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Screening of charcoal rot resistance in m 6 generation of sorghum (*Sorghum bicolor* (L.) Moench)

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Abstract
Total 100 mutants and 7 checks were used to study the charcoal rot resistance in the present experiment. This study was carried out in augmented design during *Rabi* 2022 at Agriculture research station, Hagari. Charcoal rot disease is caused by *Macrophomina phaseolinana* (Tassi) Goid. It appears in severe form on the improved varieties in hot dry weather with soil moisture stress. The screening results revealed that 28 mutant lines shown moderate resistant reaction compared to the resistant check DSV-4 (0.72) and E-36-1 (0.37), among them six mutants had exact only one node crossed by the pathogen. These mutants' lines exhibited comparatively lowest number of mean nodes crossed. 40 mutant lines shown moderate resistant response to charcoal rot index trait. These resistant lines can be used for further confirmation and also for future resistant breeding programmes.

Keywords: Mean node cross, charcoal rot index, sorghum

Introduction
The fifth most important cereal crop in the world, Sorghum [*Sorghum bicolor* (L.) Moench], commonly known as Jowar, is a tropical cultivated diploid (2n = 20) cereal grass plant. It belongs to the Poaceae family and is a monocotyledon plant of tropical origin (Nagara, 2017) [15]. Among the major sorghum producing countries in the world, India ranks first in acreage and second in production next to United States of America. In India, it is grown over an area of almost 4.82 million ha, with a production of over 4.77 million tonnes and a productivity of 989 kg/ha. It is grown in 8.2 lakh ha in Karnataka, with a production of 9.8 lakh tonnes and a productivity of 1194 kg/ha (INDIASTAT, 2022) [13]. Kalaburgi, Raichur, Koppal, Belagavi, Ballari and Vijayapur are the major sorghum growing districts in Karnataka.
The process of mutation is recognized as one of the driving forces of evolution. Induced mutation breeding is a relatively quick method of creating variability in quantitatively inherited traits between plants (Camargo *et al*., 2000) [5]. Both physical and chemical mutagens induce genetic variability, of which gamma radiation is an important tool for inducing mutants with potential to enhance yield and yield contributing traits (Thapa, 2004) [18]. Sorghum is treated with 1% sodium azide to improve germination rate, root length, shoot length, bold seeds, and yield attributing traits (Dahot *et al*., 2011) [7].
Charcoal rot is a major disease in the dry sorghum-growing regions of Asia, Africa, Americas and Australia. It is caused by *Macrophomina phaseolina* (Maubl.) was first reported in India by Uppal (1931) [19], which has been further reported as *Macrophomina phaseolina* (Tassi) Goid (Edmunds, 1962) [11]. It is a complex disease associated with a variety of symptoms including root rot, soft stalks and premature drying stalks, lodging and poorly developed panicles with small and inferior quality grains. The disease is soil borne and causes high loss of grain and fodder, relatively more severe and destructive on high yielding sorghum cultivars when grain filling coincides with low soil moisture in hot dry weather. Depending upon the cultivars, weather conditions and disease severity it causes significant yield losses upto 64% in India (Das *et al*., 2007) [9]. Recently, it also recorded 31% of charcoal rot index (CRI) at Dharwad during *Rabi* 2018-19.

Materials and Methods
I. Inoculum preparation
The pathogen was cultured (Rao *et al*., 1980) [17] on wooden tooth-picks in honey- peptone medium (peptone 1 g, honey 5 ml, distilled water 94 ml).

Tooth-picks were packed into 100 ml conical flasks along with the 20 ml of media and were sterilized at 15 psi for 20 minutes. A loop full of mycelial-sclerotial from stock cultures of *Macrophomina phaseolinana* was seeded into each flask of sterilized cooled honey peptone medium. The flasks were incubated at 35 ºC for 7 days at which time the tooth picks were covered with mycelia (Plate 1.) and sclerotia of the charcoal rot fungus and ready for use in inoculation.

II. Field inoculation procedure

Plants were inoculated at 50% flowering. Irrigation was withheld before the lines were at the boot leaf stage. A fungus infected tooth pick was inserted obliquely into a hole made with an iron pocher into each stalk at its second internode from ground level (Plate 2). Care was taken to ensure that the tooth pick did not emerge through the other side of the stem, for this would promote rapid drying of the inoculum.

