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Estimation of amylose, protein and moisture content stability of rice in multi locations

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Regarding rice grown in five different locations, rice quality is measured using an auto grain analyzer. Auto grain analyzer works on the Near-Infrared transmittance (720 - 1100 nm). Protein, amylose and moisture contents of the rice samples of nine (9) fine lines were tested. Different environment tested entries were evaluated and found that all the values have highly significant effect of environment and genotypes. The environment and genotype ranking in the Additive Main effect and Multiplicative Interaction (AMMI) model were studied and PK8680-13-3-1 and check variety Basmati 515 were found to be most stable lines in most micro environment with respect to amylose contents and moisture contents. Protein contents were studied in PK8892-4-2-1-1 and PK3810-30-1 and are best suited in all the environments. The results indicated that grain analyzer may be used for amylose and protein contents along with effect of different locations on these traits in early breeding generations for quality control in the food industry.

Key words: Rice, environment, amylose, protein, auto grain analyzer.

INTRODUCTION

For over half of the world's population, rice is the main food. Quality of the grain is of much importance with respect to the rice scientists, producers as well as consumers. In bid to spread the rice genetic base, due to which there is possibility to breed for the purpose of improved crop yield, crosses have been made between distant parents (e.g. *Indica* × *Japonica* crosses) (Wu et al., 1996; Zhuang et al., 1997). Additionally, in spite of the total poorer agronomic phenotypes detected in wild species, they have been a valid basis of favorable genes from the start of modern breeding. Among the diverse modules factors of agronomy packages for rice cultivation, transplanting is one of the significant features of rice quality impacts (Mahajan et al., 2015). The introgression of wild rice alleles has been effectively used as an actual method in cultivated rice breeding plans for additional development of agronomic traits like quality (Thomson et al., 2003; Aluko et al., 2004; Fasahat et al., 2012; Xiao et al., 1998; Septiningsih et al., 2003). The cooking excellence of rice is significantly affected by the two attributes amylose contents and protein contents in the rice (Champagne et al., 1998). The amylose content (AC) is strictly associated by the sensual possessions of the freshly cooked rice (Champagne et al., 2004);

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although, the protein content (PC) dictates to the consistency (texture) of the cooked rice by hindering absorption of the water and starch puffiness during the cooking (Xie et al*.,* 2008).

Rice is consumed primarily as a full grain and the texture of the whole grain is a matter of prime significance. Cultivars of waxy and non-waxy rice are generally categorized conferring to their amylose content, grain sizes, amylograph reliability, gelatinization possessions of the take out starches and of the texture (hardness and sensory dimensions) of the cooked rice (Juliano, 1985). Texture is a significant characteristic of food acceptance by the consumers and also a dire step in the assessment of quality of rice. Texture is proposed as it is the sensory appearance of the food arrangement and the style in which that arrangement responds to the applied force; thus, amylose content in rice variety affects the rice texture (Szczesniak, 1968).

The greatest essential aspect inducing the cooking and processing appearances of rice is amylose content which is considered to be one of them. It is normally used as an objective index for the texture of cooked rice (Webb, 1991). Low amylose contents are linked with cohesive, tender, and glossy rice on the cooking. On the contrary, high levels of amylose content cause rice to absorb extra water and accordingly expand more throughout the cooking; also, the cooked grains tend to be dry, fluffy and detached (Juliano, 1971). Rice breeders are consistently anxious regarding having new rice lines with suitable amylose content and protein contents. Rice is a vital source of protein, delivering additional 50% of the entire protein consumed in the more or less countries. Even a modest rise in protein contents levels in rice would provide an important nutritional enhancement to the hundreds of millions of people who rely on it. In the selection of each variety and market value, determination of rice quality is very much important in many countries (Fitzgerald et al*.,* 2008; Champagne et al*.,* 1999).

G × E interaction is common when genotypes (G) are verified crosswise on different environments (E). Based on the range of the interface, classification of genotypes can differ through the environments. Several approaches have been projected to analyses the genotype-by-environment relations, with illustrations being the combined analysis of variance (ANOVA). The combined ANOVA can check the significance of interactions and main effects, which again does not aid clarification of the arrays of the G × E interaction. To this purpose, AMMI is the classical model of first choice when main effects and the interactions are together essential (Zobel et al*.,* 1988). Dissimilarities in nutrient readiness and soil moisture, ambient temperature and atmospheric composition also affected starch functionality which ultimately impacts on amylose contents (Beckles and Thitisaksakul, 2014). This technique assimilates ANOVA and principal component analysis (PCA) into a combined method.

