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RESEARCH ARTICLE

Genotype × environment interaction for seed amylopectin content in waxy sorghum [*Sorghum bicolor* (L.) Moench]

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Abstract

Waxy sorghum flour can be put to multiple industrial uses. Thirteen waxy and three non-waxy sorghum genotypes were grown across four environments to assess relative genotype (G), environment (E) and genotype × environment (GEI) effects on amylopectin, grain yield and other morphological traits. The amylopectin content (APT) among the high amylopectin sorghum (HAS) lines across the locations ranged from 96.7 (HAS 9) to 99.1 (HAS 3, HAS 5, HAS 6 and HAS 12), while among the checks, it ranged from 78.1 to 81.8%. The G, E, GEI were significant for all the target traits. The genetic component explained more than 90% of the total variance for APT and the environment component for grain yield (GY). The APT and GY correlated significantly and negatively (r = -0.55; p <0.05). The HAS lines produced smaller seeds in size than checks. The HAS 4 with red pericarp and HAS 5 with cream pericarp showed high GY and APT and HAS 4 is suitable for wider adaptation. The HAS lines yielded more at location Hagari, whereas HAS 6 (cream pericarp) and HAS 12 (red pericarp) were specifically adapted.

Keywords: Waxy sorghum, G × E, amylopectin, pericarp colour, grain yield

Introduction

Starch is a significant energy source for humans and is the main storage carbohydrate in plants (Perez-Maldonado and Rodriguez 2007). Starch consists of two macromolecular components: amylose, which is a linear polymer of glucose units linked together by α-1→4 glycosidic bonds and amylopectin, a branched structure, in which the linear α-1→4 linked glucose chains are connected through α-1→6 branching at every 20 to 25 units (Rooney and Pflugfelder 1986). The relative proportion of these two components of starch may vary between genotypes. In cereal starches, typical levels of amylose are 25 to 28% and amylopectin from 72 to 75%, although high amylose (up to 70% amylose) and waxy (<1% amylose) genotypes also exist. As in most other cereals, starch forms the major sorghum grain component, amounting to approximately 70% of the dry weight (Khoddami et al. 2023). In normal sorghum genotypes, the grain starch is comprised of about 20 to 30% amylose and 70 to 80% amylopectin. In waxy genotypes the starch is comprised of < 1% amylose, while the heterowaxy sorghum genotypes contains up to ~14% amylose (Chen et al. 2019). According to Ring et al. (1982), accessions grouped as heterowaxy contain lower amylose content than non-waxy grains (20%) but display many of the physical attributes of non-waxy grains. Wang et al. (2008) reported that waxy and heterowaxy sorghum varieties have higher ethanol yields than non-waxy varieties, at the same starch level. Sorghum is cultivated across tropical and subtropical regions across the world. In Africa, sorghum is less expensive than Teff and hence preferred for producing injera and other flat bread type bakery products. The amylose to amylopectin ratio has implications in the functional properties of starch and hence end uses of sorghum grain, as these two components have different physicochemical properties. Low peak viscosity, peak pasting temperature and pasting enthalpy was reported in sorghum starch with high amylose content. Waxy sorghum flour is preferable to produce fermented

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flat bread-type products with good textural functionality and improved injera textural quality due to the slower retrogradation and better water-holding of amylopectin starch (Mezgebe et al. 2020).The waxy (high amylopectin) trait is highly associated with high hot water extract in sorghum malt (Mezgebe et al. 2018). The improved malt quality due to the better starch granule swelling property of amylopectin could have been facilitated by greater hydrolysis by amylases and proteases (Tester and Morrison 1990). As a result, waxy starch is more easily hydrolyzed by α-amylase (Wu et al. 2010). The inclusion of waxy wheat flour (Ma et al. 2017), high amylopectin corn and potato starch (Witczak et al. 2019) has produced softer bread with reduced staling. Tortilla (unfermented wheat bread) made from waxy wheat flour had greater extensibility even after three days of storage (Guo et al. 2003). Besides food, waxy starches are used in different industrial applications as a thickening agent or stabilizer in manufacturing adhesives and lubricants (Reddy and Ratnavathi 2019). Also, sorghum grains with low amylose content were found to have better ethanol conversion efficiency (Wu et al. 2007). Furthermore, waxy cereal starches have potential food and industrial applications as they produce gels with unique textures (Rooney and Pflugfelder 1986).

