Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 6 Special issue [3] 2017: 378-381

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804
NAAS Rating 4.95

FULL LENGTH ARTICLE **OPEN ACCESS**

To Assess The Relationship Between Parents And Hybrids For Grain Mold Resistance In Sorghum (*Sorghum bicolor.* L)

V.Thirumala Rao, P. Sanjana Reddy, B.V.S.Reddy and V.Venkanna
ANGRAU, Rajendranagar

ABSTRACT
The improvement of grain mold resistance in sorghum (Sorghum bicolor (L). Moench) has been difficult, presumably because of the complex inheritance of the trait. The objective of this study is to determine the relationship of parents and hybrids. probability of getting grian mold resistant hybrid is very high by crossing two resistant parents. The hybrid having more than 50% glume coverage, loose panicle, brown glume colour, red grain colour, low ergosterols, high flavn-4-ols, high grain density is desirable.

Received 23.07.2017 Revised 21.08.2017 Accepted 30.08. 2017

INTRODUCTION

In many regions of the world where sorghum is produced, grain mold is serious disease that reduces grain quality and utilization. This disease is especially severe when grain development coincides with wet and warm weather conditions. In Africa absence of grain mold resistance has been cited as a constraint to adoption of improved cultivar (Mukuru, 1992). Aflatoxin, which can cause harmful toxic effects to humans, has been associated with grain moulds. Hence, breeding for grain mould resistant cultivars may help to improve sorghum production and profitability, extend the use of improved cultivar to new areas, as well as contribute to enhanced health to humans. Several workers were studied to develop grain mould resistant sorghum hybrids and varieties by identification of useful resistance gene(s), or defining other sources of such genes.

Breeding to improve grain mold resistance in sorghum had limited success because of an incomplete understanding of the genetics of the sorghum grain mold resistance. An attempt was made to understand the relationship between parents and hybrids for grain mold resistance and other associated characters.

MATERIALS AND METHODS

The hybrids (168) developed by crossing eight male sterile lines with 21 restorer lines were screened for grain mold resistance at Patancheru, ICRISAT and Rajendranagar, ANGRAU during *kharif* 2004 and 2005. The screening nursery was sown in mid June so that the crop experiences high probability of rains and high humidity during grain-filling and maturity stages in September. Adequate level of grain mold development for good screening was realized by providing sprinkler irrigation. Sprinklers were arranged in a sequence grid pattern, the shortest distance between any two sprinklers being 12 m. The nursery was sprinkled for one hour each at 10.00 A.M and 4.00 P.M. on rain free days to ensure high humidity from flowering to grain maturity (Bandyopadhyay and Mughogho, 1988).

Grain mold damage was evaluated as a Panicle Grain Mold Rating (PGMR) at physiological maturity stage and Threshed Grain Mold Rating (TGMR) after harvest on ten randomly selected plants of each genotype in each replication by using 1-9 scale, where 1 = no mold, 2 = 1-5% of grains colonized by grain mold fungi, 3 = 6-10% of grains colonized by grain mold fungi, 4 = 11-20% of grains colonized by grain mold fungi, 5 = 21-30% of grains colonized by grain mold fungi, 6 = 31-40% of grains colonized by grain mold fungi, 7 = 41-50% of grains colonized by grain mold fungi, 8 = 51-75% grains colonized by grain mold fungi, 9 = >75% of grains colonized by grain mold fungi. The computed average values of PGMR and TGMR in both parents and hybrids from different environments were subjected to statistical analyses. Based on response of grain mold infection in pooled analysis the hybrids are classified as a resistant (mean PGMR scores, 1.0-3.0) and susceptible (mean PGMR > 3.0). The hybrids were grouped into R × R, R

