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Stability for grain oil content in sorghum (*Sorghum bicolor*)

SUJAY RAKSHIT[1,2], ARUNA C[1]*, PRADUMAN YADAV[3], PARASHURAM PATROTI[4], G GIRISH[5], GANAPATHY K N[1], RATNAVATHI C V[1], PADMAJA P G[1] and BAHADURE D M[1]

ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad, Telangana 500 030, India

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ABSTRACT

Oil content in sorghum [*Sorghum bicolor* (L.) Moench] grains is an important component in determining sorghum as a functional food. Sorghum has unique advantage in terms of its high tolerance to drought and high temperatures. With its ability to yield high with low inputs, it can be used as an alternative oil source with clinical advantages. Breeding efforts towards identifying end use of specific cultivars are required for increased profitability to the farmers. In the present study, oil content was estimated in 19 genotypes over three locations (Gulberga, Solapur and Hyderabad) which were sown in randomized block design with three replications during post rainy season of 2016-17. Oil content ranged from 3.14–4.76% with the highest value in IS 30466. Germplasm lines, IS 31681, IS 1212, IS 30536, IS 30507, IS 603 and IS 30466 were found better adapted to all test environments with stable mean oil content more than population mean. Significant effects of both genotype and environment were observed for oil content, while genotype × environment interaction was not significant indicating that the genotypes do not show differential response to the environment. Oil content had significant correlation with germ size (r = 0.484), while the associations with 100-seed weight and grain hardness were non-significant. The information generated and the genotypes identified will help in breeding sorghum with high oil content thus enhancing the demand for sorghum as an industrial crop.

Keywords: Correlations, Germ size, Grain hardness, Oil content, Sorghum, Stability

Grain sorghum [*Sorghum bicolor* (L.) Moench] is receiving greater attention because of its use in gluten free food applications, and also its less resource requirement. It is extensively cultivated world over in the semiarid tropics, predominantly as a source of food, feed, fodder and as biofuel. Sorghum grain is a good source of energy, proteins, carbohydrates, vitamins and minerals (Afify *et al*. 2011, 2012), and it is one of the most important weaning foods in both low and high income countries (Shallan *et al*. 2010). As compared to starch and protein content, sorghum grains have low oil content (about 3–5%). However, because of its high tolerance to drought and high temperatures, and its ability to yield high with low inputs, it can be used as an alternative oil source with clinical advantages (Mehmood *et al.* 2008). Sorghum varieties can be additional sources of edible oil due to the presence of clinically important saturated and high concentration of unsaturated fatty acids (Afify *et al*. 2012). Sorghum oil is reported as the most potent component to serve as a possible heart health ingredient in functional foods by modulating cholesterol, most likely by inhibiting absorption (Lee *et al*. 2014) suggesting a greater potential as a heart health functional food ingredient. Kaplan *et al*. (2018) suggested that seed oil obtained from different genotypes of sorghum could be alternative source of edible oil due to presence of all saturated and unsaturated fatty acids required for human health and also due to absence of lauric, myristic, eicosenoic and erucic acids. Such an advantage would provide other opportunities for grain sorghum development for targeted markets. In spite of the potential of sorghum oil, variability in oil content in sorghum grains is least investigated. The objective of the current study was to determine genotype × environment (GE) interaction and stability of sorghum genotypes for oil content and to identify stable lines with higher oil content. Assessment of stability for oil content helps in identifying genotypes with good and consistent oil content and their deployment in breeding programs.

MATERIALS AND METHODS

Genotypes and their evaluation: Nineteen sorghum genotypes were used in this study on oil content. The material consisted of 16 germplasm lines and three improved sorghum cultivars (Table 1). Among the improved lines, M 35-1 is a very popular *rabi* sorghum variety, while CSV 27 is a

Present address: [1]ICAR-Indian Institute of Millets Research, Rajendranagar, Hyderabad, Telangana; [2]ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana, Punjab; [3] ICAR-Indian Institute of Oilseeds Research, Rajendranagar, Hyderabad, Telangana; [4]Center on Rabi Sorghum, Solapur, Maharashtra; [5]Regional Agricultural Research Station, Hagari. *Corresponding author e-mail: aruna@millets.res.in.