Unlike resource-demanding crops like wheat, corn, and rice, grain sorghum can be grown economically with low inputs in the world's semi-arid regions. Sorghum grain has a wide range of uses, such as food, feed, brewing, and grain-based ethanol production (Ashok Kumar et al. 2011). The ethanol industry is increasingly utilizing sorghum in the United States after corn. About 95% of U.S. fuel ethanol is produced from corn and ~4% is from sorghum grain, which holds 30–35% of the total sorghum production in the United States (Kubecka 2011). Starch content in the grains is a good predictor of ethanol yield. The presence or absence of amylose may influence ethanol yield and conversion efficiency. Wu et al. (2007) reported that low amylose content in sorghum grain may be associated with increased ethanol conversion efficiency. The majority of sorghum cultivars are bred for food and feed applications (Mace and Jordan 2010). Thus, genetic improvement of grain quality in sorghum for ethanol production could increase the utilization of sorghum for ethanol production. From this perspective, a germplasm search in 4000 lines identified 13 waxy sorghum lines, which were stabilized and characterized at the Indian Institute of Millets Research (IIMR), Hyderabad. Understanding the influence of the genetic factors, environment and their interactions is very basic for genetic exploitation of the identified lines on a large scale. Therefore, the current investigation was framed to study the genotype × location interaction effects on grain amylopectin content, identifying the stable waxy sorghum lines with good grain yield and understanding the association between the target traits.

Materials and methods

Plant Material

The plant material is derived from 4000 sorghum germplasm lines obtained from the gene bank of ICAR-Indian Institute of Millets Research located at Rajendranagar, Hyderabad. The 4000 lines were multiplied during 2013 *rabi* at ICAR-IIMR, Hyderabad. The grain samples were subjected to rapid iodine staining technique given by Pedersen et al. (2004) to identify waxy phenotypes. From among them, 13 lines were identified to possess high amylopectin content (90 to 100%) based on the purple coloration with iodine staining. These include IS 23964, Hattigudur cross 2, IS 5624, IS 17994, IS 18020, IS 22119, IS 33815, IS 33887, IS 641, IS 829, IS 2269, IS 24346 and IS 27021 (Reddy and Ratnavathi 2019). These lines were advanced with single plant selection from 2014 to 2016 and assessed in agronomic trials at ICAR-IIMR during 2016 and 2017. These were further advanced with mass selection for uniformity in agronomic traits from 2018 to 2020 and designated as HAS 1 to HAS 13. The lines were photoperiod sensitive and suited for rabi adaptation. Hence, these selections were evaluated along with three checks, CSV 22, a high-yielding *rabi* sorghum variety, M 35-1, a widely grown *rabi* sorghum variety and C 43, a parent of popular *kharif* hybrid, CSH 16. The resultant 16 genotypes were used for multi-location evaluation (Table 1).

Table 1. Plant material taken from the ICAR-IIMR gene bank for the present study

Genotype	Pedigree
HAS 1	IS 641-2-2-1-1-B
HAS 2	IS 829-1-2-1-1-B
HAS 3	IS 2269-2-2-1-1-B
HAS 4	IS 3387-3-3-1-1-B
HAS 5	IS 5624-3-2-1-2-B
HAS 6	IS 17994 -2-2-1-1-B
HAS 7	IS 18020-1-2-1-2-B
HAS 8	IS 23964-1-3-1-1-B
HAS 9	IS 24346-3-2-2-1-B
HAS 10	IS 27021-4-1-2-1-B
HAS 11	IS 33815-3-2-2-1-B
HAS 12	H cross 2 -3-2-1-2-B
HAS 13	IS 22119-3-2-1-1-B
CSV 22	Postrainy varietal check
C 43	Rainy season check
M 35-1	Post-rainy varietal check

Table 2. Test locations used in the study

Location	Soil	pH	Latitude	Longitude	Altitude
Akola	Medium Black	7.8	21°18′N	77°30′E	307 m
Hagari	Black	8.0	15°14′N	76°92′E	466 m
Hyderabad	Sandy Loam	7.2	17°39′N	78°49′E	536 m
Vijayapur	Shallow Black	8.7	16°50′N	75°43′E	594 m

Experimental design and location

The trials were conducted in the four testing locations during the 2021 postrainy season. The chosen locations were from sorghum-growing states of India, such as Akola (Maharashtra), Hagari and Vijaypur (Karnataka), and Hyderabad (Telangana). The experiment was laid in a randomized block design with three replications. Each genotype had a plot size of two rows of 4m in length each, with 0.6 m between rows and 0.15 m between hills in each row. The experiment was planted in each location during the post rainy season (September last week-October first week). All the recommended crop production and protection practices were followed to raise a crop with good plant stand. Standard crop management practices were followed across all locations.

Observations recorded

Agronomical traits

The observations recorded for genetical analysis were**:** Days to flower (DF) (Number of days to attain 50% anthesis in 50% of the plants in each plot; Average plant height in cm (PHT) (From the base to the tip of the panicle at physiological maturity on 10 plants); Average panicle length in cm (PL): (Measured from the base to the tip of the panicle on 10 plants in each plot at the time of harvest); Average panicle width in cm (PW): (Measured at the broader part of the panicles of 10 plants per plot at the time of harvest); Grain yield (GY): (The plot yield was extrapolated to hectare and expressed as tons per hectare); Fodder yield (FY): (Fodder yield per plot was also extrapolated to hectare and expressed as tons per hectare) and Hundred-grain weight in gram (GWT): (The weight of 100 grains (g) averaged from 10 plants).

