costly and toxic chemicals resulting in safe and healthy environment. Resistance to shoot fly is governed by various component traits of which the most important three traits viz., non-preference for oviposition (antixenosis), antibiosis and tolerance (Gorthy *et al.*, 2017). There is a huge barrier in transferring host plant resistance through traditional breeding and field screening is also a laborious method. Hence, developing shoot fly resistance cultivar through traditional breeding has been taking a long time. Alternative method for developing a shoot fly resistance cultivar could be MAS (Marker Assisted Selection) where molecular markers tightly linked to genomic region which impart the resistance to shoot fly will be used. QTL's associated with shoot fly resistance has been identified by several scientists (Satish *et al.*, 2009; Apotikar *et al.*, 2011; Aruna *et al.*, 2011; Satish *et al.*, 2012; Kiranmayee *et al.*, 2016; Gorthy *et al.*, 2017). The aim of this study is to screen the BC_1F_3 generation of the cross CO(S) 28 X IS 18551 using the molecular markers for sorghum shoot fly resistance along with phenotypic confirmation.

Materials and Methods

The segregating population of BC_1F_3 generation of the cross CO(S) 28 x IS18551 were raised with spacing of 45 x 15 cm along with both resistant and susceptible parents. It was screened at New Area field no. 4 C during *Kharif* 2018 under the Department of Millets, TNAU, Coimbatore at the Latitude 11.0136 ^{0}N and longitude 76.9378 ^{0}E with an altitude of 436.8m. As per Crop Production Guide (2012), general agronomic practices were followed except the insecticide spray. CO(S) 28 was developed from the cross CO 25 X SPV 942 in TNAU, Coimbatore. It matures in 100 to 105 days. It was moderately resistant to grain mold disease but it was susceptible to shoot fly (Veerabadhiran *et al.*, 2002). IS18551 belongs to "durra" race from Ethiopia and is one of the important international check varieties for shoot fly. It has a narrow, pointed and shiny leaves, ear head with straw colored grains and tall stature (Bantilan *et al.*, 2004).

Molecular Markers:In our study, for screening the back cross progenies, marker associated with the shoot fly resistant QTL's as reported by Satish *et al.* (2012) was used. 4 major QTL's associated with shoot fly resistance was found to be in two chromosome viz., SBI-5 (QTL 1) and SBI-10 (QTL 2 – 4). Simple Sequence Repeat (SSR) markers associated with this 4 QTL's are listed in table 1.

In field, individual plants were tagged separately and leaf samples were collected from each tagged plants for DNA isolation. DNA extraction was carried out by CTAB (Cetyl Tri methyl Ammonium Bromide) method from 20 days old seedlings. The concentration of the isolated DNA was verified with Nano drop and it was diluted to the final concentration of 50ng/µl. PCR (Polymerase chain reaction) was carried out with isolated DNA using different markers associated with shoot fly resistance imparting QTLs. In PCR, initial denaturation was carried out at 94°C for 5 mins, denaturation at 94°C for 1 min, annealing at 55°C or 58°C for 1 min depending on the marker, extension at 72°C for 1 min, cycles were repeated from denaturation to extension for 35 times and final extension was carried out at 72°C for 7 mins with hold temperature of 4°C. PCR products were resolved in 3.5% agarose gel and progenies were scored based on their parent's polymorphism.

Phenotyping for shoot fly resistance: Observations were recorded on different component traits of shoot fly resistance. Glossiness is an important trait for multiple insect resistances as they affect the quality of light reflected from the leaves. Leaf glossiness was recorded at 14 DAE in early morning hours with scale of 1 – 5. Score of 1 is considered as a glossy with light green, shiny, narrow and erect leaves whereas 5 being non glossy with dark green, dull, broad and drooping leaves (Chamarthi *et al.*, 2011).

Seedling vigor imparts shoot fly resistance to the seedlings by making them to grow rapidly thus preventing the 1st instar larvae from the reaching the central whorl. It was scored at 14 DAE on a scale of 1-5 where 1 stands for high vigor and 5 for low vigor i.e., plants with maximum height, full expansion of leaf and robust (Chamarthi *et al.*, 2011).

Trichome density on the adaxial and abaxial surface was recorded by taking 2 cm^2 from the central portion of the fifth leaf. It was placed in 2:1

acetic acid and alcohol solution in glass vial for 24 h. It was transferred to 90% lactic acid for overnight to clear the chlorophyll content. Thereafter trichomes were counted using stereomicroscope at a magnification of 10 X and results were expressed in no. of trichomes per sq. cm.

