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Screening of Sorghum (*Sorghum bicolor* (L.) Moench) introgressed lines (BC2F4) for Shoot fly (*Atherigona soccata* Rondani) Resistance

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Abstract

In the present study, we evaluated and identified sorghum backcross populations for shoot fly resistance in augmented block design at AICRP on Sorghum, MARS, UAS, Dharwad, during late *Kharif* 2021 using interlard fishmeal technique. A total of 304 BC2F4 backcrosses introgressed lines (ILs) were obtained by crossing 3 susceptible recurrent parents *viz.*, SPV2217, BJV44, and SVD0806 with 2 shoot fly resistant donors *viz.*, J2614-5 and J2799. The screening results revealed 37 highly resistant ILs in six crosses (SPV2217 x J2614-5, SPV2217 x J2799, BJV44 x J2614-5, BJV44 x J2799, SVD0806 x J2614-5, and SVD0806 x J2799). These introgressed lines exhibited a comparatively lower number of eggs per plant, minimum dead heart percentage, high glossiness and high vigour. These lines would be greater assets in future shoot fly tolerance breeding programs followed by further confirmation study.

Keywords: Sorghum, BC2F4, *Sorghum bicolor* (L.), *Atherigona soccata* Rondani

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is an annual tropical C4 grass of the Andropogoneae tribe in the Poaceae family. It is a diploid species ($2n = 2x = 20$) with a haploid genome of ~730 Mbp. It is one of the first domesticated and most important multipurpose cereal crops used for food, fodder, fibre, fuel, and building materials. It ranks fifth (after wheat, maize, rice, and barley) in terms of both production and area planted globally (<http://www.fao.org>). Sorghum withstands many abiotic stresses, such as drought and waterlogging, salt, lack of nutrients, heat, and cold, better than other major cereals. These characteristics make sorghum a widely cultivated crop in semi-arid tropics and has become an important staple food for millions of rural families (Paterson *et al.*, 2009) ^[15].

Several biotic and abiotic stresses adversely affect post-rainy sorghum production. Among the biotic stresses, shoot fly (*Atherigona soccata*) is the most damaging pest in Asia and also in parts of Africa and Americas restricting the sorghum production (Sharma *et al.*, 2003) ^[19]. About 50% of grain loss has been reported in India by Jotwani (1979), but sometimes more severe damage up to 90% can occur depending on the shoot fly population (Rao and Gowda, 1967) ^[16]. Sorghum is highly vulnerable to shoot fly damage in the initial stages of crop growth, particularly the late-planted crop, in the rainy season and early-sown crop during the post-rainy season. The seedlings are generally attacked by shoot flies within 5–25 days after germination. Generally, the female shoot fly lays single white colored, cigar-shaped eggs on the lower surface of the newly emerged leaves parallel to the midrib. The larvae, after hatching, crawl to the base of the leaf whorl and cut the growing tip, resulting in dead-heart formation. Infestation causes dead hearts in seedlings as well as in tillers of older plants, resulting in considerable damage to the crop (Mohammed *et al.*, 2016) ^[11].

Over the years, various methods have been developed for managing the shoot fly and the most notable among them is chemical control. Though chemical control is effective, the use of chemicals by small farmers is not a feasible option because of their prohibitive cost, limited availability, and the toxicity they pose to the environment. Therefore, it is important to develop host-plant resistance (HPR) in sorghum to impart resistance against shoot fly. The cheap and sustainable option for managing shoot fly is the use of resistant cultivars (Sharma *et al.*, 2003) ^[19]. Three components govern shoot fly resistance in sorghum namely non-preference for oviposition, antibiosis, and tolerance (Sharma *et al.*, 1997) ^[14]. As mentioned in earlier studies, the main factor for shoot fly resistance is non-preference for oviposition also known as antixenosis (Dhillon *et al.*, 2006) ^[4].

Other characteristics of shoot fly resistance include glossiness, trichomes on both adaxial and abaxial surface of leaves, seedling vigour, and epicuticular wax (Nwanze *et al.*, 1992)^[13].