Determination of starch content

The starch content of sorghum grain samples was determined as per Southgate (1976). The flour (75 mg) was dispersed in ethanol and 9 mL of distilled water and autoclaved for starch gelatinization. This was followed by treatment with amyloglucosidase enzyme in sodium acetate buffer (pH 4.8) for 2 hours in a shaking water bath at 55ºC. This solution was made up to 250 mL using distilled water and further diluted 10 times. One ml aliquots were then taken for the determination of sugars using the phenol-sulphuric acid method as per Dubois et al. (1956).

Amylose and amylopectin contens in grains

William et al. (1958) determined the sorghum grain samples' amylose content. Briefly, the sorghum flour sample (100 mg) was soaked in 1-mL of 95% ethyl alcohol and 9 ml of 1N NaOH in 100 mL volumetric flask and allowed to stand overnight at 40ºC. After incubation, distilled water was added to bring the solution to 100 mL. Then 5 mL aliquot was taken and 40 mL distilled water was added and the pH was adjusted to 10.2 with 0.1 N HCl followed by the addition of 1-mL iodine reagent (I_2/KI). This solution was then made up to 100 mL using distilled water and the absorbance was measured using a UV-vis spectrophotometer (M/s Radwag, India). The amylose contents were calculated based on the standard procedure.

100 – Amylose content (% of starch) gave the amylopectin content as percent of starch

Statistical analysis

The replicated data from each location is subjected to ANOVA using Genstat 12th edition (Genstat 2012). The homogeneity of error variances for the 4 environments was checked by Bartlett's test using MS Excel (https://www.agroninfo.com/excel-tools/). The non-significance of Bartlett's statistics for all traits revealed the homogeneity of error mean-variance across all locations for further statistical analysis. The data of all four locations were subjected to ANOVA was carried out to test location (L), genotype (G), and their interaction effects (G × L). Trait variability and correlations were calculated using replicate means. The replication-wise data were analyzed for ANOVA using Genstat 12th edition (Genstat 2012). Pearson's correlation coefficients were calculated using R version 4.0 (R Core Team, 2020). Heritability (h^2b) is calculated for the target traits as the ratio of genetic variance to the phenotypic variance (the total genetic and environmental variance). For pooled data, the broad-sense heritability was calculated as: $h^2 = \sigma^2_g / (\sigma^2_g + \sigma^2_{gl} / l + \sigma^2_e / lr)$, where σ^2_g is the genotypic variance, σ^2_{gl} is the interaction variance of genotype with location, σ^2_e was the error variance, l was the number of locations, and r was the number of replicates. The estimates of σ^2g, $\sigma^2_{gl,}$ σ^2_e were obtained from an analysis of variance with environment considered a random effect, as Xie et al. (2020) mentioned. The levels of the broad-sense heritability (h^2bs) are categorized as low (<0.3), moderate (0.3–0.6), and high (>0.6), according to Robinson et al. (1949).

The GGE biplot analysis was employed to interpret the genotype by environment interactions for GY as the amylopectin content (as % of starch) was not significantly influenced by G×E interaction. The statistical theory of GGE methodology was considered by Yan (2001). For obtaining genotypes with high yield and stability using the "mean vs. stability" option, genotype-focused singular value partitioning (SVP = 1) was used, and for evaluating locations, environment focused SVP = 2 was employed (Yan 2001) and a single location was observed using the "examine an environment" option. The data was analyzed using R version 4.0 (R Core Team, 2020)

Results and discussion

The crop improvement programs until now have focused on yield improvement addressing the constraints affecting economical yields. Recently, the focus has shifted towards grain nutritional content and value addition for creating market demand. One such sorghum type is waxy sorghum that is bound to have less than <1% amylose of total starch composition in the grains. Thirteen HAS lines and 3 checks were evaluated at 4 locations to decipher if GEI (genotype × environment interaction) existed for this trait, identify promising genotypes, and derive inputs for the breeding program.

Analysis of variance (ANOVA)

The data of 16 lines evaluated across four locations were subjected to individual as well as pooled analysis. The non-significance of Bartlett's statistics for all traits revealed the homogeneity of error mean-variance across all locations for further statistical analysis.

The results of ANOVA are presented in Table 2. The pooled ANOVA (Table 2A) showed that the genotype (G), location (L) and genotype × location (G×L) interaction effects were significant for all the traits ($p < 0.01$) except G×L for panicle length and panicle width. However, the G×L was found to be very low, about 1% of total variation for APT and 2.7% for GY. The APT was majorly controlled by genetic factors as explained by 96% of total variation. The selection for this trait at the breeding location would be rewarding. Trikoesoemaningtyas et al. (2015) also reported higher variances due to amylose content genotypes. For grain yield, G×L variance was half that of variance due to G pointing towards the importance of identification of stable genotypes for grain yield. Table 2B depicted individual location ANOVA for grain yield and amylopectin content (as % of starch) for two important traits. There were significant differences among the genotypes across the locations for these two traits except Akola and Hagari for grain yield.

