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RESEARCH PAPER

Development and utilization of susceptibility index for screening maize germplasm against shoot fly, *Atherigona naqvii*

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Abstract Host plant resistance is an effective and economical approach for the management of shoot fly, *Atherigona naqvii*, a major pest of spring maize in north India. In the current study for identifying reliable resistant sources to shoot fly from large germplasm, a new scale for screening i.e. "Susceptibility Index" has been used on 19 test maize inbreds. This index is based on the egg count, per cent plant oviposited, percent deadhearts incidence and per cent leaf injury incidence. The genotypes were divided into three categories, six inbreds *viz.* DMSC 28, HK12–6–2–4, SO1SHYQBBB13B, CM 143, Winpop 8, and CML 420 as resistant group and five inbreds *viz.* LM 16, SE 563, CM 140, (CM 140 × LM 20)-2-3-1-1-1 and S87P66QBBB 30 as susceptible group falling on the left and right side of mean susceptibility index ± Standard deviation (S.D.), respectively. Eight genotypes falling within the range of mean susceptibility index ± S.D. including IIMRPPBT POOL, (CM 140 × LM 20)-1-1-1-2-1, (CM 140 × LM 20)-2-1-1-1-1, (CM 140 × LM 20)-1-2-2-1-1, IIMRSBT POOL, LM 20, (CM 140 × LM 20)-1-2-1-1-1 and CLQRCYQ 42 constituted moderately resistant group. So, this index can categorize the germplasm into discrete susceptibility classes in comparison to the overlapping classification scale (1-9) based on percent dead hearts alone. Moreover, as the screening for shoot fly is carried out under natural infestation of the pest and considering relative nature of plant resistance, this index overcomes the limitation of the variable incidence of the shoot fly over the years and locations.

Keywords: *Atherigona naqvii* • Deadhearts • Maize • Host plant • Shoot fly • Susceptibility index

Introduction

Shoot fly, *Atherigona naqvii* is the key pest of spring-sown maize in north India (Sarup *et al.,* 1984; Marwaha *et al.,*1984). Sixteen shoot fly species have so far been reported on maize in Africa and Asia. The six species i.e. *A. bidens, A. falcata, A. naqvii, A. orientalis, A. punctata* and *A. soccata* are from India (Panwar and Sarup, 1985). Apart from maize, *A. naqvii* also infests wheat (Steyskal, 1966; Kundu and Kishor, 1973) and barley (Srivastava *et al,*1969). It is responsible for the losses of about 1268 million USD in India (Dhaliwal *et al.,* 2015). The shoot fly lays white rice grain-shaped eggs near the base of the seedlings. The maggots, after hatching from the egg, penetrate the leaf whorl and cut the growing point. This leads to the drying of the central shoot and results into the dead hearts formation. It can cause up to 85% dead hearts in the maize crop (Sajjan and Sekhon, 1985).

The host plant resistance has been one of the most promising measures to control the shoot fly in maize (Rao and Panwar, 2001a,b; Kumar *et al.,* 2014b) and sorghum (Dhillon *et al.,* 2005; Chamarthi *et al.,* 2011; Riyazzadin *et al.,* 2015). These workers have identified various morphological and biochemical traits that contribute resistance to shoot fly. Leaf glossiness, leaf surface wetness, trichomes, phenols, tannins, sugars, and chlorophyll have been reported to be involved in the antixenosis and antibiosis mechanisms of resistance.

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Genotypes possessing these traits can be identified from a large germplasm only if a standardized screening technique and scale for categorization of germplasm is available.

The screening against shoot fly is carried out under the natural infestation by using moistened fish meal, which attracts the shoot fly. It is broadcasted over field at seedling emergence. To assess the relative plant damage done by the shoot fly in the test germplasm, a fixed scale of 1-9 (1 = <10%; 2 = 11–20%; 3 = 21–30%; 4 = 31–40%; 5 = 41–50%; 6 = 51–60%; 7 =61–70%; 8 = 71–80%; and 9 = >80% plants with dead hearts) is used (Sharma *et al.,*1992). Although per cent dead hearts gave quick and easy result, which also indicates the losses in yield due to reduction in the plant stand. However, this method does not give a statistically discrete grouping of genotypes into different susceptibility classes because of the quantitative nature of resistance. Moreover, due to the variable incidence of shoot fly in the field over the years, this fixed scale does not give a clear differentiation of the relative resistance among the test germplasm. These results in the masking of the differential response of the genotypes to shoot fly infestation. Therefore, a susceptibility index (modified from Kumar *et al.,* 2014a) has been evaluated in present studies keeping in view categorizing the test germplasm into more discrete categories even under the variable natural incidence of the pest over the years. This index categorizes the germplasm into three main classes namely resistant, moderately resistant, and susceptible.