Table 1 Pooled mean sum of squares for oil content in sorghum

Source of variation	df	Oil content
Rep within Env.	6	0.005
Genotypes	18	0.973 ***
Environments	2	0.301 ***
Env.+ (Gen × Env.)	38	0.027 *
Gen × Env.	36	0.012
Environments (Lin.)	1	0.602 ***
Gen × Env. (Lin.)	18	0.011
Pooled deviation	19	0.012 *
Pooled error	108	0.006
Total	56	0.331

kharif dual-purpose variety. BJV 74 is an improved variety from Bijapur, Karnataka. These genotypes were planted at three locations, viz. Regional Agricultural Research Station, Gulberga; Centre on *Rabi* sorghum, Solapur and ICAR-Indian Institute of Millets Research (ICAR-IIMR), Hyderabad during *rabi* 2016–17. In all the locations, the material was sown in randomized block design with three replications, each with two rows of 4-m length. The rows were 0.6 m apart with plant-to-plant spacing of 15–20 cm. Good crop growth and stand were ascertained by following the recommended agronomic practices. Data on oil content was recorded for the samples from all the three locations to study the stability of oil content, while the data on other grain traits were recorded on the samples from ICAR-IIMR, Hyderabad. The following grain traits were recorded for the study:

100-grain weight (HGW): Weight of 100 grains from each panicle was recorded and expressed in grams.

Grain hardness (GHR): The grain hardness was tested with a digital hardness tester (Pharmag Instruments Ltd) by using force gauge to measure tension/compression force applied on single seed. The single seed hardness of 10 randomly selected seeds per plant with five plants per replication from each sample was tested and the mean value was expressed in Newtons (N).

Germ size: Germ size was determined by visual assessment on 10 grains in each plant as per the DUS guidelines of sorghum (http://www.plantauthority.gov.in/pdf/ Revised-Sorghum.pdf). It was scored on 1-9 scale, where, 1= very small; 3=small; 5= medium; 7= large and 9= very large.

Oil content: Oil content was estimated by the benchtop Nuclear Magnetic Resonance Spectrometer (NMR) by the modified method of Yadav and Murthy (2016). A bench-top pulsed NMR-MQC-5 analyser (Oxford, London) supplied with preloaded 'easy cal' software was calibrated for estimation of oil content. Before making the calibration curve, seeds were dried by keeping them in a hot-air oven at 80°C for 8 h. The calibration was performed with 40-mm diameter sample probe, 5 MHz operating frequency, four scans, one sec recycle delay and 40°C magnetic box temperature. NMR room temperature was maintained at 23 ± 2°C. The calibration was made by conditioning the samples in a heating block for 90 min at 40°C temperature.

Data analysis: Oil content data of each location and grain data of one location were subjected to a one-way ANOVA using OPSTAT (http://14.139.232.166/opstat/default.asp). Stability analysis was carried out using the three location data using Eberhart and Russell stability model (1966) and the analysis was carried out using Windostat (Indostat Services, Hyderabad, India). This model is based on two stability parameters: linear regression and deviation from regression, which divides the genotype × environment interaction (GEI) into linear (the response of the genotypes to varying environmental index) and non-linear (the unexplained deviations from regression) deviations. Both these give an estimate of the genotype response to varied environments, which helps in understanding the potential of the genotypes across environments. Pooled error was used to test the significance of mean squares. The significance of deviation of b from 1.0 was tested by t test using the standard error of regression value (t = 1±b/SE) (bi), and to test significance of deviations from regression, F test was used, which compares the mean squares due to deviations from regression with pooled error mean squares.