Non preference for oviposition (antixenosis) is the primary mechanism associated with shoot fly resistance. Thereforth, No. of eggs laid by the female shoot fly at 21 and 28 DAE were recorded and oviposition percentage was derived using the following formula

$$\text{Oviposition}(\%) = \frac{\text{No. of seedlings with egg}}{\text{Total no. of seedlings}} x100$$

Dead heart is the direct measure of shoot fly resistance. Its percentage was calculated using the formula

$$\text{Dead heart}(\%) = \frac{\text{No. of seedlings with dead heart}}{\text{Total no. of seedlings}} x100$$

Results and Discussion

In BC_1F_3 generation, total of 100 plants were screened using foreground SSR markers. Individual plants were genotyped and scored for polymorphism based on the parents P_1 [CO(S) 28] and P_2 [IS18551]. Lines which are showing band similar to parent P_1 are scored as A, P_2 as B (A, B – homozygous) whereas lines showing band similar to both P_1 and P_2 are scored as H (heterozygous) (Fig. 1). Evaluation was carried out for shoot fly component traits which revealed that plants harboring 4 QTLs recorded very less oviposition and dead heart % with high glossiness and seedling vigor. It is in accordance with the results observed by Dhillon *et al.* (2006) and Gorthy *et al.* (2017).Significantly, higher leaf glossiness (1.56) is found in lines harboring 4 QTLs. This is on par with results obtained by Chamarthi *et al.* (2011). Seedlings with rapid growth make plants less preferred to shoot fly (Bhagwat *et al.*, 2011). Present study also revealed that plants with high seedling vigor have reduced oviposition and dead heart %. Plants which have more trichome density have reduced shoot fly infestation and this is in accordance with results obtained by Sajjanar (2002).

Plants with increased no. of eggs have prominent dead heart symptom i.e., oviposition and dead heart are found to be positively correlated which is similar to the study made by Dhillon *et al.* (2005). Line showing resistance to shoot fly are highly vigorous with high glossy leaves consisting of more trichomes especially in lower surface of the leaves. From the study, it is clear that glossiness, seedling vigor, trichome density are negatively correlated with the oviposition % and dead heart % (table 2). No. of side tillers are found to be high in the plants/lines having increased oviposition. Observations made in this study are similar with the previous studies made by Apotikar *et al.* (2011). Over all, lines which harbor 4 QTLs are performing well against shoot fly and the resistance get hold when the no. of QTLs are reduced. From the results, it is also evident that back crossed lines with even less no. of QTL perform better than the susceptible parent CO (S) 28.

Phenotypic observations like plant height (161.07 and 160.25 cm), days to flowering (72.28 and 70.91 days), leaf length (54.21 and 57.16 cm), leaf width (5.24 and 5.40 cm), stem girth (3.93 and 4.51 cm), panicle length (17.48 and 18.06 cm), panicle width (5.11 and 5.69 cm), and grain yield per plant (15.42 and 20.53 g) were recorded in BC_1F_3 and BC_1F_4 respectively (Fig. 2). Lines harboring 4 QTLs in BC_1F_3 generation had very good agronomic performance in BC_1F_4 generation with increased grain yield per plant because of reduced shoot fly attack. Homozygous lines harboring 4 QTLs can be further used in plant breeding programme to develop a shoot fly resistant varieties/ hybrid.

Acknowledgement

The authors are thankful to the DBT for providing necessary funds under scheme (Dr. N. K. (BT/PR10928/AGII/106/954/2014 dated 29.06.2015)), Department of Millets, TNAU for providing field and Centre for Plant Molecular Biology and biotechnology (CPMB&B) for providing the needed facilities.

References

Apotikar, D., D. Venkateswarlu, R. Ghorade, R. Wadaskar, J. Patil, and P. Kulwal. 2011. "Mapping of shoot fly tolerance loci in sorghum using SSR markers." *Journal of Genetics* **90** (1):59-66.

Aruna, C., V. Bhagwat, R. Madhusudhana, V. Sharma, T. Hussain, R. Ghorade, H. Khandalkar, S. Audilakshmi, and N. Seetharama. 2011. "Identification and validation of genomic regions that affect shoot fly resistance in sorghum [Sorghum bicolor (L.) Moench]." *Theoretical and Applied Genetics* **122** (8):1617-1630.

Bantilan, M., C. Gowda, B. Reddy, A. Obilana, and R. Evenson. 2004. *Sorghum genetic enhancement: research process, dissemination and impacts*: International Crops Research Institute for the Semi-Arid Tropics.