Table 1. List of evaluated genotypes under study.

S/N	Designation	Variant code
1	PK8892-4-2-1-1	FV1
2	PK8647-11-1-1	FV2
3	PK8431-1-2-1-2-4	FV3
4	PK8430-1-2-1-3	FV4
5	PK8431-6-1-1-1	FV5
6	PK8667-8-5-1	FV6
7	PK8680-13-3-1	FV7
8	Basmati 515 (Check)	FV8
9	PK3810-30-1	FV9

METHODOLOGY

Nine basmati lines of rice PK8892-4-2-1-1, PK8647-11-1-1, PK8431-1-2-1-2-4, PK8430-1-2-1-3, PK8431-6-1-1-1, PK8667-8-5-1, PK8680-13-3-1, Basmati 515, PK3810-30-1 were categorized as fine variants FV1, FV2, FV3, FV4, FV5, FV6, FV7, FV8, FV9, respectively (Table 1) grown in five major rice producing areas (Farooqabad, Gujranwla, Faisalabad, Shorkot and Kala Shah Kaku) of Punjab, Pakistan during the year 2014 in Regional Adaptability Yield Trial (RAYT-14). The sample population was collected for the amylose, protein and moisture content in the form of milled rice. Auto Grain Analyzer is used for measurement of the characters evaluated. The measurement system is Near-Infrared Transmittance (720-1100 nm) and was used to define the amylose content, proteins contents and moisture contents in the rice.

The AN-900 Near infrared microscopy is accomplished for calculating moisture content, protein and amylose contents in the short, long brown rice as well as milled rice. Elements were calculated based on the transmittance of the light. Measurements are happening by simply loading a sample into the sample case. This method permitted rapid, simple and non-destructive constituent examination of the traits. Paralleled to the infrared reflectivity measurement method, the Near-Infrared Transmittance method engaged by the AN-900 is quite affected by the shape or color of the sample, and therefore, excellent measurement characteristics. To access a sample, 60 ml of the rice sample (milled) was simply filled in sample case and the rice sample case was inserted into the slot on top of the AN-900. In less than 30 s, all of the measurement components were displayed on the large digital screen and output is taken on computer or optional printer.

Statistical analysis

For the statistical analysis of data, attained software Statistx 8.1 and GENSTAT 12.1 were used. Additive Main effect and Multiplicative Interaction (AMMI) model of stability was applied to study the genotype and environment interactions.

RESULTS AND DISCUSSION

The mean values of nine genotypes grown in five different environments were calculated. Apart from the environments, the genotypes and all others traits were found to be highly significant. Different locations were studied for the quality traits of rice with different environmental temperature, rainfall pattern and soil. In

Faisalabad, PK8892-4-2-1-1 have higher amylose contents (25.3%) compared to other locations and was also higher from the check Basmati 515 approved cultivated variety. While in Farooqabad, the Basmati 515 performed at higher level of amylose contents with the value of 25.6% and the fine variant PK3810-30-1 also produces the amylose contents 25.3%. In Gujranwala, the 25.5% amylose contents were produced in another fine variant named PK8680-13-3-1, whereas in Kala Shah Kaku, 26% highest value was obtained for the amylose contents by the same variant who performed in the Faisalabad PK8892-4-2-1. In Shorkot variant, line PK8680-13-3-1 again performed at highest level for amylose contents with the mean value of 25.05%. So it is assumed that despite the different environmental effects, 3 lines along with checks produced highest level of amylose contents in different environments. However, in case of other fine variants there is similar expression. The lowest amylose contents 21.9% were produced in the PK8647-11-1-1 in Farooqabad location and the same line produced the same pattern of amylose contents production in different environments.

Like amylose, protein content was affected by the environmental parameter when it was checked in different locations. At two locations, viz; Faisalabad and Farooqabad, the maximum value of protein content was 8.3% produced by the variants PK8430-1-2-1-3 and PK8892-4-2-1-1. Fine variant PK3810-30-1 with 8.4%, PK8647-11-1-1 with 8.35%, PK8892-4-2-1-1 with 8.2% produced maximum protein content in the locations Kala Shah Kaka, Gujranwala and Shorkot, respectively. The minimum value for protein content (7.5%) was observed in the PK8667-8-5-1 in Farooqabad and Shorkot. In case of protein contents, it was studied that the PK8892-4-2-1 is the most stable line in different environments.