Materials and methods

Nineteen diverse maize inbreds were evaluated for their relative response to shoot fly infestation. These include nine inbreds procured from Winter Nursery Centre, Hyderabad named CLQRCYQ 42 (S 14), CML 420 (S 30), HK12–6–2–4 (S 44), SO1SHYQBBB13B (S 57), S87P66QBBB 30 (S 58), IIMRPPBT POOL (S 61), IIMRSBT POOL (S 62), DMSC 28 (S 64) and Winpop 8 (S 68)), five advance breeding lines (F_6 generation) selected from a cross of moderately tolerant lines CM 140 × LM 20 ((CM 140 × LM 20)-2-3-1-1-1 – E1, (CM 140 × LM 20)-1-2-1-1-1 – E2, (CM 140 × LM 20)-1-2-2-1-1 – E3, (CM 140 × LM 20)-2-1-1-1-1 – E4 and CM 140 × LM 20)-1-1-1-2-1 – E5, parental lines (CM 140 and LM 20) along with resistant check (CM 143) and susceptible checks (SE 563 and LM 16). The experiment was conducted at maize experimental area, Punjab Agricultural University, Ludhiana in spring season. The test inbreds were sown in treatment plots containing two rows of 2 m length with 60 cm row to row and 20 cm plant to plant spacing. The seeds were sown on the southern side of the ridge with two seeds per hill. The thinning was performed after the emergence of seedlings to maintain only one plant per hill. The experiment was performed in a randomized complete block design with three replications.

The shoot fly infestation was ensured by applying moistened fish meal technique. The moistened fish meal @ 50 gm^{-2} was broadcasted at the seedling emergence to attract the shoot fly (Jindal *et al.,* 2007). The relative reaction of different test inbreds to shoot fly incidence was evaluated by taking observations on number of eggs laid per plant, the number of plants oviposited, leaf injury incidence and dead hearts incidence. The eggs per plant and plants oviposited were recorded at 7 and 10 days after germination (DAG). The percent leaf injury and percent dead heart incidence were recorded at 10 and 21 DAG. The tolerance or recovery was also assessed in terms of the proportion of dead hearts out of total incidence (leaf injury + deadhearts) at 21 DAG. The per cent plants oviposited, per cent leaf injury, per cent dead hearts incidence and proportion of deadhearts out of total incidence from these observations, were calculated separately as follows:

$$\text{Plant oviposited (\%)} = \frac{\text{Number of plants with shoot fly eggs}}{\text{Total number of plants observed}} \times 100$$

$$\text{Leaf injury incidence (\%)} = \frac{\text{Number of plants with leaf injury}}{\text{Total number of plants observed}} \times 100$$

$$\text{Deadhearts incidence (\%)} = \frac{\text{Number of deadhearts}}{\text{Total number of plants observed}} \times 100$$

Susceptibility index

The susceptible index (modified from Kumar *et al.,* 2014a) used in present studies to categorise the inbreds into different susceptibility groups is as follows:

$$\text{Susceptibility index} = A_{x1} + \frac{A_b}{A_{x2}} + \frac{A_d}{A_n}$$

Where, $A_{x1} = \frac{\text{No.of plants oviposited}}{\text{Total number of plants observed}}$

$A_{x2} = \frac{\text{Total no. of eggs laid}}{\text{No. of plants oviposited}}$

$A_b = \frac{\text{No. of deadhearts}}{\text{No. of plants oviposited}}$

A_d = Per cent deadhearts

A_n = Per cent plants infested (plants with leaf injury + plants with deadhearts)

The germplasm within mean susceptibility index± standard deviation (S.D.) range are moderately resistant, while germplasm on the left and right side represents resistant and susceptible germplasm, respectively.

In this susceptibility index, the expression A_{x1} indicates the antixenosis, $\frac{A_b}{A_{x2}}$ indicates the antibiosis, and $\frac{A_d}{A_n}$ indicates the tolerance mechanism of resistance. So, it gives a far more reliable and discrete classification of test germplasm irrespective of shoot fly infestation level as opposed to a fixed 1-9 scale based on per cent dead hearts.