All the studies utilized a common shoot fly resistance donor germplasm line *viz.*, IS18551, originated from Ethiopia. These QTLs were introgressed into elite sorghum maintainer lines, *viz.*, 296B and BTx623, using marker-assisted backcrossing (Deshpande *et al.*, 2010)^[2]. The effects of these introgressed QTLs on the shoot fly resistance were confirmed by field-level evaluation of several versions of introgression lines (ILs) for each QTL per genetic background over multiple seasons. For each QTL, most stable versions, *i.e.*, J2658-6, J2698-7 from SBI- 01, J2714-3, J2743- 3 from SBI-07, J2614-3, J2614-5 from SBI-10 and J2833-11, J2799 from SBI-05 of introgressions, confirmed for presence of shoot fly resistance alleles from donor line IS18551 in BTx623-background (a shoot fly susceptible, elite B-line) were used as donors. However, none of these ILs are adapted to major sorghum growing areas (Gorthy *et al.*, 2017)^[7]. Therefore, we have undertaken introgression of QTLs controlling shoot fly resistance component traits (oviposition non-preference, seedling vigour, glossiness, dead heart percent), present on three different chromosomes *viz.*, SBI- 05 (Donor line- J2614-5), SBI-07 and SBI-10 (Donor line- J2799), into the elite shoot fly susceptible post-rainy season sorghum varieties (SPV2217, BJV44 and SVD0806) grown in northern Karnataka, using marker-assisted backcross breeding (MABC).

With this background, in the present study, 304 BC2F4 introgressed lines derived from 6 crosses *viz.*, SPV2217 x J2614-5, SPV2217 x J2799, BJV44 x J2614-5, BJV44 x J2799, SVD0806 x J2614-5, and SVD0806 x J2799 through marker-assisted backcross breeding are screened for shoot fly resistance through interlard fish meal technique.

Materials and Methods

A total of 304 BC2F4 introgressed lines derived from six crosses *viz.*, SPV2217 x J2614-5 (IL 1 to IL 52), SPV2217 x J2799 (IL 53 to IL 111), BJV44 x J2614-5 (IL 112 to IL 129), BJV44 x J2799 (IL 130 to IL 194), SVD0806 x J2614-5 (IL 195 to IL 251), and SVD0806 x J2799 (IL 252 to IL 304), were planted along with six checks *viz.*, SPV2217, BJV44, SVD0806 (Recurrent parents), J2614-5, J2799 (Donor parents), and DJ6514 (susceptible check). The materials were raised in augmented block design. Each introgressed line and checks were planted in a two-row plot (2m) with row to row and plant to plant spacing of 45 and 15cm, respectively. The sowing was carried out on 30th July 2021 for screening in the late *Kharif* season at AICRP on Sorghum, MARS, UAS, Dharwad.

To attain uniform shoot fly pressure under field conditions the interlard-fish meal technique (Nwanze, 1997)^[14] was followed for screening resistance (Fig 2). A susceptible check (DJ 6514) was sown 20 days before sowing the test material. This was done to allow for multiplication of shoot fly for one generation. Seven days after the seedling emergence, moistened fish meal was spread uniformly in blocks covering all the test material to attract the emerging shoot flies from *Infester* rows. The plant protection measures were avoided until the shoot fly infestation period was complete.

Observations recorded

Data were recorded on leaf glossiness, seedling vigour, number of eggs per plant, and dead heart percentage. Evaluation for leaf glossiness was performed according to the scale given by Sharma and Nwanze (1997)^[14]. Seedling vigour (a combination of height, leaf growth, and robustness) was evaluated on a 1 to 5 scale at 9 Days after emergence (DAE) according to Sharma *et al.* (1997b)^[18]. Both glossiness and vigour scores were normalized using the square root transformation method for statistical analysis. The number of eggs per plant was calculated at 14 DAE (Sekar *et al.*, 2018)^[17]. The dead heart percentage was recorded by calculating the ratio of number of plants with dead heart to total number of plants and multiplying with 100 at 21 DAE. The rating scales were 1 = \leq 10% infestation (highly resistant); 2 = 11 to 20% infestation (resistant); 3 = 21 to 30% infestation (moderately resistant); 4 = 31 to 50% infestation (susceptible); 5 = \geq 50% infestation (highly susceptible) given by Nimbalkar *et al.*, (1987)^[12]. The percent values of dead hearts were normalized using arcsine transformation method for statistical analysis. The data was subjected to statistical analysis using augmented RCBD package in R Studio for analysis of variance.