Mean across locations and broad sense heritability

The mean values, ranges of trait expression, and broad-sense heritability among the 16 genotypes across four locations and in pooled data is presented in Table 3. The genotypes flowered early at Hagari and Vijaypura (67 days) followed by Akola (74 days) and were late at Hyderabad (83 days). The h^2b was high for this trait across all the locations. The genotypes were shorter at Akola (170 cm) and grew taller at Hagari (240 cm) and more than 200 cm at the other two locations. The heritability was moderate at Vijaypura and high at other locations for plant height. The average panicle length of the genotypes was 15.0 cm at Hagari, which also showed moderate heritability for this trait. At other locations, the heritability was high with the Hyderabad location having highest average panicle length of 19.4 cm. The panicle width ranged from 4.4 (Hagari) to 5.7 cm (Akola). The heritability estimates varied widely for this trait from low (Hagari), to moderate (Akola, Vijaypura) to high (Hyderabad). For the economically important trait, grain yield, the Vijaypura location had the lowest grain yield of 1.31 t/ha with high heritability for the trait. The Hagari location had the highest grain yield 3.08 t/ha with low heritability. The Akola and Hyderabad locations had a grain yield of 2.3 to 2.54 t/ha. Similar trends were noted for fodder yield that ranged from 3.41 (Vijaypura) to 15.15 t/ha (Hagari) with moderate (Akola) to high (Hagari, Vijaypura, Hyderabad) heritability for this trait. Similar trends were noted for grain size as calculated from 100-grain weight that ranged from 2.39 (Hagari) to 2.84 g (Vijaypura) with moderate (Akola) to high (Hagari, Vijaypura, Hyderabad) heritability for this trait. For the grain amylopectin content expressed as% of starch, the values ranged from 94.4 (Hyderabad) to 95.9 (Akola), with very high heritability for this trait denoting high repeatability. Thus, the broad-sense heritability was high for days to flower, plant height, panicle length, fodder yield,

Table 2a. ANOVA for different traits studied across four locations during the post-rainy season of 2021

Source	df	DF	PHT	PL	PW	GY	FY	GWT	APT
Genotype	15	128.54**	5627.80**	83.90**	2.48**	0.99**	11.61**	0.43**	478.56**
Location	3	1876.23**	26832.20**	133.66**	14.45**	17.44**	824.14**	1.11**	13.71**
Genotype × Location	45	18.82**	990.80*	10.46	1.08	0.51**	4.02**	0.14**	5.03**
Residual	63	6.30	590.10	6.86	0.75	0.21	1.68	0.02	1.10

*$p \leq 0.05$, **$p \leq 0.01$, DF = Days to flower, PHT = Plant height, PL = Panicle length, PW = Panicle width, GY = Grain yield, FY = Fodder yield, GWT = weight of 100 grains and APT = Grain amylopectin content

Table 2b. ANOVA for grain yield and amylopectin (% of starch) in individual locations during the post-rainy season of 2021

Source		Grain Yield (t/ha)				Amylopectin (% of starch)			
	df	Akola	Hagari	Hyderabad	Vijaypura	Akola	Hagari	Hyderabad	Vijaypura
Replication	1	0.11	0.03	0.10	0.05	0.35	2.55	3.41	7.62
Genotype	15	0.26	0.38	0.97**	0.89**	87.67**	117.79**	170.55**	117.64**
Residual	15	0.30	0.37	0.06	0.12	1.20	1.36	0.58	0.79

*$p \leq 0.05$, **$p \leq 0.01$

Table 3. Mean performance of high amylopectin genotypes at different locations during the post-rainy season of 2022

Centre	Trait	Range	Mean	Lsd (5%)	h^2bs
Akola	DF	71–79	74.3	1.4	0.96
	PHT	149–185	170	14.6	0.81
	PL	9.0–21.5	16.3	4.9	0.8
	PW	4.0–7.5	5.7	2.4	0.51
	GY	1.75–2.96	2.30	1.18	NE
	FY	5.35–8.30	6.67	1.71	0.47
	GWT	2.48–2.76	2.55	0.17	0.48
	APT	79.3–99.5	95.9	2.3	0.99
Hagari	DF	56–76	67.3	8.8	0.83
	PHT	125–290	240	70	0.76
	PL	8–20	15.0	7.9	0.35
	PW	3.5–6	4.4	1.8	0.06
	GY	2.43–3.83	3.08	1.29	0.05
	FY	9.26–18.52	15.15	5.10	0.64
	GWT	1.90–2.89	2.39	0.40	0.73
	APT	77.2–99.3	95.2	2.5	0.99
Hyderabad	DF	75–89	83.5	5.58	0.85
	PHT	150–245	203	27	0.88
	PL	9.5–27.5	19.4	4.2	0.92
	PW	3.25–5.9	4.6	1.4	0.67
	GY	1.18–3.49	2.54	0.50	0.94
	FY	4.00–8.15	6.17	0.61	0.98
	GWT	1.55–3.28	2.56	0.21	0.97
	APT	75.1–99.5	94.4	1.6	0.99
Vijaypura	DF	61–73	67.3	5.2	0.81
	PHT	148–254	210	73	0.54
	PL	10.6–21.7	18.7	4.01	0.83
	PW	4.25–7.25	5.6	1.9	0.36
	GY	0.36–2.32	1.31	0.74	0.87
	FY	1.67–5.93	3.41	1.40	0.87
	GWT	1.78–3.39	2.84	0.38	0.92
	APT	76.1–99.1	94.8	1.9	0.99
Pooled data	DF	66–77	73.1	2.5	0.85
	PHT	145–238	206	24.3	0.82
	PL	9.7–21.8	17.3	2.62	0.88
	PW	3.9–6.1	5.1	0.86	0.57
	GY	1.67–2.91	2.31	0.45	0.48
	FY	5.1–9.2	7.85	1.30	0.65
	GWT	2.0–2.9	2.6	0.14	0.67
	APT	78.1–99.1	95.1	1.05	0.99