The following parameters were recorded to assess charcoal rot incidence:

1. Lodging percentage due to charcoal rot

The number of plants lodging due to charcoal rot among the infected plants was recorded and lodging percentage was calculated. Lodging of plant due to charcoal rot (Plate 3).

$$\text{Lodging (\%)} = \frac{\text{Number of plants lodged due to charcoal rot}}{\text{Number of plants infected}} \times 100$$

Based on charcoal rot percentage and mean length of spread of lesion, Disease reaction of each genotype was determined using the CRI scales (Das *et al.*, 2018) [8].

CRI value	Reaction
≤5	Highly resistant
6-10	Resistant
11-25	Moderately resistant
26-40	Susceptible
>40	Highly susceptible

CRI = (Lodging % × 0.4 + Mean length Spread × 0.6)

2. Mean number of nodes crossed

The number of nodes crossed by the pathogen from the point of infection was recorded. Based on mean number of nodes crossed by charcoal rot disease the genotypes were graded using 1- 5 scale where 1=no internode crossed and 5=>4 internode crossed (Das *et al*., 2007) [9]

3. Mean length of spread (cm)

The length of spread of disease from the point of infection to the tip of disease spread was recorded in centimeter.

Results and Discussion

A total of 100 mutant lines were screened for resistance to charcoal rot in the present study. As compared to the resistant check DSV-4 (0.72), IS-2312 (0.56) and E-36-1 (0.37), six mutants had exact only one node crossed by the pathogen, namely IS925-7-1-1 (1), IS925-132 (1), IS925-RD-44 (1), PV-29 (1), PV-RD-53 (1) and PV-RD-45 (1). It indicates moderate resistance to charcoal rot in these mutants. The Mutants IS925-RD-37 (4.5) and PV-1-1 (4.5) showed the highest number of mean nodes crossed by the pathogen in comparison to the susceptible check SPV-86 (3.99). The charcoal rot disease is highly susceptible to these two mutants.

Mutant lines were graded using a 1-5 scale based on the mean number of nodes crossed by pathogens (Das *et al*., 2007) [9]. According to Table 1, three 3 mutant lines were resistant, 28 mutant lines were moderately resistant, 46 mutant lines were moderately susceptible, 28 mutant lines were susceptible and 2 mutant lines were highly susceptible. Badigannavar *et al*. (2018) [4] and Vinayaka *et al*. (2019) [20] have also reported similar results.

Among mutant lines screened, IS925-7-1-1 (9.9cm) and PV-RD-22 (9.5 cm) showed least mean length of spread of charcoal rot disease, when compared to the resistant check DSV-4 (17.98cm) and E-36-1 (11.94cm). PV-52 (49.4cm), and PV- (47.cm) mutant lines showing highest mean length of spread of disease compared to susceptible check SPV-86 (39.21cm). These results are in accodance with the findings of Jahagirdar *et al*. (2002) [14] and Girish *et al*. (2016a) [12].

Table 1: Classification of M_6 sorghum mutant lines based on mean number of nodes crossed by charcoal rot infection

Grade Scale	Disease reaction	Mutants		Total mutants
		IS925	Phule Vasudha	
1	Resistant (< 1 node crossed)	E-36-1, DSV-4, IS-2312.	-	3+0
2	Moderately resistant (1 node crossed)	IS925-120, IS925-132, IS925-RD-16, IS925-RD-140, IS925-83, IS925-RD-44, IS925-RV-2, IS925-109, IS925-23-1, IS925-7-1-1, IS925-7, IS925-22, GS-23, DJ-6514.	PV-RD-6, PV-1, PV-RD-33, PV-29, PV-RD-53, PV-RD-7, PV-RD-31, PV-RD-45, PV-16, PV-35, PV-11-1, PV-13-1, PV-17-1, PV-19,	14+14
3	Moderately Susceptible (2 nodes crossed)	IS925-3, IS925-RD-49, IS925-39, IS925-101, IS925-85, IS925-105, IS925-136, IS925-44, IS925-137, IS925-RV-41, IS925-29, IS925-RD-98, IS925-1, IS925-45, IS925-138, IS925-RV-4, IS925-15, IS925-6, IS925-RD-31, IS925-8, IS925-21-1, IS925-21, IS925-5, IS925-2-1, M-35-1.	PV-RD-62, PV-RD-20, PV-RD-41, PV-RD-13, PV-RV-62, PV-48, PV-37, PV-10, PV-62, PV-45,PV-RD-51, PV-60, PV-6-E, PV-2-1, PV-42, PV-8, PV-14, PV-5, PV-18,	25+19
4	Susceptible (3 nodes crossed)	IS925-89, IS925-RV-3, IS925-54, IS925-41, IS925-131, IS925-71, IS925-70, IS925-RD-21, IS925-20, IS925-58, IS925-RV-6, IS925-23-1, IS925-14, SPV-86.	PV-RD-25, PV-33, PV-RD-54, PV-RD-22, PV-RD-11, PV-30, PV-RD-44, PV-RD-27, PV-9, PV-13, PV-52, PV-RD-1, PV-50, PV-18, PV-6-1,	14+15
5	Highly Susceptible (4 and > 4 nodes crossed)	IS925-RD-37,	PV-2, PV-1-1,	1+2