Results and Discussion

Analysis of augmented design

Augmented block design (Federrer, 1956)^[6] is a method of choice to undertake initial evaluation of a large set of germplasm accessions to select genotypes suitable for different aspects of crop breeding. This is all the more important in cases where initial seed is limited in quantity to undertake replicated experiments as well as our failure to ensure comparably homogenous experimental units which is a basic requirement of standard field designs. The design makes use of a procedure wherein a large number of test entries to be screened are evaluated along with standard checks, with the checks being replicated randomly in all blocks. The data from checks is used to adjust mean values of test entries to make them comparable and also provide an estimate of experimental error.

In the present study, 304 test entries along with six checks were evaluated in an augmented block design for shoot fly resistance. The trail mean values of eggs per plant, glossiness score, vigour score and dead heart % @ 21DAE were 0.95, 2.69, 1.30 and 39.76% respectively and the range for these traits was 0.20-2.40, 1.00-5.00, 1.00-5.00 and 5.00 – 100%, respectively. The highest value of coefficient of variation (C.V) was found in case of number of eggs per plant (12.45%) followed by dead heart % @ 21DAE (10.7%) while vigour (9e-13%) and glossiness (2.1e- 13%) had very low C.V values (Table 1).

The analysis of variance (ANOVA) (Table 2) revealed a significant mean sum of squares for all traits. The block effects (unadjusted) and the treatment effects (adjusted as well as unadjusted) were significant for all the traits. Similarly, the effects due to checks and varieties were also significant. However, the adjusted block effects were non-significant for all traits indicating homogeneity of evaluation blocks. Likewise, the mean square due to checks *v/s* varieties was significant for all the traits, implying that the test entries were significantly different from checks. The dead heart symptom

recorded in susceptible check DJ6514 was 83.24 indicating sufficient pest load in the screening plot. Based on the analysis of dead heart symptoms among the introgressed lines, a total of 37 lines were identified as highly resistant and 35 were resistant for shoot fly. Among the remaining ILs, 63, 85 and 84 were moderately resistant, susceptible and highly susceptible, respectively (Table 3). Similar results were also observed by Kamatar *et al.*, (2010)^[8] and Kiran (2014)^[10].

In the cross, SPV2217 x J2614-5, among 52 introgressed lines screened, four introgressed lines each were highly resistant (0-10% dead heart) and resistant (11-20% dead heart), three lines were moderately resistant (21-30%), 15 lines were susceptible (31-50%) and 26 lines were highly susceptible (>50%) against shoot fly (Table 3). The percent dead heart formation observed in four resistant lines was between 8 to 10 percent in IL 13, IL 25, IL 41 and IL 46 (Table 4, Fig 3b) which was significantly low compared to recurrent parent SPV2217 (68.23% dead heart). The line IL 13 exhibited less number of eggs per plant (0.6 eggs per plant) with a glossiness score of 2 and vigour score 1. The remaining resistant lines *viz.*, IL 25, IL 41 and IL 46 showed 0.8 eggs per plant, with a glossiness score of about 2, 2 and 3 and vigour score of about 1, 1, and 2, respectively (Table 4).

A total of 59 introgressed lines derived from the cross, SPV2217 x J2799, were screened for shoot fly resistance. Three lines are found highly resistant (0-10% dead heart), seven lines are found resistant (11-20%) and four lines were moderately resistant (21-30%) (Table 3). The three highly resistant lines namely, IL 85, IL 102, and IL 103 revealed a percent dead heart formation of 10, 9.09, and 9.52, respectively (Table 4, Fig 3c). The lines IL 102 and IL 103 showed comparatively less number of eggs per plant (0.8 eggs per plant) than IL 85 (1 eggs per plant). Further, the resistant lines IL 102, IL 103 and IL 85 bagged scores 2, 2 & 3, and 1, 2 & 2 for other resistance attributing traits *viz.*, glossiness and vigour, respectively (Table 4) indicating their contribution to lower dead heart and shoot fly resistance.

The recurrent parent SPV2217 showed more number of eggs per plant (1.2), higher dead heart percent (68.23%), low glossiness (score 5) and vigour (score 2) as compared to the highly resistant lines derived from the crosses SPV2217 x J2614-5 and SPV2217 x J2799 (Table 4).