Statistical analysis

The analysis of variance (ANOVA) was performed to compare the treatment means as per least significant difference (LSD) at p= 0.05 using IBM SPSS 25.0 (IBM Corp, 2017).

Results and discussion

Ovipositional non-preference

Mean egg counts: The number of eggs laid per plant by shoot fly varied non-significantly among the test inbreds during 2018 and 2019 (Table 1). This indicated that no preference for oviposition among the test inbreds was displayed by shoot fly. The mean egg count at 7 days after germination (DAG) ranged from 1.02 to 1.45 and 0.46 to 0.71 eggs plant^{-1} during 2018 and 2019, respectively. At 10 DAG, the mean egg count varied from 0.53 to 0.80 and 0.18 to 0.31 eggs plant^{-1} during 2018 and 2019, respectively. The mean egg count at 7 DAG was comparatively more than 10 DAG during both years (Table 1).

Plants ovipoisted: The percent plants oviposited also varied non–significantly among test inbreds. Plants oviposited (%) at 7 DAG varied from 45.90 to 70.87 and 40.60 to 56.48 percent during 2018 and 2019, respectively (Table 1). At 10 DAG, the plants oviposited varied from 24.83 to 36.08 and 23.42 to 39.25 percent during 2018 and 2019, respectively (Table 1). Similar to egg count, the plants oviposited (%) were also relatively higher at 7 DAG than the 10 DAG. The non–significant variation in the mean egg counts and percent plants oviposited indicated the absence of ovipositional non–preference (antixenosis) against shoot fly in test inbreds.

Relative damage by shoot fly, A. naqvii on the test inbreds

Shoot fly incurs damage by leaf injury and deadheart formation and both parameters varied significantly among the inbreds, indicating that the test genotypes showed relative degree of resistance. During 2018, at 21 DAG, higher leaf injury was observed on S 58, E1, LM 20, LM 16, and E4 (43.68–48.22%) which was at par with the susceptible check SE 563 (48.22%). The lower leaf injury at this stage was observed on S 64, S 57, S 44, S 30, S 68 and E3 (30.08 –34.74%) being at par with resistant check CM 143 (33.14%). Similarly, during 2019, at 21 DAG, significantly higher leaf injury was observed on susceptible inbreds LM 16 and SE 563 (41.19–42.48%). Whereas, minimum leaf injury was observed on S 68 followed by inbreds S 30 and S 44 (24.21–26.84%) (Table 2).

Significantly higher dead hearts incidence at 21 DAG, was observed on susceptible checks SE 563 and LM 16 (42.19–44.06%) during 2018, showing highest susceptibility to *A. naqvii*. Whereas, inbreds S 44, S 57, S 64, CM 143, and S 30 (13.81–18.77%) exhibited maximum resistance to *A. naqvii*. The inbreds S 61, S 62, E3, E5, E2, E4, E1, LM 20, S 14, CM 140, and S 58 (20.39–31.96%) were observed to be intermediate regarding their susceptibility to shoot fly. In 2019 also, inbred S 57 being at par with S 68, S 44, CM 143 and S 64 (10.45–12.34%) constituted the resistant group of the inbreds. The susceptible group was made by SE 563 and LM 16 (34.59–36.55%), showing maximum deadhearts incidence. The inbreds E5, S 30, S 61, E3, S 14, LM 20, S 61, E4, CM 140, E2, E1 and S 58 (15.29–27.84%) being at par with one or the other, comprised moderately resistant group (Table 2).

Further in pooled analysis of leaf injury and deadhearts incidence of both the years at 21 DAG, a similar grouping of the inbreds was observed. In pooled analysis, the maximum leaf injury was observed in SE 563 being at par with LM 16, LM 20, E1and S 58 (41.97- 44.70 %). The minimum leaf injury was observed in resistant inbreds S 44, S 68, S 57, S 64, S 30 and CM 143 (28.73–31.27%) being at par with each other (Table 2).