RESULTS AND DISCUSSION

Diversity for oil content and other grain parameters: Oil content of the genotypes ranged between 3.14% and 4.76% (Table 1). IS 30466 (4.76%), IS 30536 (4.48%) and IS 1212 (4.46%) recorded highest oil content. The lowest oil percentage (3.14%) was found in IS 29914. The hundred-seed weight (HSW) ranged from 0.89 g (IS 603) to 4.94 g (IS 31714). Average seed harness was 6.32N, which ranged from 4.17 N (IS 31681) to 8.41 N (IS 1212). The germ size in different genotypes varied from small (Score 3) in case of IS nos. 21512, 23348, 29914, 31714 to large (score 7) in IS nos. 1212 and 31681. Oil content revealed significant correlation with germ size (r = 0.484) only while the associations with grain hardness and hundred-grain weight are non-significant (data not shown). This supports the earlier reports suggesting that oil content of sorghum mostly occurs in the germ fraction (Glew *et al.* 1997).

Genotype × environment interaction for oil content: In the present study, oil content data on 19 sorghum genotypes from three locations were subjected to a pooled analysis of variance (Table 1). The mean sum of squares due to environments and genotypes were significant for oil content indicating that environment had influence on the expression of the trait and there is a substantial amount of variation in the genetic material. Rakshit *et al*. (2012, 2014) and Aruna *et al*. (2015) also reported higher contribution of environment and genotype in explaining the variation in multi-location data in sorghum. Significant level of variation for oil content in sorghum was reported by Osman *et al*. (2000), Mehmood *et al*. (2008), Liu (2011), while Afify *et al*. (2012) reported oil content of 3.58 to 3.91% in sorghum.

Moreau *et al*. (2016) reported a variation of 3.21 to 4.29% in 18 samples, while Kaplan *et al*. (2018) reported a range of 2.32 to 5.74%. High influence of both genetic structure and environmental factors on oil content and quality of oil crops was reported earlier (Baenziger *et al*. 2001). The GEI was not significant for oil content indicating that the genotypes do not show differential response to the environment for oil content. Highly significant mean squares due to environment (linear) indicated that variation among environments was linear and had predominant effects on oil content. Significant pooled deviation for this trait indicated predominance of non-linear components in the manifestation of the GEI for the trait. Non-significant GEI with significant pooled deviation, as observed in this study, implies that the variation in the performance of genotypes was entirely unpredictable and depended on the changes in the environment. The mean square values due to environments plus genotype × environment were significant for oil content which suggested the distinct nature of environments and GEI in phenotypic expression. Significant variance due to Environment (linear) showed the presence of larger environmental differences among the environments for oil content. It is also suggested that the genetic differences between the genotypes for their regression on the environmental index were highly significant. The higher magnitude of mean squares for E (linear) compared to G × E (Linear) indicated that linear response of environment accounts for the major part of the total variation for oil content and may be responsible for high adaptation.

Stability for oil content: Environmental indices indicate the favourability of an environment for the trait and can provide the basis for identifying a favourable environment for the potential expression of a genotype. Environmental means and indices (Table 2) for oil content were highest in Hyderabad. However, the range in environmental values was not much for oil content. According to Eberhart and Russell (1966), a stable cultivar is characterized by a regression coefficient (b value) not different from unity

Table 2 Performance of genotypes for seed traits and mean and stability parameters for oil content in sorghum