Among 18 introgressed lines screened from BJV44 x J2614-5, five lines were highly resistant (0-10%), one line was resistant (11-20% dead heart), 5 lines were moderately resistant (21-30%), 6 lines were susceptible (31-50%), and a single line noted as highly susceptible (>50%) which are presented in the Table 3. The line IL 123 showed least dead heart per cent (5%) (Fig 3d), least number of eggs per plant (0.2 eggs per plant), high glossiness (Score 1) and vigour (score 1). The percent dead heart formation and number of eggs per plant in the remaining 4 resistant introgressed lines *viz.*, IL 115, IL 117, IL 122, IL 129 were (9.52, 0.8), (9.09, 0.8), (8, 0.4) and (8.33, 0.4), respectively. IL 122 and IL 129 showed high vigour (Score 1) and glossiness (Score 1) whereas IL 115 and IL 117 showed vigour and glossy score of 2 (Table 4).

Of the 65 introgressed lines screened from the cross BJV44 x J2799, 10 lines were highly resistant (0-10%), eight lines were resistant (11-20% dead heart), 13 lines were moderately resistant (21-30%) against shoot fly (Table 3). The percent dead heart formation observed in the 10 highly resistant lines (IL 138, IL 144, IL 157, IL 162, IL 164, IL 168, IL 172, IL 179, IL 181, and IL 193) was below 10 percent (Table 4, Fig

3e). IL 138 and IL 144 were having the least number of eggs per plant (0.2) followed by IL 157 (0.6) and IL 162 (0.8). The lines IL 138, IL 144, IL 157 and IL 164 were highly vigorous with a glossiness score of 1 while the remaining resistant lines, IL 162, IL 168, IL 179 and IL 181 scored 2 for both vigour and glossiness traits. The line IL 172 bagged least scores 2 and 3 for glossiness and vigour, respectively among the resistant lines (Table 4). These results indicate the component traits for shoot fly resistance are introgressed from donor (J2799) and the ILs were resistant to shoot fly with lower dead heart symptoms compared to recurrent parent, BJV44 (50.27% dead hearts). These lines also exhibited high resistance compared to their respective donor parents J2614-5 and J2799 (Table 4).

A total of 57 introgressed lines from the cross SVD0806 x J2614-5 were screened against shoot fly. Of which, seven lines were highly resistant, 10 lines were found resistant, 18 lines were moderately resistant, 15 lines were susceptible and 7 lines were highly susceptible. The resistant lines IL 198, IL 199, IL 204, IL 224, IL 241, IL 245, and IL 251 showed percent dead heart formation of 6.9%, 8%, 9.09%, 8.33%, 6.25% (least), 6.25% (least), and 10%, respectively (Table 4, Fig 3f). Among 7 resistant lines IL 241 and IL 245 showed least number of eggs per plant (0.4 eggs per plant), lowest dead heart % (6.25%), high glossiness and high vigour (score 1). Highly vigorous and glossy resistant lines IL 199 and IL 204 had 0.8 eggs per plant while, remaining resistant lines *viz.*, IL 198, IL 224, and IL 251 exhibited 0.6, 0.8 and 0.8 number of eggs per plant with a glossiness score of about 2, 3 and 2, respectively. All the resistant lines showed high vigour with score 1 (Table 4).

In the cross, SVD0806 x J2799, among the 53 introgressed lines screened, eight introgressed lines were highly resistant, five lines were resistant, 20 lines were moderately resistant, 14 lines were susceptible and six introgressed lines were highly susceptible. About 7.69%, 7.69%, 6.67%, 10%, 7.41%, 8.33%, 8.33% and 9.09% of dead heart formations were observed in the eight highly resistant lines IL 259, IL 278, IL 280, IL 288, IL 291, IL 292, IL 300 and IL 302, respectively (Table 4, Fig 3g). Among the resistant lines, IL 280 showed least number of eggs per plant (0.4 eggs per plant) with high glossiness score 1 while, remaining resistant lines *viz.*, IL 259, IL 278, IL 288, IL 291, IL 292, IL 300 and IL 302 exhibited 0.6, 0.6, 1, 0.6, 0.8, 0.8 and 1 number of eggs per plant respectively. IL 259, IL 278, and IL 29 scored 1 for both glossiness and vigour. IL 292 and IL 300 showed glossiness score 2 with vigour score 1 and 2 respectively whereas, IL 288 and IL 302 showed glossy score of 3 with vigour score of 3 and 1 respectively (Table 4).

Among six checks, DJ6514 showed highest dead heart percentage (83.24%), low glossiness and vigour with score 5 followed by SPV2217, SVD0806, BJV44, J2799 and least is J2614-5 (Table 4).