Table 1. Oviposition response of shoot fly, *A. naqvii* on maize inbreds under natural conditions during spring season 2018 and 2019

Genotypes	2018				2019			
	Egg plant^{-1}		Plants oviposited (%)		Egg plant^{-1}		Plants oviposited (%)	
	7 DAG	10 DAG	7 DAG	10 DAG	7 DAG	10 DAG	7 DAG	10 DAG
S 57	1.07	0.59	45.90	26.04	0.49	0.21	42.95	26.14
S 68	1.17	0.59	56.30	28.27	0.46	0.18	43.91	26.71
S 44	1.02	0.55	46.12	25.57	0.48	0.20	40.60	27.03
CM 143	1.05	0.62	57.06	26.70	0.50	0.22	41.13	29.39
S 64	1.13	0.67	53.57	24.83	0.47	0.20	42.27	25.09
E5	1.23	0.78	69.14	33.49	0.50	0.21	45.43	25.19
S 30	1.20	0.53	47.52	27.70	0.58	0.18	45.67	30.89
S 61	1.10	0.62	52.26	28.95	0.49	0.25	42.54	23.42
E3	1.12	0.63	56.91	26.16	0.50	0.22	55.67	29.58
S 14	1.26	0.67	62.63	28.70	0.71	0.30	56.37	31.55
LM 20	1.36	0.69	65.72	33.84	0.70	0.30	48.31	32.07
S 62	1.11	0.68	61.36	30.17	0.62	0.31	55.24	31.89
E4	1.36	0.71	58.54	24.90	0.55	0.31	52.55	25.05
CM 140	1.25	0.55	57.90	30.77	0.58	0.31	50.51	24.14
E2	1.32	0.71	70.87	30.88	0.62	0.26	51.40	28.08
E1	1.21	0.67	65.07	28.25	0.59	0.30	56.48	30.47
S 58	1.16	0.76	62.92	34.60	0.67	0.30	51.56	28.71
LM 16	1.24	0.58	61.41	36.08	0.66	0.26	46.80	34.88
SE 563	1.45	0.80	57.19	29.95	0.69	0.30	55.21	39.25
Mean	1.20	0.65	58.34	29.26	0.57	0.25	48.66	28.92
LSD (p= 0.05)	NS	NS	NS	NS	NS	NS	NS	NS
P value	0.258	0.365	0.062	0.357	0.176	0.060	0.058	0.079

*Values are the means based on whole plot basis in three replications; DAG: Days after germination

The deadhearts incidence in the pooled analysis was observed to be highest in SE 563 and LM 16 (38.39–40.31%), showing higher susceptibility to the shoot fly and comprising the susceptible group. The inbreds S 57, S 44, S 64, CM 143 and S 68 (12.37–14.99%), all being at par with each other, displayed higher degree of resistance against shoot fly and thus comprised resistant group of inbreds. The remaining inbreds which exhibited intermediate infestation in terms of deadhearts incidence, comprised a moderately resistant group including S 30, E5, S 61, E3 and S 62 (17.36–26.23%) (Table 2).

Categorization of genotypes into susceptibility classes using susceptibility index

Based on percent deadhearts incidence, the inbreds may be categorized in different susceptibility groups over the years due to natural variation in shoot fly infestation level during different years. The susceptibility index (S.I.) offered a definite categorization of test germplasm into different susceptibility groups. During 2018, S.I. varied from 1.02 to 1.48 with mean and S.D. of 1.21 and 0.14, respectively. The genotypes falling on the left of mean susceptibility index ± S.D. including S 64, S 44, S 57, CM 143, S 68 and S 30 (1.02-1.07) constitutes resistant group. The genotypes falling on the right side of mean susceptibility index ± S.D. include LM 16, SE 563, CM 140 and S 58 (1.35-1.48) were categorized as susceptible. The genotypes falling within the range of mean susceptibility index ± S.D. including S 61, E5, E4, E3, S 62, LM 20, E2, S 14 and E1 (1.07-1.35) constitute moderately resistant group of inbreds. Almost similar grouping was observed in the year 2019, in which susceptibility index varied from 0.94 to 1.69 with mean and standard deviation of 1.26 and 0.24, respectively. Resistant genotypes with on the left of mean susceptibility

Table 2. Relative damage (leaf injury and deadhearts incidence) by shoot fly on maize inbreds under natural conditions during spring season