× S, S × R and S × S categories based on grain mold infection response of parents. The number of grain mold resistant hybrids belonging to each of the groups and the conditional probability that given a grain mold resistant hybrid, the probabilities that it is derived from R × R, R × S, S × R and S × S groups were estimated. Morpholgical Characters such as glumes coverage was recorded as coverage of the grain with glumes on a 1 to 4 scale, 1 = 0- 25% of grain covered, 2 = 26-50% of grain covered, 3 = 51-75% of grain covered, 4 = > 75% of grain covered, Panicle compactness was recorded on a 1-4 scale as 1 = semi compact, 2= compact, 3 = loose and 4 = very loose, Color of the glumes was recorded on a 1-3 scale as 1 = white,2 = red and 3 = brown, Color of the grains was recorded on a 1-3 scale as 1 = white,2 = red and,3 = brown and Presence or absence of testa was recorded as P = present and A = absent

Based on mean grain yield, PGMR and TGMR scores, 25 hybrids were selected. In which 15 hybrids (10 hybrids with white grain colour and 5 hybrids with red grain color) were grain mold resistant with high yielding ability and 10 hybrids (5 hybrids with white grain colour and 5 hybrids with red grain colour) were grain mold susceptible. These 25 hybrids and their parents along with check Bulk Y from Patancheru location were utilized in the estimation of ergosterol, flavan-4-ols, grain density and germination percentage.

Ergosterol was determined according to the modified method of Jambunathan *et al.* (1991). From each entry 10 panicles were chosen at random and dried. Threshed grains from ten dried panicles were collected and mixed thoroughly. A 25 g sample of the mixed grains was ground in a Udy Cyclone mill (U.D. Corp. Boulder, CO) to pass through a 0.4 mm screen. Duplicate 10 g samples of ground grain were weighed in polythene screw cap bottles (125 ml capacity), 50 ml of methanol (MeOH) was added, and bottles were shaken vigorously on a reciprocating shaker for 60 min at room temperature. The mixture was allowed to settle and 25 ml of clear extract was transferred into a screw-capped test tube containing 3 g of KOH and was shaken till the KOH dissolved. Ten milliliters of n-hexane was added and the mixture was incubated at 75^{o} C in a water bath for 30 min and then allowed to cool to room temperature. Distilled water (5 ml) was then added, the solution was mixed thoroughly, and the top hexane layer was removed with the help of Pasteur pipette and transferred to a 50 ml beaker. To the remaining aliquot in the test tube, 10 ml of hexane was added and mixed vigorously and hexane layer was removed carefully and pooled with the earlier extract. This procedure was repeated twice and all the pooled hexane extracts in the beaker were evaporated to dryness in a hot-water bath. The residue was redissolved in 5 ml methanol (HPLC grade) and filtered through a 0.45 mm filter (Millex, HV, Millipore Corp. Bedford, MA) and the filtrate was used for ergosterol analysis.

Ergosterol was determined in a SHIMADZU LC-6A High Performance Liquid Chromatograph (HPLC) with manual loading. The extract was loaded on a reverse-phase column [3 µm particle size, 6 mm × 8 cm] consisting of two 4 cm Zorpax Reliance Cartridges (Du Pont). The mobile phase was methanol-water (96:4 v/v) at a flow rate of 1.2 ml min^{-1}. The column temperature was maintained at 50^{o}C, and the absorbance of eluted ergosterol was detected at 282 nm. The standard ergosterol (Sigma) had a retention time of 8.3 min. Along with the experimental sample, a sample of standard grain mould susceptible check (Bulk Y) was also analyzed every time to maintain the accuracy of the procedure. The standard ergosterol was loaded in 2.5, 5.0, 7.5, and 10.0 mg concentration for computation of the instrument every time. The instrument was calibrated for standard ergosterol and directly gave ergosterol content of the sample in ppm.

The procedure of Butler (1982) was followed for estimation of flavan-4-ols in grains. Grains were collected from 10 panicles from each replication and bulked. Five grams of grain was ground in Udy Cyclone Mill. The grain powder was defatted with hexane and air dried. Duplicate of 200 mg of defatted grain powder was taken in screw cap test tubes and 5ml methanol was added. Tubes containing the defatted seed material were placed on Stuart tube rotator (TR-2) and mixed for one hour. The tubes were then centrifuged for 10 min and the supernatant was decanted into vials. The two methanol extracts from each sample were pooled separately to form methanol extract. Five milliliters of methanol-HCL was added to the remaining residue and repeated same procedure to form methanol-HCL extract of each sample.