Glossiness of seedling leaves may possibly affect the quality of light reflected from leaves and influence the orientation of oviposition of shoot flies towards their host plant. The presence of glossy surface on adaxial and abaxial surface causes in the fall down of eggs to soil before hatching and results in reduction in maggot population. The intensity of leaf glossiness at the seedling stage is positively associated with the level of resistance to shoot fly in the form of low number of eggs and dead heart. (Aruna *et al.*, 2011; Dhillon *et al.*, 2006; Gorthy *et al.*, 2017)^[1, 4, 7].

Kamatar *et al.*, (2010)^[8] observed positive relations between vigor of the plant and its escape from shoot fly attack. When the shoot fly eggs hatched on the vigorous seedlings, maggots need longer time to reach meristematic tissue. Sekar (2018)^[17] concluded that rapid seedling growth and long thin seedling leaves make plants less susceptible to shoot fly. Seedling vigor was significantly and negatively associated with dead hearts and oviposition.

In conclusion, among 37 highly resistant introgressed lines, IL 13 (SPV2217 x J2614-5), IL 102 (SPV2217 x J2799), IL 123 (BJV44 x J2614-5), IL 138 (BJV44 x J2799), IL 241 and IL 245 (SVD0806 x J2614-5) and IL 280 (SVD0806 x J2799) are best introgressed lines for shoot fly resistant which are

selected based on low dead heart percentage, less number of eggs per plant, high glossiness and vigour. These lines can be used for further conformation and used in future tolerance breeding programs.

Table 1: Descriptive statistics of shoot fly resistance components traits in 304 introgressed lines of sorghum

Trait	Mean	Min	Max	CV (%)
Number of eggs per plant	0.95	0.20	2.40	12.45
Glossiness	2.69	1.00	5.00	2.1e-13
Vigor	1.30	1.00	5.00	9e-13
Dead heart (%) @ 21DAE	39.76	5.00	100	10.7

Table 2: Analysis of variance of augmented block design for four shoot fly resistant component traits in 304 introgressed lines along with six checks of sorghum

Source	Df	DH	EG	GL	VIG
Treatment (ignoring Blocks)	309	246.69 **	0.17 **	0.14 **	0.06 **
Treatment: Check	5	2666.34 **	1.72 **	2.15 **	1.52 **
Treatment: Test vs. Check	1	1608.74 **	0.17 **	0.61 **	2.42 **
Treatment: Test	303	202.26 **	0.15 **	0.11 **	0.03 **
Block (eliminating Treatments)	7	10.69 ns	0.01 ns	3.4e-31 ns	8.2e-29 ns
Residuals	35	16.94	0.01	6.4e-30	1.6e-28

ns $p > 0.05$; * $p \leq 0.05$; ** $p \leq 0.01$

Table 3: Classification of sorghum introgressed lines based on percent Dead heart formed due to shoot fly incidence

Sl. No	Cross	Resistance Score					Total
		1 - Highly Resistance (0-10%)	2 - Resistance (11-20%)	3 - Moderately Resistance (21-30%)	4 - Susceptible (30-50%)	5 - Highly Susceptible (>50%)	
1	SPV2217 x J2614-5	4	4	3	15	26	52
2	SPV2217 x J2799	3	7	4	10	35	59
3	BJV44 x J2614-5	5	1	5	6	1	18
4	BJV44 x J2799	10	8	13	25	9	65
5	SVD0806 x J2614-5	7	10	18	15	7	57
6	SVD0806 x J2799	8	5	20	14	6	53
	Total	37	35	63	85	84	304