Table 2: Classification of M_6 sorghum mutant lines along with checks based on charcoal rot index (CRI)

Disease reaction	Mutants		Total mutants
	IS925	Phule Vasudha	
Highly resistant (≤-5)	-	-	-
Resistant (6-10)	-	-	-
Moderately resistant (11-25)	IS925-120, IS925-132, IS925-RD-140, IS925-44, IS925-137, IS925-83, IS925-RD-44, IS925-RV-2, IS925-138, IS925-RV-4, IS925-109, IS925-15, IS925-23-1, IS925-7-1-1, IS925-21-1, IS925-5, IS925-7, IS925-22, E-36-1, DSV-4, GS-23, IS-2312, DJ-6514.	PV-RD-33, PV-RD-29, PV-RD-53, PV-48, PV-RD-7, PV-RD-31, PV-RD-45, PV-16, PV-60, PV-42, PV-35, PV-11-1, PV-13-1, PV-14, PV-17-1, PV-19, PV-18,	23+17
Susceptible (26-40)	IS925-3, IS925-49, IS925-39, IS925-101, IS925-16, IS925-105, IS925-136, IS925-29, IS925-RD-21, IS925-58, IS925-RD-98, IS925-1, IS925-45, IS925-6, IS925-RV-6, IS925-RD-31, IS925-8, IS925-14, IS925-2-1, M-35-1.	PV-RD-62, PV-RD-20, PV-RD-6, PV-1, PV-RD-13, PV-RV-62, PV-RD-22, PV-37, PV-10, PV-62, PV-45, PV-RD-51, PV-6E, PV-2-1, PV-8, PV-5,	20+15
Highly Susceptible (> 40)	IS925-RD-37, IS925-89, IS925-85, IS925-RV-3, IS925-54, IS925-41, IS925-131, IS925-41, IS925-71, IS925-70, IS925-20, IS925-23-1, IS925-21, SPV-86.	PV-RD-25, PV-RD-41, PV-33, PV-RD-54, PV-RD-11, PV-30, PV-RD-44, PV-RD-27, PV-9, PV-2, PV-13, PV-52, PV-RD-1, PV-50, PV-RD-50, PV-18, PV-1-1, PV-6-1,	14+18

Using lodging percent and mean length of spread, the charcoal rot index (CRI) was calculated and mutant lines are classified into groups based on CRI scales 1-5 (Das *et al*., 2018) [8]. Among the 100 mutant lines studied, none of the mutant highly resistant or resistant to charcoal rot disease, 40 mutant lines were moderately resistant, 35 lines were susceptible, and 32 lines were highly susceptible Table 2. The similar result was also reported by Chattannavar and Vinayaka (2020) [6].

According to the study, Honnutagi local, Kannolli local, and Muddehali jola genotypes showed lower charcoal rot levels. According to previous reports, Jahagirdar *et al*. (2002) [14], Avadhani and Ramesh (1979) [3], Anahosur *et al*. (1974) [2] and Girish *et al*. (2016a) [12] the genotypes resistant to the disease is the result of delayed senescence, accompanied by slow drying at physiological maturity and a stay green trait, According to Anahosur and Naik (1985) [1], resistant genotypes contain more sugar than susceptible genotypes. Similarly, Nalawade *et al*. (2008) [16] also found genotypes with higher levels of sugar and phenolic compounds were formed to be resistant to charcoal rot. This was also resulted in our study, the mutant exhibited high level of resistance as well as a high level of vulnerability to disease. Mean performances of 100 M6 sorghum mutant lines for charcoal rot incidence is represented in Table 3.