In both Farooqabad and Gujranwala locations, the moisture content was maximum (14.5%) in PK8667-8-5-1; however, in other locations: Faisalabad, Kala Shah Kaku and Shorkot, the moisture content was stable and less than 13% which are more desirable. The ANOVA revealed highly significant G × E interactions as well as significant differences among genotypes and among environments for all traits. For the significance of analysis of variance, the data was normalized to apply AMMI analysis and the variability among genotypes for different environments was checked in initial data analysis.

AMMI-1 biplot display

To further examine the main and interaction effects across genotypes and environments, biplots were constructed (Figure 1). The genotype and environment means are plotted on the x-axis, while the IPCA1 scores for the same genotypes and environments are on the y-axis. Displacement along the x-axis shows differences in the main effects; whereas displacement along the y-axis reflects differences in the interaction effects. When a cultivar and an environment have the same sign on IPCA1, their interaction is positive; if the sign is different, their interaction is negative. Genotypes with dissimilar interaction scores have dissimilar interaction effects across environments, while genotypes with interaction scores close to zero have negligible interaction effects. FV4 was found more suitable for the Faisalabad environment along with FV3, FV9 and FV7 with the strong positive interaction. In Gujranwala, FV5, FV8, FV6 and FV1 interacted positively and strongly. While in Farooqabad, FV6, FV7, FV8, FV9 performed at their suitable level of amylose content. Gujranwala, Kala Shah Kaku and Shorkot are the best suited genotype locations (Table 2). For protein and moisture content, the mean and variances of genotype and environment in AMMI ranking were studied and found to be the best suited environment for all the variants (Tables 4, 5 and 6).

DISCUSSION

Before releasing a new variety on a commercial basis, plant breeders grow different varieties in different environments over several years to evaluate the magnitude of G × E interactions for confirming the stability of the variety across various environments (Sabaghnia et al*.,* 2008). The AMMI model is suitable for the analysis of the G × E interaction different location trials (Zobel et al*.,* 1988). The analysis of variance of the AMMI model showed that G × E interactions were highly significant for all traits. Firmness and fluffiness of rice grain on cooking depends upon amylose content whether it will be, or it will turn sticky and glutinous. The average amylose content of rice grown in five diverse environments was high under the present study, which outcomes in elongation during cooking and cooked rice showed soft texture (Juliano and Pascual, 1980). The rice grain quality traits such as amylose and protein content were readily affected by various environmental factors including solar radiation, temperature and location of the field in various studies (Sharifi et al*.,* 2010; Bao et al*.,* 2002; Tian et al*.,* 2005). Comparable to the grain yield, the confirmed grain quality parameters were entirely significantly influenced by genotype, environment and G × E interaction by Nagarajan et al. (2010). The AMMI analysis produced highly significant principal components for protein content and amylose content (Table 3). Variable properties of growing temperatures on amylose content of the rice cultivars had been described (Singh et al*.,* 2014). However, rice has a low quantity of protein content (that is, between 5.8 and 9.4%) in the milled rice, though rice is used as the main source of protein in numerous rice-consuming countries of world. Therefore, protein content is significant from a dietary perspective.

In this study, protein content was quite high (> 8%) for all the genotypes. Protein content was affected by

