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Research Article

Gamma ray induced genetic improvement of sorghum landraces for grain yield and charcoal rot tolerance

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Abstract
Sorghum is an important cereal crop used for food, livestock feed and biofuel. In order to improve rabi sorghum (post-rainy season), which are mainly used for human consumption, physical and chemical mutagens are used to create genetic variability for quantitative traits. Two advanced sorghum mutant populations, TJP 1-5 and TC-2 were subjected to gamma ray irradiation (300 Gy) and treated with 0.2% EMS. Selections were carried out for grain yield and charcoal rot tolerance from M2 generation onwards along with check varieties. In M3 generation, 300 mutant lines were selected and screened for charcoal rot disease using tooth pick method. Based on the field screening, TCM-38, TCM-95, TJM-6-1 and TJM-35 showed less sclerotial growth (1.92, 2.08, 1.92 and 1.61 nodes crossed respectively) with high grain yield. Among all the mutants screened, TJM-35 had least mean length of fungal growth (13.47 cm) which translated into less lodging (21.74%) and better grain yield (2583 kg/ha) compared to check. Wide genetic variability was created for grain yield (354-2874 Kg/ha as against SPV-86 check, 1499 kg/ha) with mean value of 1833 Kg/ha). Among all the mutants studied, TJM-35, TJM 6-1 and TCM-95 were not only high yielding but also possess tolerance to charcoal rot disease.

Keywords
Induced mutations, Gamma rays, Sorghum landraces, Charcoal rot, Grain yield,

Introduction

Sorghum (*Sorghum bicolor* L Moench) is fifth most important crop grown worldwide for food and feed. African and Asian countries are the major sorghum producers with predominance of local landraces/ historical varieties. India has the largest area of 7.06 million ha with production of 7.45 million tonnes and productivity of 960 kg/ha (Rakshit et al., 2014). The wide genetic variability present in the Indian sorghum genotypes have been the driving force for the development of high yielding hybrids and varieties suitable for kharif and rabi seasons. In tropical and subtropical regions, charcoal rot (*Macrophomina phaseolina*) disease has been a greatest concern due to depleting residual moisture. The types of crop losses may be reduced grain yield and seed quality due to stunted plants, smaller stalks, and poor crop stand due to seedling blight, destruction of lodged plants by termites or other pests. The charcoal rot pathogen invades and destroys root tissue and subsequently rots the stalk, reducing its strength, finally leading to lodging (Uppal, 1936). Even improved high yielding cultivars under good management practices tend to be susceptible to this disease (Mughogho and Pande, 1984). It has been reported in epidemic form in winter season sorghum causing severe crop loss (Parameshwarappa et al., 1976). The indirect loss computed due to charcoal rot alone amounts to 23-64 per cent (Mughogho and Pande, 1984). Even charcoal rot infected fodder is unfit for animal consumption (Mayek-parez et al., 2001). Hence there is need to induce tolerance to charcoal rot disease in a high yielding background using traditional breeding or mutation breeding methods. Various physical and chemical mutagens have been used to induce mutations in qualitative and quantitative characters. Gamma rays have been used to improve quantitative traits in sorghum, such as panicle size, grain yield, number of grains per panicle, seed weight and disease resistance (Reddy and Rao, 1981; Soeranto et al., 2001). In this context, the following experiment was conducted to screen two advanced sorghum mutant populations for grain yield and charcoal rot resistance.

Popular landraces of northern Karnataka region, Chincholli-2 and JP-1-5 were initially subjected to gamma rays (300 Gy) and then treated with Ethyl Methane Sulphonate (0.1%) to improve grain yield and tolerance to charcoal rot disease. In M_2 and M3 generations, favorable mutants with altered morphology, earliness, panicle size and seed weight were identified. In M4, 300 progenies in each of these landrace were evaluated for yield traits and screened for charcoal rot (CR) by tooth pick method. Based on percent lodging and mean node crossed, 40 mutant progenies (TJM of JP 1-5 and TCM of Chincholli-2 derived mutants) tolerant to CR were selected as compared to checks, M-35-1, DSV-4, E-36-1 (resistant to charcoal rot) and SPV-86 (susceptible to charcoal rot) including their parents. These mutant lines were grown in randomized complete block design (RCBD) with two replications, at Agriculture Research Station, Gulbarga, Karnataka during the post rainy season of 2016-

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17. Observations on grain yield and disease reaction were recorded on five plants in each replication.