h^2bs = broad-sense heritability

grain size and amylopectin content in most of the cases, while it was moderate to high for grain yield and variable in case of panicle width depicting the active interaction of the environment with the genotype in individual locations leading to low repeatability for the traits showing low heritability in specific locations.

Mean performance of genotypes

The mean performance among the genotypes is studied for two economically important traits, grain yield and amylopectin content. The pooled results showed that the grain amylopectin content (% of starch) among the high amylopectin sorghum (HAS) lines across the locations ranged from 96.7 (HAS 9) to 99.1 (HAS 3, HAS 5, HAS 6 and HAS 12) while among the checks, it ranged from 78.1 (M 35-1) to 81.8% (CSV 22). The differences between the 13 HAS lines were non-significant and these were significantly different from the checks. Similarly, at individual locations, non-significant differences were observed among the HAS lines clearly differentiating waxy sorghum from non-waxy sorghum. When individual locations were considered, the amylopectin content was above 97.5% in all HAS genotypes across all the locations (Table 4) except HAS 9 (93.1%) evaluated at Hyderabad location. The waxy sorghum lines had three pericarp colors. The lines HAS1, HAS 3, HAS 4, HAS 8, HAS 9, HAS 11, HAS 12, and 13 had red pericarp while HAS 2 had brown pericarp. The lines HAS 5, HAS 6, HAS 7 and HAS 10 had cream colored pericarp. The grain yield among the HAS lines ranged from 1.67 (HAS 2) to 2.59 (HAS 4) while

Table 4. Performance of high grain amylopectin lines across four locations during rabi 2021

Entry	Amylopectin content (% of starch)					Grain yield (t/ha)					Grain pericarp colour
	Akola	Hagari	Hyderabad	Vijaypur	Pooled	Akola	Hagari	Hyderabad	Vijaypur	Pooled	
HAS 1	99.2	98.0	99.3	98.2	98.7	1.75	3.04	2.21	1.28	2.07	Red
HAS 2	99.2	98.8	99.1	97.7	98.7	2.21	2.92	1.18	0.36	1.67	Brown
HAS 3	99.2	99.2	99.5	98.4	99.1	2.00	2.97	1.81	2.06	2.21	Red
HAS 4	99.2	99.0	98.7	97.9	98.7	2.05	2.84	3.49	1.98	2.59	Red
HAS 5	98.7	99.2	99.5	98.9	99.1	2.92	3.60	3.27	0.43	2.56	Cream
HAS 6	98.9	99.3	99.1	99.0	99.1	2.29	3.63	2.77	1.03	2.43	Cream
HAS 7	98.9	99.0	99.0	97.9	98.7	2.63	2.43	2.45	0.63	2.03	Cream
HAS 8	98.9	98.1	99.0	98.9	98.7	1.83	2.51	3.42	2.12	2.47	Red
HAS 9	98.0	98.2	93.1	97.6	96.7	2.34	2.67	2.33	1.56	2.22	Red
HAS 10	98.6	97.5	98.6	97.6	98.1	1.92	2.62	2.21	0.44	1.80	Cream
HAS 11	99.1	99.2	99.3	97.9	98.9	2.17	2.98	2.80	1.93	2.47	Red
HAS 12	99.5	98.9	98.8	99.1	99.1	2.25	3.27	2.85	0.95	2.33	Red
HAS 13	98.5	99.1	98.9	98.6	98.8	2.25	3.25	1.25	0.74	1.87	Red
CSV 22	85.5	84.1	77.5	80.1	81.8	2.96	3.73	3.25	1.69	2.91	Cream
C 43	79.3	78.7	75.1	82.3	78.9	2.58	3.83	2.59	2.32	2.83	Cream
M 35-1	83.5	77.2	75.4	76.1	78.1	2.59	2.91	2.75	1.46	2.43	Cream
Mean	95.9	95.2	94.4	94.8	95.1	2.30	3.08	2.54	1.31	2.31	
LSD (5%)	2.3	2.5	1.6	1.9	1.1	1.18	1.29	0.5	0.74	0.45	