Methanol extract (0.5 ml) was taken and 7 ml water saturated butanol and HCL (70:30) was added. Simultaneously, a blank was prepared by mixing water saturated butanol, methanol and 0.1N acetic acid in a 70:15:15 ratio, respectively. The tubes along with the blank were rotated in the test tube rotator for 1h. The same procedure was followed for methanol-HCL extract. The absorbance was read at 550 nm. in spectrometer (Spectronic 21, Bausch & Lamb, USA). All the results were calculated as A_{550} g^{-1} dry sample.

The method used by Pendelton *et al.,* 2005 was utilized to determine the density of the grain. Grain sample of 20 g from each genotype was taken in duplicate. These samples were put with 70 ml of water into a 100 ml glass graduated cylinder. The amount of water displaced by the grain was used as the

volume of the grain. The weight of the grain was divided by volume of the grain to determine the density of the grain in g ml^{-1}.

Germination percent in mold infected grains was estimated by taking hundred grains from each of the 10 panicles of each replicate, which were scored for TGMR, were incubated in petri dishes lined with wet filter paper for four days at 30°C and the number of germinated grains was counted.

RESULTS AND DISCUSSION

Grain mold is a one of the major biotic constraints in sorghum production. It is very important to understand the relationship between parents and hybrids to delineate the methods of producing GMR hybrids. A novel attempt was made to find the relationship between parents and hybrids for grain mold resistance. The hybrids were grouped into R × R, R × S, S × R and S × S categories based on grain mold infection response of parents (Table 1). In R × R category among 12 hybrids, 8 hybrids found resistant to grain mold infection. Similarly in R × S category 21 hybrids were resistant to grain mold out of 51 hybrids. In S × R category 12 hybrids were grain mold resistant out of 20 hybrids. Five hybrids registered resistance in S × S category out of 85 hybrids.

The probabilities of getting resistant hybrids are high in R × R (0.66) category followed by S × R (0.60) category compared to other categories indicating crossing of both the parents with resistance is promising to obtain resistant hybrids. Most of the resistant hybrids are in R × S (21) and S × R (12) categories. It is very interesting to get resistant hybrids (5) by crossing both susceptible parents. This shows that the diverse and complementary mechanisms, each with small effect may be acting synergistically in hybrids leading to higher levels of resistance.

Table 1: Distribution of GMR sorghum hybrids in different categories of crosses

Category of the crosses	Total no of hybrids	Mean PGMR score	No of GMR hybrids	Conditional probability of GMR hybrids
R × R	12	3.29	8	0.66
R × S	51	3.33	21	0.41
S × R	20	3.14	12	0.60
S × S	85	4.61	5	0.05
Total	168	-	46	-

Hybrids were classified based on glumes coverage, panicle shape, glumes colour and grain colour(Table 2). Based on glumes coverage, 28 hybrids were found with 25 % of glumes coverage and 18 hybrids with 50 % of glumes coverage, showed resistant reaction to grain mold infection. Based on panicle compactness 27, 1 and 18 hybrids were resistant, and had semi-compact, compact and loose panicles, respectively. Based on glumes colour, one hybrid with white, 26 hybrids with red and 19 hybrids with brown glumes colour showed resistant reaction to grain mold infection. Based on grain colour, eight hybrids with white colour and 38 hybrids with red grain colour and showed resistance to grain mold. The probabilities of getting GMR hybrids based on glumes coverage, panicle shape, glumes colour and grain colour are estimated. Chances of getting GMR hybrids are high with 50 % of glumes coverage (0.31) compared to 25 % of glumes coverage (0.25). The hybrids with loose of panicle (0.32) are desirable compared to other types *viz*., semi-compact (0.28) and compact (0.06) nature of panicle. The hybrids with brown glumes colour (0.36) are good followed by red glumes colour (0.24) and white glumes colour (0.10). The hybrids with red grain colour (0.52) are desirable compared to white grain colour (0.08) for grain mold resistance.