Macrophomina phaseolina inoculum was cultured in honey peptone medium (peptone 1g, honey 5ml, distilled water 94 ml) (Rao et al., 1980). Two loops of a mycelial-sclerotial suspension made from stock cultures of the pathogen were seeded into each 100 ml of sterilized cooled honey peptone medium and poured into wide-mouthed bottles (about 20 ml/ bottle) containing the sterile tooth picks. The bottles were incubated at 35^{o}C for 7 days at which, tooth picks were covered with mycelia and *sclerotia* of the charcoal rot fungus and ready for inoculation. Plants were inoculated about two weeks after 50 per cent flowering. Irrigation was with held when the majority of the lines were at the boot leaf stage. A fungus infected tooth pick was inserted obliquely into a hole made with an iron poacher into each stalk at its second internode from ground level. Three parameters were recorded on charcoal rot inoculated plants *viz.*, i) lodging per cent due to charcoal rot ii) mean number of nodes crossed (MNC) iii) mean length of spread in centimeters (MLS). Based on the percentage lodging and soft stalk, the genotypes were graded using 0 - 9 scale (Mayee and Datar, 1986) and grouped into respective categories. Based on mean number of nodes crossed by charcoal rot disease the genotypes were graded using 1- 5 scale (Das et al., 2007).

In the present study, TJP 1-5 and TC-2 derived from JP 1-5 and Chincholli-2 landraces were initially gamma ray irradiated at 300 Gy and then treated with 0.1% EMS. M1, M2 and M3 generations were raised at Experimental and Gamma Field Facility, BARC, Mumbai during 2012-15. Selections were done for earliness, high grain and seed quality traits retaining 25% of the populations superior over parent and checks. As many as 300 mutant lines selected for yield contributing traits were subjected to charcoal rot screening in the hot spot area, ARS, Gulbarga, Karnataka during 2015-16. Based on the grain yield and reaction to charcoal rot disease, 40 mutant lines have been identified in comparison to checks (Table 1). In order to declare a mutant line as resistant/susceptible to CR disease, vertical growth of sclerotia across the nodes and its spread leading to lodging of the plant has to be assessed. In the present study, elite mutant lines were screened accordingly and MNC varied from 1.17-2.75 nodes as against 2.75 in the susceptible check (SPV-86). TJM-3-1 recorded less sclerotial growth (1.17 MNC) as against its parent, JP 1-5 (2.17) and check, SPV-86. With respect to mean length of slerotial growth (MLS), the range was 13.47-32.53 cm among mutant lines with mean of 19.10 cm. TJM-35 recorded least MLS (13.47 cm) as against its parent 15.67 cm and check, SPV-86 (32.53 cm). The sclerotial growth has directly transformed into lodging with range of 16.11-38.06 % among the mutant lines as against 60.57% in susceptible check. Among the mutant lines, TCM-95 recoded least lodging (16.11%) as against its parent Chincholli-2 (27.26%). Most of the root infections were initially in primary roots, when the temperatures were high with low water potential, extensive fungal growth was observed (Odvody and Dunkle 1979). In addition, several anatomical and physiological plant characters have been associated with CR resistance. The non-senescence trait has been significantly associated with lodging resistance (Rosenow, 1980).

The present study focused on efficiency of radiation induced genetic variability for charcoal rot tolerance in sorghum landraces. Based on the number of nodes crossed by the sclerotial growth and percent lodging, elite mutant lines were classified based on the disease reaction (Table: 2a and 2b). Among the JP 1-5 derived mutants, TJM-35 and TJM-6-1; and among Chincholli-2 derived mutants, TCM-38 and TCM-95 were found moderately resistant for both mean number of nodes crossed, percent lodging and grain yield as against their respective parents and susceptible check, SPV-86. These moderately resistant mutants were also superior in grain yield, which ranged from 2041-2583 Kg/ha as against SPV-86 (1499 Kg/ha). A total of 9 and 17 mutant lines have been identified as moderately resistant to CR based on the MNC and percent lodging, respectively. The disease reaction is conditioned by post flowering water stress and it has immense influence on the fungal development (Diourte et al., 1995). There are number of control measures that have been advocated, including resistant cultivars, straw mulch and *Trichoderma* seed treatment which could reduce lodging and nodal infection (Jamdar and Desai, 1996). Some of the germplasm lines such as Dagadi Solapur, RS-29, GRS-1 and BCR-9 were moderately resistant to CR when compared to CSV-8R and also these lines showed desirable grain yield and seed quality traits (Srinivas and Shankar, 2017; Prabhu et al., 2012).