Genotype	Details	HGW	GHR	GS	Oil content (%)							
					Gulberga	Solapur	Hyderabad	Mean	βi	Rank	S²Di	Rank
BJV 74	Improved variety	3.06	7.41	5	3.09	3.35	3.23	3.22	0.527	13	0.017	17
IS 1212	Germplasm line	2.06	8.41	7	4.41	4.35	4.64	4.46	0.928	2	0.013	16
IS 14090	Germplam	3.14	5.71	5	3.04	3.14	3.27	3.15	0.915	3	-0.006	12
IS 20298	Germplasm	2.52	5.66	5	4.35	4.35	4.46	4.39	0.428	14	-0.005	7
IS 21512	Germplasm	2.11	6.43	3	3.28	3.17	3.23	3.23	-0.178*	18	-0.001	2
IS 23348	Germplasm	3.23	5.69	3	3.27	3.29	3.35	3.30	0.307	17	-0.006	10
IS 29654	Germplasm	2.43	5.63	5	3.85	4.20	4.50	4.19	2.579**	19	-0.006	8
IS 29914	Germplasm	2.17	7.52	3	3.02	3.18	3.22	3.14	0.816	6	-0.004	4
IS 30383	Germplasm	3.30	6.62	5	3.94	4.24	4.29	4.15	1.392	11	0.005	6
IS 30443	Germplasm	2.46	6.00	5	4.29	4.07	4.68	4.34	1.584	15	0.103***	19
IS 30451	Germplasm	2.40	6.11	5	4.25	4.42	4.67	4.45	1.64*	16	-0.006	9
IS 30466	Germplasm	1.95	7.09	5	4.60	4.77	4.92	4.76	1.256	8	-0.006	13
IS 30507	Germplasm	2.07	7.56	5	4.32	4.41	4.52	4.42	0.821	5	-0.006	14
IS 30536	Germplasm	2.40	5.74	5	4.37	4.48	4.60	4.48	0.9	4	-0.006	15
IS 31681	Germplasm	2.21	4.17	7	4.08	4.28	4.33	4.23	0.986	1	-0.002	3
IS 31714	Germplasm	4.94	4.47	3	3.93	4.25	4.12	4.10	0.722	9	0.027 *	18
IS 603	Germplasm	0.89	5.10	5	4.07	4.24	4.38	4.23	1.256	7	-0.006	11
M 35-1	Improved variety	3.69	7.32	5	3.11	3.28	3.28	3.22	0.654	10	-0.001	1
CSV 27	Dual-purpose variety	1.91	7.50	5	3.29	3.41	3.66	3.45	1.461	12	-0.004	5
Mean		2.57	6.32	4.79	3.82	3.94	4.07	3.94				
Environmental indices					-0.125	-0.003	0.127					
CV		18.5	20.7	18	4.14	3.21	3.18					
CD (P=0.05)		1.01	1.89	2	0.26	0.21	0.21					

HGW: 100-grain weight (g), GHR: Grain hardness (N), GS: Germ size score and deviation from regression close to zero. The mean and deviation from the regression of each genotype were considered for stability, and linear regression was used for testing the genotypic response. In the current study the mean oil content (xi) among the genotypes ranged from 3.14% (IS 29914) to 4.76% (IS 30466) with population mean of 3.94% (Table 2). Two genotypes were unstable as indicated by the significant σ^2_{di}. Genotypes, IS 31681, IS 1212, IS 30536, IS 30507, IS 603 and IS 30466 were found better adapted to all environments with their bi=1 and mean more than population mean. These genotypes had moderate to hard seed with the germ size ranging from medium to large. Usually, harder grains have best storage properties. Grains of IS 1212, IS 30466, IS 30507 were harder and also recorded high oil content (>4.4). These genotypes can be potential candidates while breeding for high oil content with better storability. These genotypes could be of more value in breeding as they are suitable across environments. Genotypes with high mean, bi >1 with non-significant σ^2_{di} are considered below average in stability. Genotypes, IS 30451 and IS 29654 were better adapted to the favourable environment (bi>1 and xi>x). Such genotypes tend to respond favourably to better environments but give poor oil in unfavorable environments.

Sorghum is receiving attention as a source of health promoting component for foods. In the present study, evaluation of 19 sorghum genotypes across three environments showed considerable variations for oil content. Mean performance and coefficient of regression (bi) were used as response indices while deviation from regression (σ^2di) was used as a stability index. IS 31681 was the most stable genotype with high mean performance, followed by IS 1212, IS 30536, IS 30507, IS 603 and IS 30466. These genotypes are recommended for use in the breeding programs aiming for high oil content. These can also be used directly for characterization of the lipid profile for use as functional food. Contrasting genotypes identified in the study may be used to study the genetics of oil content in sorghum.

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