Plate 1: Toothpicks cultured with *Macrophomina phaseolinana*

Plate 2: Toothpick inoculation to sorghum stalk

Plate 3: Lodging due to charcoal rot

Table 3: Mean performances of M_6 mutant lines for charcoal rot disease

Sl. No	Mutants	Lodging %	MNC	MLS	CRI
P+C (IS925)					
1	IS925-23-1	60	3.3	42	49.2
2	IS925-8	40	2.8	32	35.2
3	IS925-7-1-1	20	1	9.9	13.94
4	IS925-21-1	20	2.6	24.8	22.88
5	IS925-14	40	3.8	38	38.8
6	IS925-21	40	2.9	44.6	42.76
7	IS925-5	20	2.8	18.8	19.28
8	IS925-2-1	40	2.9	35.8	37.48
9	IS925-7	20	1.5	20.6	20.36
10	IS925-22	20	1.5	18.5	19.1
P+C (PHULE VAUDHA)					
1	PHULE VAUDHA-1-1	80	4.5	55.4	65.24
2	PHULE VAUDHA-11-1	20	1.8	22.5	21.5
3	PHULE VAUDHA-13-1	20	1.9	22.2	21.32
4	PHULE VAUDHA-8	40	2.6	31.2	34.72
5	PHULE VAUDHA-14	20	2.5	26.4	23.84
6	PHULE VAUDHA- 5	40	2.8	29.8	33.88
7	PHULE VAUDHA-17-1	20	1.7	22.9	21.74
8	PHULE VAUDHA- 19	20	1.7	20.8	20.48
9	PHULE VAUDHA-6-1	40	3.7	47.9	44.74
10	PHULE VAUDHA-18	20	2.4	28.2	24.92
Sl. No	**Mutants**	**Lodging %**	**MNC**	**MLS**	**CRI**
P (IS925)					
1	IS925- 3	40	2.7	39	39.4
2	IS925-RD-49	40	2.8	28.5	33.1
3	IS925- 39	40	2.8	38	38.8
4	IS925-RD-37	80	4.5	61.4	68.84
5	IS925- 120	20	1.8	24.2	22.52
6	IS925-132	20	1	22.8	21.68
7	IS925-89	60	3.4	38.6	47.16
8	IS925- 101	40	2.6	35	37
9	IS925-85	40	2.8	40.8	40.48
10	IS925- RD-16	20	1.6	28.4	25.04
11	IS925- RV-3	60	3.6	42.8	49.68
12	IS925- 105	40	2.8	35.5	37.3
13	IS925-54	60	3.4	44.2	50.52
14	IS925-RD-140	20	1.7	21.2	20.72
15	IS925-136	40	2.6	27.2	32.32
16	IS925-41	40	3.8	51.8	47.08
17	IS925- 44	40	2.8	38.8	39.28
18	IS925- 131	40	2.6	30.6	34.36
19	IS925- 137	20	2.6	24.2	22.52
20	IS925- RV-41	20	2.8	43.2	33.92
21	IS925- 71	60	3.8	45.6	51.36
22	IS925-83	20	1.9	27.9	24.74
23	IS925- 70	60	3.6	47.9	52.74
24	IS925-29	20	2.4	24.3	22.58
25	IS925- RD-21	20	3.6	52.8	39.68
26	IS925- 20	60	3.8	53.2	55.92
27	IS925- RD-44	20	1.6	27.2	24.32
28	IS925- 58	40	3.7	36.7	38.02
29	IS925 –RD-98	20	2.7	44.6	34.76
30	IS925-RV-2	20	1.9	12.3	15.38
31	IS925- 1	40	2.8	38.9	39.34
32	IS925-45	20	2.70	35.1	29.06
33	IS925- 138	20	2.6	27.6	24.56
34	IS925-RV-4	40	3.6	38.9	39.34
35	IS925- 109	20	1.8	24.8	22.88
36	IS925-15	20	2.7	24.9	22.94
37	IS925- 6	40	2.7	35.4	37.24
38	IS925-23-1	20	1.9	25.1	23.06
39	IS925- RV-6	40	3.6	38.8	39.28
40	IS925-RD-31	20	2.8	37.9	30.74