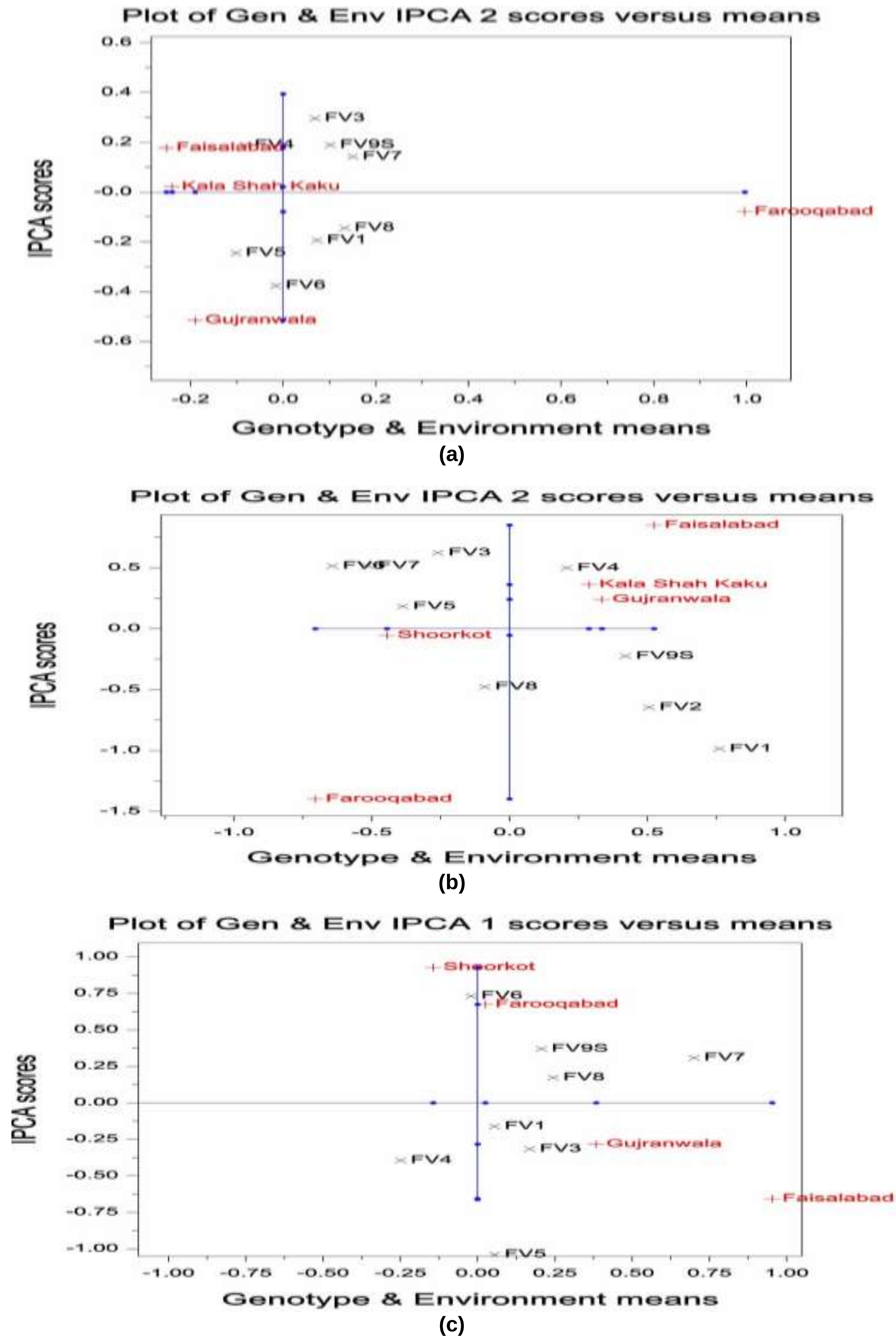

Figure 1. AMMI-1 biplot showing the means of Genotypes (G) and Environments (E) against their respective IPCA1 scores. a) Amylose content %, b) Protein content %, c) Moisture Content.

Table 2. Combine analysis of variance from AMMI model for evaluated traits in experimental environments.

Source	df	Protein Content		Amylose Content		Moisture Contents	
		SS	MS	SS	MS	SS	MS
Total	134	134.00	1.000	134	1	134.00	1.000
Treatments	44	129.93	2.953**	38.9	0.884**	126.93	2.885**
Genotypes	8	29.40	3.676**	2.78	0.347**	30.55	3.818**
Environments	4	31.45	7.862^{ns}	33.74	8.435**	69.25	17.313**
Block	10	0.45	0.045**	94.56	9.456^{ns}	0.46	0.046**
Interactions	32	69.07	2.159**	2.38	0.074**	27.13	0.848**
IPCA1	11	28.56	2.596**	1.49	0.135**	15.41	1.401**
IPCA2	9	24.64	2.738**	0.63	0.07**	7.84	0.871**
Residuals	12	15.87	1.322	0.26	0.022	3.89	0.324
Error	80	3.62	0.045	0.54	0.007	6.60	0.083

ns = Non-significant; ** = highly significant at 0.01 and 0.05 levels of significance,

Table 3. Environment means and variances and AMMI ranking for Amylose contents.