Genotypes	2018				2019				Pooled mean* incidence of 2018 & 2019 at 21 DAG	
	Leaf injury* (%)		Deadhearts* (%)		Leaf injury* (%)		Deadhearts* (%)			
	10 DAG	21 DAG	10 DAG	21 DAG	10 DAG	21 DAG	10 DAG	21 DAG	LI (%)	DH (%)
S 57	18.94	30.41	10.99	14.29	10.91	28.53	6.78	10.45	29.47	12.37
S 68	15.05	33.57	11.57	19.19	8.53	24.21	7.34	10.79	28.89	14.99
S 44	17.21	30.62	9.82	13.81	8.89	26.84	7.04	11.05	28.73	12.43
CM 143	16.48	33.14	12.49	16.78	10.23	29.40	6.27	11.93	31.27	14.35
S 64	16.66	30.08	12.50	14.64	9.73	28.91	6.44	12.34	29.50	13.49
E5	20.17	40.12	16.35	24.20	13.68	29.30	9.24	15.29	34.71	19.74
S 30	19.17	32.58	12.74	18.77	11.94	26.37	8.44	15.95	29.47	17.36
S 61	19.69	37.55	15.67	20.39	9.79	29.22	8.33	18.39	33.38	19.39
E3	20.77	34.74	16.70	23.98	12.23	31.04	10.29	20.47	32.89	22.22
S 14	24.93	38.29	17.43	28.95	14.57	34.00	11.83	21.54	36.14	25.24
LM 20	29.26	46.64	20.66	28.65	17.35	37.82	13.56	22.60	42.23	25.62
S 62	24.09	35.15	18.74	23.29	13.40	33.86	12.56	23.49	34.51	23.39
E4	30.22	43.68	22.60	27.01	16.19	36.12	11.93	24.16	39.90	25.59
CM 140	25.29	40.24	16.91	29.24	21.79	36.95	13.63	25.36	38.60	27.30
E2	28.44	42.68	18.05	25.80	15.92	34.67	13.10	26.90	38.67	26.35
E1	22.68	47.39	15.83	27.67	14.56	36.61	12.11	27.24	42.00	27.46
S 58	22.65	47.98	24.33	31.96	13.85	35.96	13.18	27.84	41.97	29.90
LM 16	30.59	46.55	22.69	42.19	22.96	42.48	14.50	34.59	44.51	38.39
SE 563	32.99	48.22	23.20	44.06	25.42	41.19	16.29	36.55	44.70	40.31
Mean	22.91	38.93	16.80	24.99	14.31	32.82	10.68	20.89	35.87	22.94
LSD(p= 0.05)	5.06	8.03	5.21	5.14	4.73	4.26	2.38	3.17	4.02	2.80
P value	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000

*Values are the means based on whole plot basis in three replications; LI: Leaf Injury, DH: Deadhearts, DAG: Days after germination

index ± S.D. including S 44, S 57, S 64, S 68 and CM 143 (0.94-1.02) represent resistant group of inbreds. The genotypes falling on the right of mean susceptibility index ± S.D. including SE 563, LM 16, E1, S 58 and LM 20 (1.50-1.69) represent susceptible group of inbreds. Moderately resistant group of inbreds falling within the range of mean susceptibility index ± S.D. includes S 30, S 61, E5, S 14, E3, S 62, E4, E2 and CM 140 (1.02-1.50) (Table 3, Figure 1).

In present studies, egg counts varied non significantly and all the genotypes were oviposited, which ruled out the presence of ovipositional non-preference in the germplasm. However, per cent leaf injury and deadhearts varied significantly among the test inbreds but it was not adequate to give a clear categorization of the genotypes due to the overlapping results. Marwaha *et al.* (1984) also found that even under a wide range of dead heart formation (1.07–25.17% and 3.30–28.30%, in 1981 and 1983, respectively), the apparent differential response of maize germplasm could not be established. A similar trend of masking the differential response at the upper extreme was also found. Conversely, the susceptibility index, considering all three mechanisms of resistance, provided a discrete classification of the inbreds into different susceptibility classes. The adequate level of shoot fly infestation was ensured using fish meal but still it varied as more infestation was observed in 2018 (13.81–44.06%) as compared to 2019 (10.45–36.55%). The susceptibility index proved more reliable in gathering trends for the relative response of test inbreds during both years as it overcomes the limitation of variable incidence of shoot fly during different years which may result in contrasting results on a fixed 1-9 scale as per Sharma *et al.* (1992).