Table 2: Classification of hybrids based on glumes coverage, panicle shape, glumes colour and grain colour

Trait		Resistant hybrids	Total hybrids	Conditional probability of getting resistant hybrids
Glumes coverage	25% of glumes coverage	28	111	0.25
	50% of glumes coverage	18	57	0.31
Panicle compactness	Semi-compact	27	95	0.28
	Compact	1	16	0.06
	Loose	18	56	0.32
Glumes colour	White	1	10	0.10
	Red	26	106	0.24
	Brown	19	52	0.36
Grain colour	White	8	95	0.08
	Red	38	73	0.52

Twenty five selected hybrids were utilized for estimation of ergosterol, flavan-4-ols, grain density and germination percentages. Resistant hybrids recorded 10.08 µg/g ergosterol, 1.80 A_{550} g^{-1} flavan–4-ols in methanol extract, 0.99 A_{550} g^{-1} flavan–4-ols in acidified methanol extract,1.19 g/ml grain density and 89.63 percent of germination whereas susceptible hybrids recorded 19.49 µg/g ergosterol, 1.20 A_{550} g^{-1} flavan–4-ols in methanol extract, 0.73 A_{550} g^{-1} flavan–4-ols in acidified methanol extract,1.10 g/ml grain density and 67.40 percent of germination (Table 3).

Comparison of resistant and susceptible hybrids based on ergosterol, flavan-4-ols, grain density and germination percentage clearly indicating that hybrids with low ergosterol, high flavan-4-ols, grain density and germination percentages are resistant to grain mold.

Table 3: Classification of hybrids based ergosterol, flavan-4-ols, grain density and germination percentage

Character	Resistant hybrids	Susceptible hybrids
Ergosterol (µg/g)	10.08	19.49
Flavan-4-ols in methanol extract (A_{550} g^{-1})	1.80	1.20
Flavan-4-ols in acidified methanol extract (A_{550} g^{-1})	0.99	0.73
Grain density (g/ml)	1.19	1.10
Germination percentage	89.63	67.40

CONCLUSION

The above study clearly indicating the probability of getting grian mold resistant hybrid is very high by crossing two resistant parents. The hybrid having more than 50% glume coverage, loose panicle, brown glume colour, red grain colour, low ergosterols, high flavn-4-ols, high grain density and germination percent are desirable for grain mould resistant.

ACKNOWLEDGEMENT

We gratefully acknowledge the assistance of the sorghum unit, ICRISAT.

REFERENCES

1. Bandyopadhyay, R., Mughogho, L.K., 1988. Evaluation of field screening techniques for resistance to sorghum grain moulds. Plant Dis. 72, 500-503.
2. Butler, L.G., 1982. Relative degree of polymerization of sorghum tannin during seed development and maturation. J. Agric. Food Chem. 30, 1090-1094.
3. Jambunathan, R., Kherdekar,M.S., Vaidya, P., 1991. Ergosterol concentration in mold susceptible and mold-resistant sorghum at different stages of grain development and its relationship to flavan-4-ols. J. Agric. Food Chem. 39, 1866-1870.
4. Mukuru S Z 1992 Breeding for grain mould resistance. p. 273-285. *In* Sorghum and Millets diseases: a second world review. W.A.J. de Milliano, R.A. Frederiksen, and G.D. Bengston (eds.), International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India.
5. Pendleton M W, Vitha S, Ellis E A, Chito F M and Pendleton B B 2005 Morphology of sorghum grain in relation to resistance to maize weevil. International Sorghum and Millets Newsletter 46: 55-57.

CITATION OF THIS ARTICLE

V.Thirumala Rao, P. Sanjana Reddy, B.V.S.Reddy and V.Venkanna. To Assess The Relationship Between Parents And Hybrids For Grain Mold Resistance In Sorghum (*Sorghum bicolor.* L). Bull. Env. Pharmacol. Life Sci., Vol 6 Special issue [3] 2017: 378-381