Bhagwat, V., G.S. Prasad, A. Kalaisekar, B. Subbarayudu, T. Hussain, S. Upadhyaya, D.

Daware, R. Rote, and V. Rajaram. 2011. "Evaluation of Some Local Sorghum Checks Resistant to Shoot Fly (Atherigona soccata Rondani) and Stem Borer (Chilo partellus Swinhoe)." *Annals of Arid Zone* **50** (1):47-52.

Chamarthi, S., H. Sharma, K. Sahrawat, L. Narasu, and M. Dhillon. 2011. "Physico-chemical mechanisms of resistance to shoot fly, Atherigona soccata in sorghum, Sorghum bicolor." *Journal of Applied Entomology* **135** (6):446-455.

Dhaliwal, G., R. Arora, and A. Dhawan. 2004. "Crop losses due to insect pests in Indian agriculture: an update." *Indian Journal of Ecology* **31** (1):1-7.

Dhillon, M., H. Sharma, B. Reddy, R. Singh, and J. Naresh. 2006. "Inheritance of resistance to sorghum shoot fly, Atherigona soccata." *Crop Science* **46** (3):1377-1383.

Dhillon, M., H. Sharma, R. Singh, and J. Naresh. 2005. "Mechanisms of resistance to shoot fly, Atherigona soccata in sorghum." *Euphytica* **144** (3):301-312.

Gorthy, S., L. Narasu, A. Gaddameedi, H.C. Sharma, A. Kotla, S.P. Deshpande, and A.K. Are. 2017. "Introgression of shoot fly (Atherigona soccata L. Moench) resistance QTLs into elite post-rainy season Sorghum varieties using marker assisted backcrossing (MABC)." *Frontiers in Plant Science* **8**:1494.

Guo, C., W. Cui, X. Feng, J. Zhao, and G. Lu. 2011. "Sorghum insect problems and Managementf." *Journal of Integrative Plant Biology* **53** (3):178-192.

Kiranmayee, K.U., P.K. Kishor, C.T. Hash, and S.P. Deshpande. 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 Biology* **9** (1):12-28.

Kishore, P., and M. Jotwani. 1982. "Integrated pest management in sorghum." *Journal of Environmental Research* 3 (1):1-7.

Mullet, J., D. Morishige, R. McCormick, S. Truong, J. Hilley, B. McKinley, R. Anderson, S.N. Olson, and W. Rooney. 2014. "Energy sorghum—a genetic model for the design of C4 grass bioenergy crops." *Journal of Experimental Botany* **65** (13):3479-3489.

Padmaja, P., R. Madhusudhana, and N. Seetharama. 2010. "Sorghum shoot fly." *Directorate of Sorghum Research, Hyderabad, India*.

Price, H.J., S.L. Dillon, G. Hodnett, W.L. Rooney, L. Ross, and J.S. Johnston. 2005. "Genome evolution in the genus Sorghum (Poaceae)." *Annals of Botany* **95** (1):219-227.

Rao, M., and S. Gowda. 1967. "A short note on the bionomics and control of jowar fly." *Sorghum Newslett* **10**:55-57.

Sajjanar, G. 2002. "Genetic analysis and molecular mapping of components of resistance to shoot fly (Atherigona soccata Rond.) in sorghum [Sorghum bicolor (L.) Moench]." University of Agricultural Sciences.

Satish, K., R. Madhusudhana, P. Padmaja, N. Seetharama, and J. Patil. 2012. "Development, genetic mapping of candidate gene-based markers and their significant association with the shoot fly resistance quantitative trait loci in sorghum [Sorghum bicolor (L.) Moench]." *Molecular Breeding* **30** (4):1573-1591.

Satish, K., G. Srinivas, R. Madhusudhana, P. Padmaja, R.N. Reddy, S.M. Mohan, and N. Seetharama. 2009. "Identification of quantitative trait loci for resistance to shoot fly in sorghum [Sorghum bicolor (L.) Moench]." *Theoretical and Applied Genetics* **119** (8):1425-1439.

Veerabadhiran, P., M. Suresh, R. Marimuthu, P. Ramasamy, N. Subbaraman, C. Surendran, B. Rajasekaran, K. Ponnuswamy, and T. Ganapathy. 2002. "CO (S) 28-A high yielding short duration sorghum variety suited for Tamil Nadu." *MADRAS AGRICULTURAL JOURNAL* **89** (10/12):683-687.