Environment	NE	Em	Variances	IPCAe [1]	IPCAe [2]	1	2	3	4
Faisalabad	1	-0.2515	0.0286	0.06976	0.17793	FV7	FV3	FV9	FV8
Farooqabad	2	0.9966	3.7499	-0.73783	-0.07846	FV8	FV9	FV6	FV7
Gujranwala	3	-0.1893	0.0287	0.28991	-0.51480	FV1	FV5	FV8	FV6
Kala Shah Kaku	4	-0.2394	0.0241	0.18796	0.02120	FV7	FV1	FV8	FV3
Shorkot	5	-0.3164	0.0249	0.19019	0.39414	FV7	FV3	FV9	FV4

Table 4. Genotypes means and scores for proteins content, amylose content and moisture.

Genotype	NG	Protein Content			Amylose Content			Moisture Content		
		Gm	IPCAg[1]	IPCAg[2]	Gm	IPCAg[1]	IPCAg[2]	Gm	IPCAg [1]	IPCAg [2]
FV1	1	0.7611	-0.42362	-0.98562	0.0734	0.12467	-0.19334	0.0549	-0.16100	0.64036
FV2	2	0.5058	0.33768	-0.64551	-0.3271	0.11957	0.14119	-1.1603	0.32778	-0.00470
FV3	3	-0.2600	-0.45738	0.62233	0.0690	-0.06908	0.29623	0.1688	-0.31622	0.07503
FV4	4	0.2080	-0.81184	0.49833	-0.0829	0.37584	0.18890	-0.2489	-0.39415	-0.68147
FV5	5	-0.3876	0.75400	0.18206	-0.1018	0.46073	-0.24477	0.0549	-1.03944	0.11592
FV6	6	-0.6429	0.63324	0.51417	-0.0156	-0.25733	-0.37567	-0.0211	0.73201	-0.07951
FV7	7	-0.5153	0.21064	0.51631	0.1502	-0.05074	0.14228	0.7004	0.30879	-0.54460
FV8	8	-0.0898	0.54213	-0.47701	0.1330	-0.33957	-0.14410	0.2447	0.17283	-0.15103
FV9	9	0.4207	-0.78485	-0.22506	0.1018	-0.36410	0.18929	0.2067	0.36939	0.63000

Table 5. Environment means and variances and AMMI ranking for protein contents.

Environment	NE	Em	Variances	IPCAe[1]	IPCAe[2]	1	2	3	4
Faisalabad	1	0.5247	0.3945	-0.50365	0.84972	FV4	FV9	FV3	FV1
Farooqabad	2	-0.7044	1.4911	0.01375	-1.39726	FV1	FV2	FV9	FV8
Gujranwala	3	0.3356	0.4943	1.31372	0.24044	FV2	FV5	FV8	FV6
Kala Shah Kaku	4	0.2884	0.3249	0.20714	0.36176	FV2	FV1	FV4	FV9
Shoorkot	5	-0.4444	1.2393	-1.03097	-0.05466	FV1	FV9	FV4	FV2

Table 6. Environment means, variance and AMMI ranking for moisture contents.

Environment	NE	Em	Variance	IPCAe[1]	IPCAe[2]	1	2	3	4
Faisalabad	1	0.9536	0.3910	-0.65675	0.28970	FV5	FV3	FV1	FV7
Farooqabad	2	0.0253	0.5972	0.67430	0.73315	FV9	FV7	FV1	FV6
Gujranwala	3	0.3840	0.2773	-0.28332	0.31474	FV7	FV5	FV1	FV9
Kala Shah Kaku	4	-1.2194	0.5861	-0.66243	-0.64542	FV7	FV5	FV4	FV3
Shoorkot	5	-0.1435	0.6388	0.92820	-0.69217	FV7	FV6	FV8	FV9

different factors, e.g. fertilization and soil salinity or alkalinity (Fasahat et al*.,* 2012; Eggum and Juliano, 1975; Juliano, 1985), and short growth periods. A great quantity of the whole variability in protein content is nonetheless to be accredited to environment (Shobha et al*.,* 2006). As a result, brown rice becomes more unaffected to cracking and breakage during abrasive milling due to the high grain protein than low protein rice of the same variety (Hatfield and Follett, 2008). The impact of different environments on protein and amylose was also studied on different transplanting date (Kaur et al*.,* 2016). Climate fluctuations caused severe deviations in rainfall patterns, with increasing temperatures and critical growing conditions. Rice yield and quality are considerably affected by weather circumstances. Studies carried out on rice established the adverse influence of such (Oteng-Darko et al*.,* 2013).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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