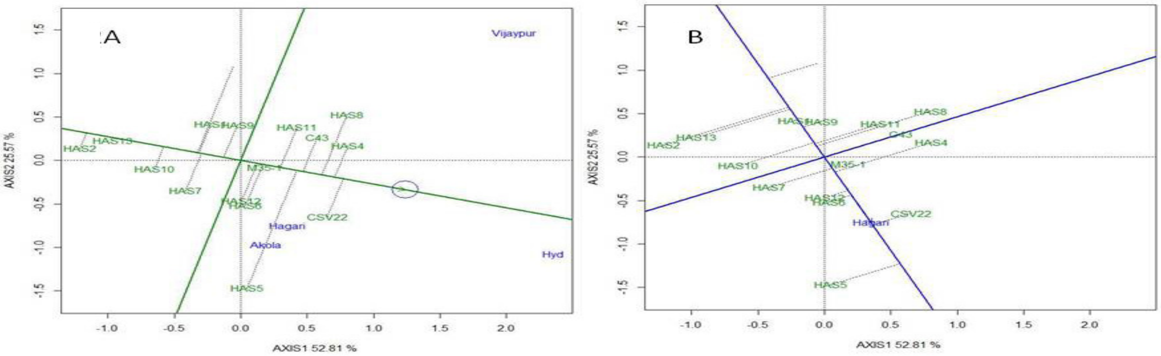

Fig. 1. GGE biplots from combined analysis of data for grain yield A) Mean vs. Stability, B) Examine an environment (Hagari)

the best check CSV 22 had a grain yield of 2.91 t/ha. Four HAS lines, HAS 4, HAS 5, HAS 8 and HAS 11 with grain yield ranging from 2.47 to 2.59 t/ha were on par with CSV 22. Along with the four lines, HAS 6 (2.43 t/ha) was on par with C 43 (2.83 t/ha). Ten of the 13 HAS lines were on par with the popular varietal check M 35-1 (2.31 t/ha). None of the HAS lines were numerically superior for grain yield as compared to CSV 22 and C 43. Though among the HAS lines, HAS 4 had the highest grain yield of 2.59 t/ha, it had 12.35% lesser grain yield than the best check CSV 22. Similarly in earlier studies, the waxy grain trait in sorghum has been reported to reduce sorghum grain yield by 17% (Rooney et al. 2005). In addition, Rooney et al. (2005) did identify waxy sorghum hybrids that yielded as high as non-waxy hybrids.Thus, HAS 4 with red pericarp and HAS 5 with cream pericarp were promising among the waxy sorghum lines that can be readily exploited as they are supposed to have good potential for grain ethanol production at an industrial scale.The anthocyanins extracted from easily available red sorghum bran would be a valuable source for antioxidant and antiproliferative activity in food industry (Kumari et al. 2013). The average grain size (100 grain weight) of the HAS lines was 2.5 g which was

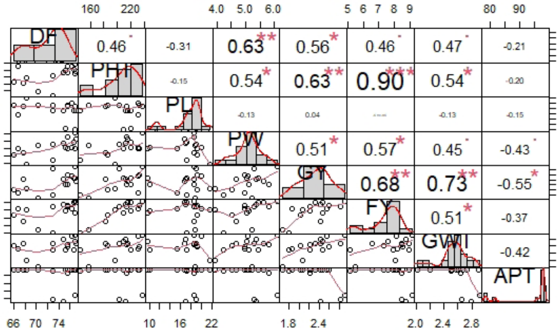

Fig. 2. **Inter-relationship among the measured traits over four locations during 2021 post-rainy season**

lesser than the average of the check lines (2.8g). Rooney et al. (2005) also reported that waxy sorghum lines have lower test weights than wild-type sorghum.

Contribution of sources of variation for grain yield and amylopectin content

The relative contribution of variances due to G, E and G × E to the total variance is calculated from Table 2A for all the traits. The location with 91.1% of the total variance was the major contributor towards the variation in grain yield followed by G with 5.2% and G × E with 2.7%. In contrast, for grain amylopectin content, the genotypic variance with 96.0% was the major contributor towards total variation followed by location (E) with 2.8% and G × E with 1.0%. The location was the major contributor for other traits viz., DF (92.4%), PHT (78.8%), PL (56.9%), PW (77%), FY (97.9%) and GWT (65.3%). Apart from GY and APT, the genotypic variance was highest was PL (35.7%), GWT (25.3%), PHT (16.5%), PW (13.2%), DF (6.3%) and FY (1.4%). The G × E component was 34% of G+GE for GY, 30.3% for PW, 25.7% for FY, 24.6% for GWT, 15.0% for PHT, 12.8% for DF, 11.1% for PL and 1.0% for APT. Further analysis is done for economically important traits, GY and APT. Since G × E component is negligible for APT, the stability of genotypes was estimated graphically through GGE biplot analysis for GY.