Table 4: List of highly resistant lines along with six checks

SL. No	Cross	IL. No	EG/PT	GL	VIG	DH % @21 DAE
1	SPV2217 x J2614-5	IL 13	0.6	2	1	8.00
		IL 25	0.8	2	1	8.33
		IL 41	0.8	2	1	10.00
		IL 46	0.8	3	2	10.00
2	SPV2217 x J2799	IL 85	1	3	2	10.00
		IL 102	0.8	2	1	9.09
		IL 103	0.8	2	2	9.52
3	BJV44 x J2614-5	IL 115	0.8	2	2	9.52
		IL 117	0.8	2	2	9.09
		IL 122	0.4	1	1	8.00
		IL 123	0.2	1	1	5.00
		IL 129	0.4	2	2	8.33
4	BJV44 x J2799	IL 138	0.2	1	1	5.88
		IL 144	0.2	1	1	6.25
		IL 157	0.6	1	1	6.25
		IL 162	0.8	2	2	8.00
		IL 164	0.6	1	1	6.67
		IL 168	0.8	2	2	8.00
		IL 172	0.8	2	3	9.09
		IL 179	0.8	2	2	8.00
		IL 181	1	2	2	10.00
5	SVD0806 x J2614-5	IL 193	0.6	1	1	7.69
		IL 198	0.6	2	1	6.90
		IL 199	0.8	1	1	8.00
		IL 204	0.8	1	1	9.09
		IL 224	0.8	3	1	8.33

		IL 241	0.4	1	1	6.25
		IL 245	0.4	1	1	6.25
		IL 251	0.8	2	1	10.00
6	SVD086 x J2799	IL 259	0.6	1	1	7.69
		IL 278	0.6	1	1	7.69
		IL 280	0.4	1	1	6.67
		IL 288	1	3	3	10.00
		IL 291	0.6	1	1	7.41
		IL 292	0.8	2	1	8.33
		IL 300	0.8	2	2	8.33
7	Checks	IL 302	1	3	1	9.09
		SPV2217	1.2	5	2	68.23
		BJV44	0.6	3	1	50.27
		SVD0806	0.8	4	2	62.50
		J2614-5	0.4	1	1	12.46
		J2799	0.5	1	1	15.58
		DJ6514	1.6	5	5	83.24



Interlard-fish meal technique



Dead heart symptom

Fig 1: Interlard fish meal technique and dead heart symptom

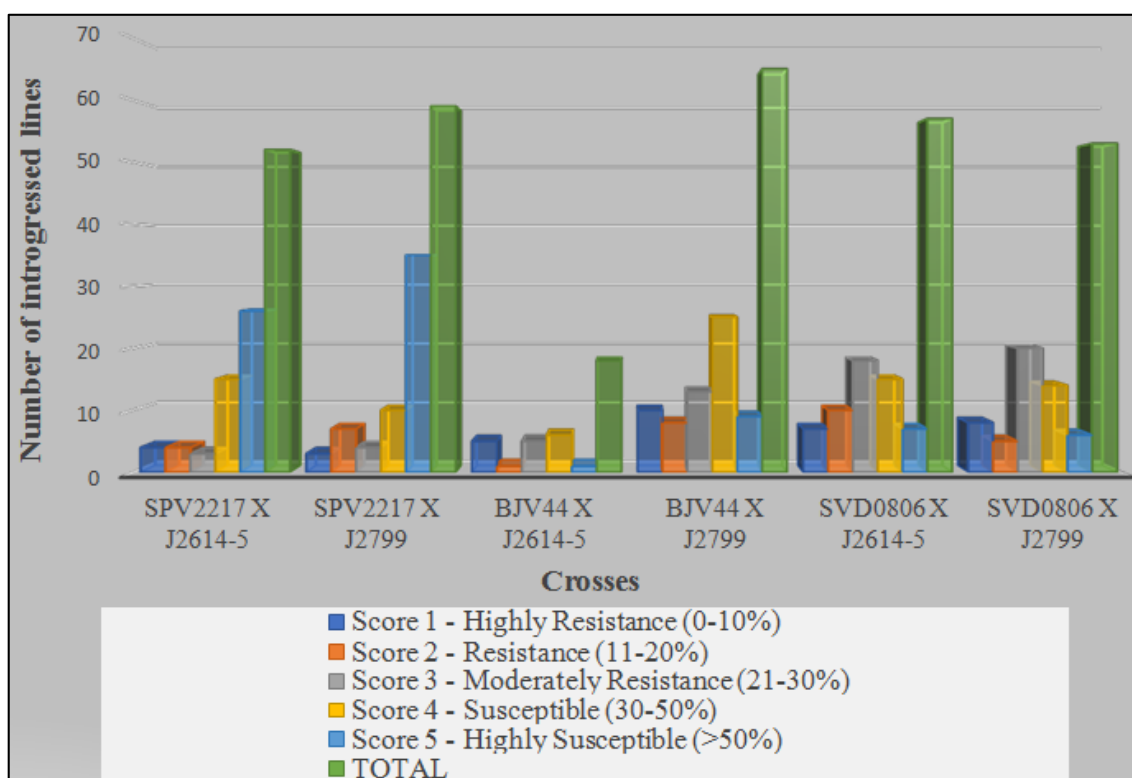


Fig 2: Resistance reaction of sorghum introgressed lines.