Sl. No	P (Phule Vaudha) Mutants	Lodging %	MNC	MLS	CRI
1	Phule Vasudha- RD-62	20	2.4	27.2	24.32
2	Phule Vasudha-RD-20	40	2.2	29.2	33.52
3	Phule Vasudha- RD-6	40	1.9	24.2	30.52
4	Phule Vasudha- RD-25	40	3	42.9	41.74
5	Phule Vasudha- RD-41	60	2.5	36.2	45.72
6	Phule Vasudha-1	60	1.5	24.2	38.52
7	Phule Vasudha-RD-33	40	1.3	14.6	24.76
8	Phule Vasudha- RD-13	40	2.3	36.4	37.84
9	Phule Vasudha- 33	60	3.2	41.2	48.72
10	Phule Vasudha- RD-29	40	1	13.2	23.92
11	Phule Vasudha- RV-62	40	2.4	29.2	33.52
12	Phule Vasudha-RD-54	20	2.6	28	24.8
13	Phule Vasudha- RD-53	20	1	26.6	23.96
14	Phule Vasudha- 48	20	2.1	26.2	23.72
15	Phule Vasudha-RD-22	40	3.2	9.5	21.7
16	Phule Vasudha- RD-7	20	1.8	23.1	21.86
17	Phule Vasudha- 37	40	2.3	35.4	37.24
18	Phule Vasudha- RD-11	60	3.4	47.6	52.56
19	Phule Vasudha- 10	40	2.6	30.4	34.24
20	Phule Vasudha- 62	20	2.2	29.4	25.64
21	Phule Vasudha- 30	60	3.3	41.2	48.72
22	Phule Vasudha- RD-44	20	3.2	44.2	34.52
23	Phule Vasudha- 45	20	2.1	33	27.8
24	Phule Vasudha- RD-31	40	1.5	14.3	24.58
25	Phule Vasudha-RD-45	20	1	22.5	21.5
26	Phule Vasudha- RD-51	40	2.6	36	37.6
27	Phule Vasudha- RD-27	40	3.6	47.8	44.68
28	Phule Vasudha- 10	60	3.5	47.2	52.32
29	Phule Vasudha- 9	40	3.2	47.3	44.38
30	Phule Vasudha- 2	40	2.2	26.8	32.08
31	Phule Vasudha- 13	60	3.2	49.6	53.76
32	Phule Vasudha- 16	20	1.5	9.6	13.76
33	Phule Vasudha-60	20	2.4	27.2	24.32
34	Phule Vasudha- 6E	40	2.3	15.4	25.24
35	Phule Vasudha-52	60	3.9	49.4	53.64
36	Phule Vasudha- 2-1	40	2.4	27.2	32.32
37	Phule Vasudha- RD-1	60	3.5	41.2	48.72
38	Phule Vasudha- 16	40	1.9	14.4	24.64
39	Phule Vasudha- 42	20	2.4	27.1	24.26
40	Phule Vasudha- 35	20	1.8	22.3	21.38
Sl. No	**Mutants**	**Lodging %**	**MNC**	**MLS**	**CRI**
Checks					
1	SPV-86	80	3.99	39.21	55.52
2	E-36-1	20	0.37	11.94	15.76
3	DSV-4	20	0.72	17.98	18.38
4	M 35-1	40	2.05	20.27	28.16
5	GS-23	20	1.2	17.58	18.55
6	IS-2312	40	0.67	15.4	24.24
7	DJ-6514	20	1.95	12.86	15.71

P+C = Physical + Chemical, P = Physical treated, PV = Phule Vasudha, MNC = Mean number of nodes crossed, MLS = Mean length of spread, CRI = Charcoal rot index

Conclusion

The present experiment was conducted to identify mutant lines, which were resistant to charcoal rot. Study revealed that among 100 mutant lines six lines *viz*., IS925-7-1-1 (1), IS925-132 (1), IS925-RD-44 (1), PV-29 (1), PV-RD-53 (1) and PV-RD-4 (1) showed moderate resistant to charcoal rot component characters *viz*., mean number of nodes crossed, mean length spread and Lodging percentage based on charcoal rot index compared to resistant check DSV-4 and E-36-1 (Resistance) under tooth pick method. These six mutant lines were promising lines to reduce shoot fly infestation, so these lines can be used for further confirmation and future tolerance breeding programs.

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