Correlations

The relationship among the traits were studied in the pooled data over locations (Fig. 2). The grain amylopectin content was negatively and significantly associated with grain yield and did not correlate with other traits. The grain yield was associated with late maturity, increase in plant height, more panicle width, higher fodder yield and larger size of grains.

GGE biplot analysis for grain yield

Mean performance and stability analysis of genotypes across environments is depicted by average environment coordination (AEC) method and presented in Fig.1A (Yan 2001). The first two PCs explained about 78.4% of variation for grain yield. In Fig.1A, the AEC abscissa is represented by a line with a single arrowhead, passes through the biplot origin and points towards higher mean values. The length of perpendicular lines to the AEC abscissa indicates stability. Accordingly, CSV 22 is the highest grain yielder, followed by HAS 4, HAS 8, C43 and HAS 5. Among the HAS lines, HAS 4 has high stability being closer to AEC abscissa. HAS 13 was poor yielder with the highest stability. Thus, the mean vs. stability graph for grain yield indicated that HAS 4 is a high high-yielding and highly stable genotype. In contrast to APT, location contributed 91.1% of total variation for GY. Hence, a good production environment is required for grain production. The Hagari location which has the highest average grain yield, is examined for suitability of genotypes. When Hagari location was examined through GGE biplot graph (Fig.1B - Examining an environment – Hagari), the genotypes HAS 6 and HAS 12 were found to be high and stable yielders for grain yield while HAS 5 and HAS 4 also yielded better. Earlier studies also reported that genetic and environmental factors affected sorghum's amylose/ amylopectin content (Beta and Corke 2001). The amylose/ amylopectin content has been reported to vary with the botanical source of the starch and is affected by the climatic and soil conditions during grain development (Boudries et al. 2009). The amylose content of rice was also reported to be affected by both environment and genotype (Juliano et al. 1964).

The HAS lines have industrial uses and may generate additional income to the growers. The current study deduced that the amylopectin content (as % starch) is a stable and a genetic trait, less influenced by the environment. Breeding for high amylopectin content may reduce grain yield and seed size, but waxy trait can be possibly bred in good agronomic and high-yielding backgrounds. Production on commercial scale can be taken up for HAS 4, which was a high and stable yielder in the current study. Suitable locations can be identified in the future by involving more locations in MLTs.

Authors' contribution

Conceptualization of research (PSR); Designing of the experiments (PSR, KNG); Contribution of experimental materials (PSR); Execution of field/lab experiments and data collection (VMM, PSR, KNG); Analysis of data and interpretation (PSR, VMM, PP); Preparation of manuscript (PSR, VMM, CTS, KNG, CVR, PP).

References

Ashok Kumar A., Reddy B.V.S., Sharma H.C., Hash C.T., Rao P.S., Ramaiah B. and Sanjana Reddy P. 2011. Recent advances in sorghum genetic enhancement research at ICRISAT. Am. J. Plant Sci., **2**: 589–600. https:// doi.org/10.4236/ ajps.2011.24070

Beta T. and Corke H. 2001. Genetic and environmental variation in sorghum starch properties. J. Cereal Sci., **34**: 261-268.

Boudries N., Belhaneche N., Nadjemi B., Deroanne C., Mathlouthi M., Roger B. and Sindic M. 2009. Physicochemical and functional properties of starches from sorghum cultivated in the Sahara of Algeria. Carbohydr. Polym., **78**: 475-480.

Chen B., Wang C., Wang P., Zhu Z., Xu N., Shi G., Yu M., Wang N., Li J., Hou J., Li S., Zhou Y., Gao S., Lu X. and Huang R.2019. Genome-wide association study for starch content and constitution in sorghum (*Sorghum bicolor* (L.) Moench). J. Integr. Agric., **18**: 2446–2456.

Dubois M., Giller K.A., Hamilton J.K., Rebers P.A. and Smith, F. 1956. Colorimetric method for the determination of sugars and related substances. Anal. Chem., **28**: 350-356.

GenStat. 2012. GenStat 12th ed. VSN International Ltd., Hemel Hempstead, UK.

Guo G., Jackson D.S., Graybosh R.A. and Parkhurst A.M.2003. Wheat tortilla quality: Impact of amylose content adjustments using waxy wheat flour. Cereal Chem., **80**: 427–436. https://doi.org/10.1094/CCHEM.2003.80.4.427

Juliano B.O., Albano E.L. andCagampang G.B.1964. Variability in protein content, amylose content and alkali digestibility of rice varieties in Asia. Philipp. Agric., **48**: 234-241.

Khoddami A., Messina V., Vadabalija Venkata K., Farahnaky A., Blanchard C.L. and Roberts, T. H.2023. Sorghum in foods: Functionality and potential in innovative products.Crit. Rev. Food Sci.Nutr., **63**: 1170-1186. https://doi.org/10.1080/10408398.2021.1960793

Kubecka B.2011. Sorghum plays role in ethanol's impact. In: Ethanol Producer Magazine, p. 18.

Kumari B., Randi K. and Sharma, R.2013. Classification of 3-red sorghum bran and deoxyanthocyanins and its biological properties. Front. Agri. Food Technol., **1**: 63-74.