Sorghum plants succumb to charcoal rot as the root cells senesce due to the reduction in carbohydrates to maintain metabolic functions, including resistance (Edmunds 1964). If the photosynthetic stress and translocation imbalance reduces carbohydrate supply to root tissue, then lower part of the stalk senesce and lose resistance to the charcoal rot pathogen. Among the different mechanisms existed for charcoal rot resistances, delayed senescence in the form of slow drying at physiological maturity (Jahagirdar et al., 2002) and low stem water depletion rate proved to be the best to tolerate infection from *M. phaseolina*. Thus, the present study has effectively induced CR tolerance in popular landraces grown in the N-Karnataka region during the post rainy season. Popularizing them directly as varietal lines or as parental lines in the recombination breeding would lead to reduced disease infestation, ultimately increasing the grain yield and fodder quality.

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Table. 1 Mean, range and variability parameters for sorghum mutant lines screened for charcoal rot disease and grain yield

Mutant	MNC	MLS (cm)	Percent of Lodging	Grain yield (Kg/ha)
TJM-3-2	2.42	18.97	34.74	2125
TJM-6-2	2.58	17.20	22.92	2000
TJM-4-1	2.58	19.50	31.62	2874
TJM-42	1.89	15.67	30.56	2583
TJM-47	2.17	14.44	34.17	667
TJM-12	2.28	16.69	28.67	2624
TJM-44	2.58	19.75	29.73	2624
TJM-6	2.33	15.97	24.33	1291
TJM-9-12	2.55	19.33	27.46	1916
TJM-7	1.80	15.42	27.15	2166
TJM-9-11	2.33	22.36	36.20	2958
TJM-9-1	2.11	17.28	38.06	1142
TJM-7-1	2.33	23.83	35.28	2541
TJM-34	2.27	18.42	35.28	2249
TJM-9	2.17	18.33	35.84	2166
TJM-3-1	1.17	14.17	27.54	667
TJM-35	1.61	13.47	21.74	2583
TJM-3	2.03	15.42	27.42	1416
TJM-6-1	1.92	17.42	34.19	2874
TJM-11	2.33	24.25	20.06	2833
TJM-33	2.00	15.70	20.74	1083
TJM-23	2.17	14.92	23.25	1916
TJM-1	2.25	18.53	22.38	1458
TJM-8	2.17	15.80	23.25	1291
TJM-5-1	2.17	17.50	18.47	1208
TCM-50	1.93	18.17	26.68	1450
TCM-78	2.67	24.83	26.36	1187
TCM-44	2.08	15.31	23.92	354
TCM-38	1.92	18.54	24.77	2041
TCM-43	2.61	21.42	25.89	979
TCM-18	2.17	20.00	21.41	855
TCM-105	2.33	18.57	29.00	2020
TCM-35	2.58	21.17	20.16	1250
TCM-66	2.69	21.80	27.00	1812
TCM-95	2.08	20.42	16.11	2437
TCM-42	1.83	18.17	30.51	1499
TCM-14	1.92	20.23	24.18	2141
TCM-28	2.33	18.17	28.19	2228
TCM-95-1	2.58	21.67	18.95	2437
TCM-39	2.5	21.83	25.99	2582
Parents				
Chincholli-2	2.00	18.42	27.26	1958
JP-1-5	2.17	15.67	20.03	1208
Checks				
SPV-86	2.75	32.53	60.57	1499
DSV-4	1.42	20.67	31.11	1541
E-36-1	1.42	20.67	24.52	2041
M-35-1	2.75	30.00	45.29	1541
Mean	2.19	19.10	28.02	1832.93
Range	1.17-2.75	13.47-32.53	16.11-60.57	354-2874
CV (%)	16.37	18.77	17.54	16.52
CD @ 5%	0.424	5.709	2.92	45.32
Std. deviation	0.35	3.79	7.71	668.74
Std Error	0.05	0.559	1.138	98.60

Table. 2a. Classification of sorghum mutant lines for CR disease based on mean numbers of nodes crossed

Disease grade	Disease reaction	Genotypes	Total number of genotypes
1	**Resistance** (<1 node crossed)	-	0
2	**Moderately resistant** (1 nodes crossed)	TJM-42, TJM-7, TJM-3-1, TJM-35, TJM-6-1, TCM-50, TCM-38, TCM-42, TCM-14	09

Table. 2b. Classification of sorghum mutants for CR disease based on lodging percentage

Disease grade	Disease reaction	Genotypes	Total number of genotypes
0	Immune	-	0
1	Highly resistant	-	0
3	Resistant	-	0
5	Moderately resistant	TJM-6-2, TJM-6, TJM-35, TJM-33, TJM11, TJM23, TJM-1, TJM-8, TJM-5-1, TCM-44, TCM-38, TCM-43, TCM-18, TCM-35, TCM-95, TCM-14, TCM-95-1	17