Table 1. SSR Markers flanking QTL's associated with Shoot fly resistance

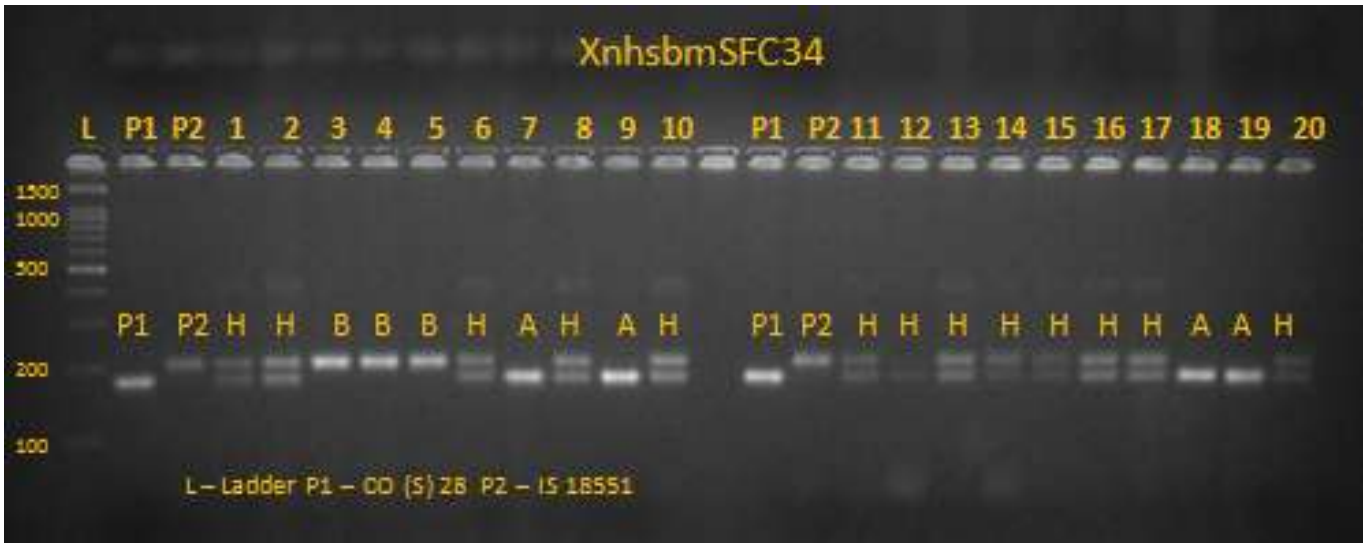
Chromosome number	QTL	Flanking Marker details	Traits with coupled with QTL
SBI-5	QTL 1	Xtxp65 – XnhsbmSFC61*	Leaf surface glossiness, dead heart % and Oviposition
SBI-10	QTL 2	Xtxp20 – Xnhsbm1011	Leaf glossiness, seedling vigour, dead heart %, oviposition and trichome density
SBI-10	QTL 3	XnhsbmSFC34* – XnhsbmSFCILP30	Leaf surface glossiness, oviposition,dead heart % and trichome density
SBI-10	QTL 4	Xtxp129 – Xtxp217	Dead heart % and leaf surface glossiness

*candidate gene marker

Table 2. BC_1F_3 generation – sorghum shoot fly resistance component traits mean performance