Table 3. Susceptibility index of maize inbreds in response to shoot fly incidence in spring season 2018 and 2019

Genotypes	Mean* Susceptibility Index during	
	2018	2019
S 57	1.04	0.96
S 68	1.06	0.99
S 44	1.04	0.94
CM 143	1.06	1.01
S 64	1.02	0.97
E5	1.16	1.12
S 30	1.07	1.04
S 61	1.14	1.08
E3	1.21	1.28
S 14	1.28	1.26
LM 20	1.25	1.50
S 62	1.24	1.28
E4	1.17	1.37
CM 140	1.37	1.45
E2	1.25	1.40
E1	1.29	1.53
S 58	1.35	1.51
LM 16	1.48	1.64
SE 563	1.43	1.69
Mean	1.21	1.26
Standard deviation (S.D.)	0.14	0.24
Mean ± S.D.	1.07–1.35	1.02–1.50

* Mean based on whole plot basis in three replications

As the insect resistance is quantitative, the polygenic nature of resistance to shoot fly is under polygenic control was evidenced by the identification of 19 putative QTLs governed by over dominance to partial dominance and additive gene actions (Vikal, 2020). The screening of a large number of germplasm based only on deadheart incidence may give a continuous pattern of statistically overlapping results. Rather it should be carried out incorporating different aspects of shoot fly infestation including egg laying, leaf injury and deadhearts. So, to group the germplasm into discrete susceptibility classes, a dynamic scale such as susceptibility index can be more useful than fixed 1-9 scale based on per cent deadhearts incidence alone. Moreover, it incorporates the antixenosis, antibiosis and tolerance components of host plant resistance in categorising the test genotypes into discrete non-overlapping classes.

Conclusion

In conclusion, the present studies found that the susceptibility index was a more reliable method for categorizing the test germplasm into statistically discrete categories as it helps in overcoming the restraints of variable incidence of shoot fly during different years by harmonically integrating all the mechanisms of resistance.

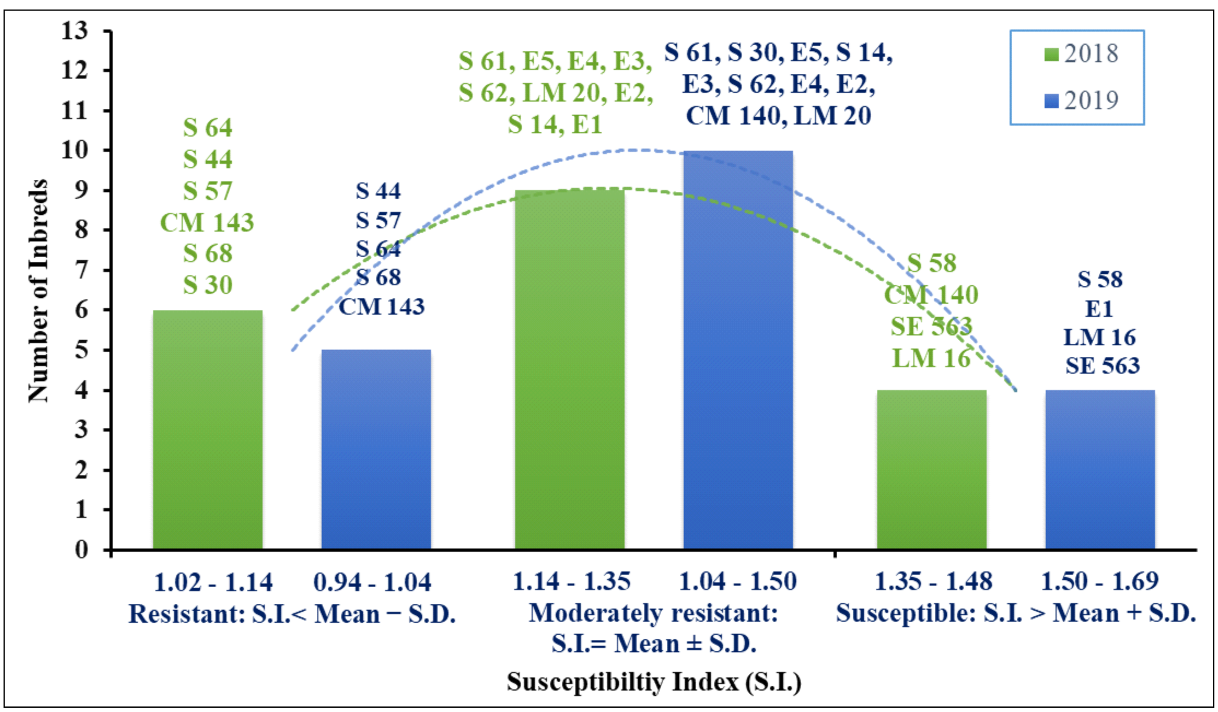

Figure 1. Categorization of maize inbreds based on susceptibility index during spring season

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