Ma F., Ji T. and Baik B.K. 2017. Quality characteristics of northern-style Chinese steamed bread prepared from soft red winter wheat flours with waxy wheat flour substitution. J. Cereal Sci., **73**: 99–107. https://doi.org/10.1016/j.jcs.2016.12.002

Mace E. and Jordan D.2010. Location of major effect genes in sorghum (*Sorghum bicolor* (L.) Moench). Theor. Appl. Genet., **121**: 1339-1356.

Mezgebe A.G., Taylor J.R.N. and de Kock H.L. 2020. Influence of waxy (High Amylopectin) and high protein digestibility traits in sorghum on injera sourdough-type flatbread sensory characteristics. Foods, **9**: 1749. https://doi.org/10.3390/foods9121749.

Mezgebe A.G., Abegaz K. and Taylor J.R.N.2018. Relationship between waxy (high amylopectin) and high protein digestibility traits in sorghum and malting quality. J. Cereal Sci.,**79**: 319-327.

Pedersen J. F., Bean S. R., Graybosch R. A., Park S. H. and Tilley M. 2005. Characterization of waxy grain sorghum lines in relation to granule-bound starch synthase. Euphytica, **144**: 151–156.

Perez-Maldonado R.A. and Rodriguez H.D. 2007. Nutritional Characteristics of Sorghums in Queensland and New South Wales for Chicken Meat Production. RIRDC, Barton, Australia.

R Core Team. 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/

Reddy P.S. and Ratnavathi C.V. 2019. Identification, characterization and evaluation of high amylopectin sorghum (S*orghum bicolor* (L.) Moench) germplasm lines. The Journal of Research PJTSAU, **47**: 9-13.

Ring S.H., Akingbala J.O. and Rooney L.W. 1982. Variation in amylose content among sorghums. *In*: Proceedings of the International symposium on sorghum grain quality. (eds. L. W. Rooney and D. S. Murty). ICRISAT, Patencheru, India: 269-279.

Robinson H.F., Comstock R.E. and Harvey P.H. 1949. Estimates of heritability and degree of dominance in corn. J. Agron., **41**: 253-259.

Rooney L.W. and Pflugfelder R.L. 1986. Factors affecting starch digestibility with special emphasis on sorghum and corn. Anim. Sci. J., **63**: 1607–1623. https://doi.org/10.2527/jas1986.6351607x

Rooney L.W., Aydin S. and Kuhlman L.C. 2005. Assessing the relationship between endosperm type and grain yield potential in sorghum (*Sorghum bicolor* L. Moench). Field Crops Res., **91**: 199–205.

Southgate D.A.T. 1976. Determination of food carbohydrates. Applied Science Publishers Ltd. London.

Tester R.F. and Morrison W.R. 1990. Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. Cereal Chem., **67**: 551-557.

Trikoesoemaningtyas, Wirnas D., Sopandie D. andTesso T.2015. Genotypes × environment interaction effect on nutritional quality of sorghum lines in Indonesia. Ekin J. Crop Breed. Genetic, **1-2**: 26-31.

Wang D., Bean S., McLaren J., Seib P., Madl R., Tuinstra M., Shi Y., Lenz M., Wu X. and Zhao R. 2008. Grain sorghum is a viable feedstock for ethanol production. J. Ind. Microbiol. Biotechnol., **35**: 313-320. https://doi.org/10.1007/s10295-008-0313-1

Williams V.R., Wu W.T., Tsai H.Y. and Bates H.G. 1958. Varietal differences in amylose content of rice starch. J. Agric. Food Chem., **6**: 47-48.

Witczak M., Korus J., Ziobro R. and Juszczak L. 2019. Waxy starch as dough component and anti-staling agent in gluten-free bread. LWT - Food Sci. Technol., **99**: 476–482. https://doi.org/10.1016/j.lwt.2018.10.009

Wu X., Zhao R., Bean S.R., Seib P.A., McLaren J.S., Madl R.L., Tuinstra M., Lenz M.C. andWang D. 2007. Factors impacting ethanol production from grain sorghum in the dry-grind process. Cereal Chem., **84**: 130-136.

Wu X., Jampala B., Robbins A., Hays D., Yan S., Xu F., Rooney W., Peterson G., Shi Y.C. and Wang D. 2010. Ethanol fermentation performance of grain sorghums (*Sorghum bicolor*) with modified endosperm matrices. J. Agric. Food Chem., **58**: 9556-9562.

Xie Y., Xu J., Tian G., Xie L., Xu B., Liu K. and Zhang X. 2020. Unraveling yield-related traits with QTL analysis and dissection of QTL×environment interaction using a high-density bin map in rapeseed (*Brassica napus*. L). Euphytica, **216**: 171. https://doi.org/10.1007/s10681-020-02708-5

Yan W.2001. GGE biplot-a Windows application for graphical analysis of multi-environment trial data and other types of two-way data. J. Agron., **93**: 1111–1118.