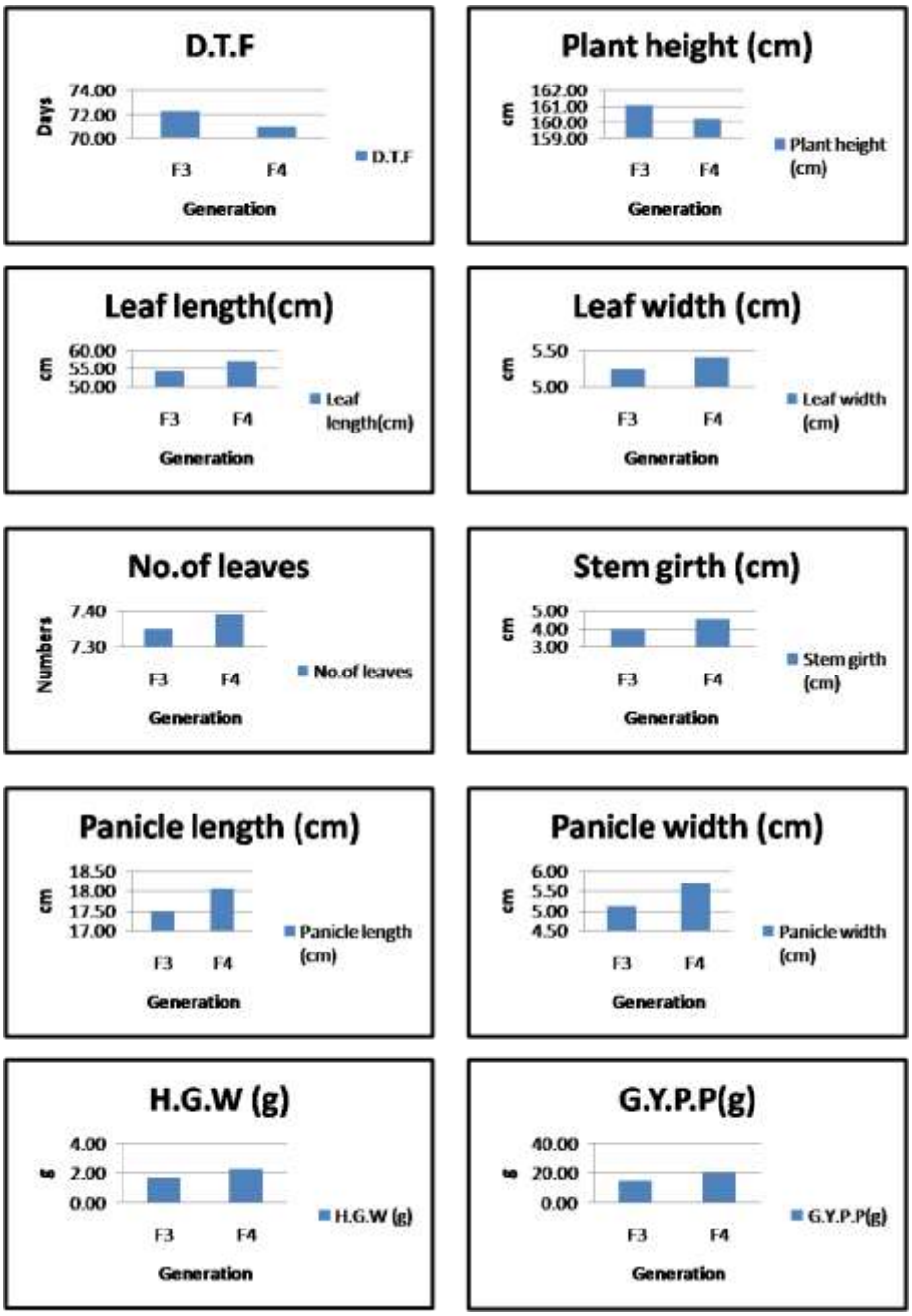
S.No	Genotype & Generation	No. of QTLs	Oviposition%	Deadheart%	No. of side tillers *	Leaf glosiness*	Seedling vigor*	TDL*	TDU*
1		4 QTL	17.24	13.79	0.78	1.56	1.67	51.74	19.69
2	BC_1F_3	3 QTL	28.57	17.14	2.11	2.22	2.00	46.28	13.27
3	generation	2 QTL	37.50	25.70	2.44	3.33	2.78	32.84	9.76
4		1 QTL	44.44	33.33	3.67	3.78	3.56	27.61	6.43
	Susceptible parent								
5	CO(S)28		54.54	36.36	1.16	4.80	4.90	24.50	4.23
	Donar parent								
6	(IS18551)		11.24	5.88	0.16	1.23	1.48	53.55	25.58

*Mean performance of 9 plants
TDL – Trichome Density Lower, TDU –Trichome Density Upper
TDL & TDU: Numbers per sq. cm



L- Ladder (NEX –GEN 100bp DNA ladder)
P1-susceptible parent (CO (S) 28)
P2-Donar parent (IS18551)
A - Progeny with homozygous allele of parent P1
B - Progeny with homozygous allele of parent P2
H- Progeny with allele from both parents P1 and P2
XnhsbmSFC34 – SSR marker flanking the QTL 3

Fig. 1. Foreground selection using SSR Marker and scoring



D.T.F – Days to flowering, H.G.W – Hundred grain weight and G.Y.P.P – Grain yield per plant

Mean performance of BC_1F_3 and BC_1F_4 of various agronomic characters like plant height, days to flowering, leaf length, leaf width, no. of leaves, stem girth, panicle length, panicle width, hundred grain weight, grain yield per panicle are compared. G.Y.P.P in BC_1F_4 was found to be high due to reduced infestation of shoot fly.

Fig. 2. Comparison of mean agronomic performance of BC_1F_3 and BC_1F_4 generations