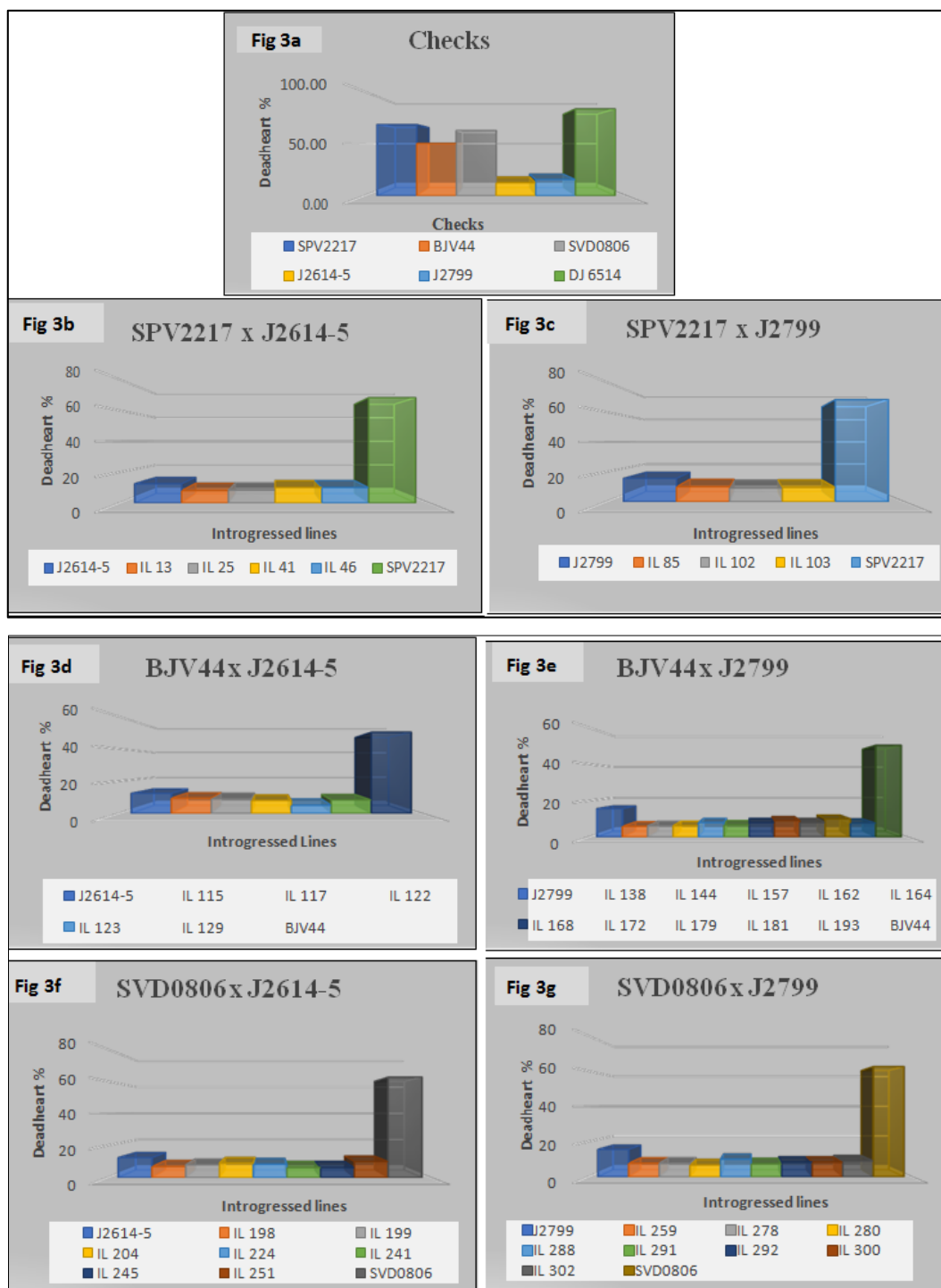


Fig 3: Dead heart percent of shoot fly resistant introgressed lines (ILs) among six cross along with checks

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