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ASSESSMENT OF PHENOTYPIC AND GENOTYPIC VARIATION IN DIVERSE RICE GENOTYPES USING AGRO-MORPHOLOGICAL AND PHYSIO-CHEMICAL GRAIN QUALITY TRAITS

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Abstract

This study aims to investigate the diversity of agro-morphological, physio-chemical, cooking and textural characteristics in twenty-one rice varieties/ advanced lines with four international reference material named PI 388273, PI 389864, PI 636803 and PI 317519, cultivated at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The findings demonstrated significant variation (p≤05) among the various quantitative and qualitative traits. The results showed highly significant agronomic diversity among the genotypes. The amylose content was ranged between 1.2-30.5% among the genotypes. The highest amylose were recorded in reference material PI.317519 (30.5%) followed by EF52xELD.B (33/4) (29.8%), Noor Basmati (25.3%) and Kisan Basmati (24.2%) which is desirable for diabetic patients due to their low value of glycemic index. Cultivar PS-2 grains were extra-long, slender, scented with high alkali digestion value (6) and high gelatinization temperature and had the highest kernel length 4.5-6.5 mm before and after cooking. DM 1-30-20 grains were short-bold, scented with low alkali digestion value (2) and low gelatinization temperature with 2 chalkiness grade along with very low amylose (5%) content and had the lowest kernel length 1.03-1.23 mm. In cooking analysis all rice genotypes showed water uptake ratio range 4.6-7.3 which indicative of good value of cooking. Principal component analysis exhibited that 94 % variation among quality and yield related traits was contributed by first two PCs. Cultivar CP-1 was observed quite distinct as compared to rest of the genotypes. This finding provides that genotypes which have been ranked high in respective grain quality and nutritive attributes can be employed in future rice cross breeding program to enhance the improvement of the traits of interest.

Key words: Basmati rice; Amylose content; Diabetes; Alkali spreading value; Gelatinization temperature; Glycemic index; Chalkiness

Introduction

Rice (*Oryza sativa* L.) is one of the foremost source of nutrition and staple food of one half of the world's population while about 90% of rice is produced and consumed in Asia (Laborte *et al*., 2017).It is second principal cereal of Pakistan and also a source of foreign exchange earnings (about $2 billion, USD) whereas Pakistan produced 7.4 million tons of rice having 3.1 % share in value-added agriculture and 0.6 % to GDP (Irshad *et al*., 2018).Globally, it is being mostly consumed by more than 2.7 billion people. The consumption of rice in the year of 2019-2020 is around 509 million tones out of which 411.7, 16.4 and 81 million tones are used for food, feed and other purposes, respectively (Lee *et al*., 2015).

Recently, climate change significantly affected rice production and quality around the globe. Joy *et al*., (2017) reported that the climate change causing increase in temperature which ultimately decreases the productivity of rice and its grain quality. Rice grain quality is a complex trait that reflects producers, processors, sellers, and consumers perspectives on the production of grain, processing, marketing, and consumption. As a result, farmers and buyers accept rice with best features because consumer taste is influenced by appearance and grain texture. The physio-chemical properties of rice grain determine its texture and have a significant impact on commercial rice production as well as on market demand (Devi *et al*., 2012).

Nutritional quality is an important criterion by rice consumer's especially diabetic patients. During recent eras, higher consumption of white rice has been involved in increasing the risk of type II diabetes and heart disease (Meera *et al*., 2019). The suitability of carbohydrate-based foods for diabetic subjects has been assessed using glycemic index (GI) studies. Rice with high amylose content is reported to raise blood glucose less than rice with higher amylopectin content. Standard rice starches contain 2-30% of amylose and 70 to 98% of amylopectin that may be effect on gelatinization temperature (Kemashalini *et al*., 2018). A high amylose rice variety slows down the digestion of starch and is often associated with a type of healthy fiber which may promote colon health (Deveraj *et al*., 2020). On the other hand, glutinous rice (high amylopectin) varieties are highly digestible which are not always favorable because it may cause an unhealthy spike

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in blood sugar, especially among the diabetics (Sivakamasundari *et al*., 2020). Bran of brown rice may be a good source of antioxidants, fiber and minerals, which may help prevent heart disease (Samaranayake *et al*., 2017). The milling cooking and eating variation has been attributed to a multitude of physio-chemical factors of rice grain such as amylose content, fiber content, physical size and form (Lahkar *et al*., 2020).Its physicochemical qualities, such as composition, gelatinization, texture, and other factors influence its use in food and non-food applications. It is categorized on the basis of physical property such as size and shape. Golam & Prodhan, (2013) classified rice into two types long grain and short grain. Long rice grains are preferred when the grains are wanted to stay separate during cooking, while short rice grains are used to give a stickier more viscous appearance.

The objective of this research is to assess the agro-morphological, physio-chemical and nutritional status of twenty one diverse rice genotypes including advanced lines, varieties and international reference material (PI 388273, PI 389864, PI 636803, and PI 317519). The proposed study will help to establish the nutritional and commercial value of candidate advance lines under the study. It will help to preempt the future cross breeding combinations with respect to grain quality improvement.

Material and Methods

Plant material and field evaluation of phenological traits: Twenty one rice genotypes named DM-1-30-34-99 x ELD(1), DM-1-30- 34-99 x ELD 4+34/2, Basmati370 mutant 20KR (33/3), EF52 x ELD-B, 201001 x ELD (33/5), EF-1-30-79 x ELD (33/6), ELD x 201001 (33/7), ELD x 201001 (33/8), ELD x 201001-3 (33/9), EF-1-30-79 x ELD (33/10), Kisan Basmati, NIAB Basmati 2016, PS-2 Kainat, Super Basmati, Noor Basmati, DM-1-20- 03-06, CP-1, PI.286178, PI.389864, PI.636803, PI.317519 (Last 4 as international reference material collected from United States Department of Agriculture ;USDA). Which were utilized to analyze the agro-morphological, physio-chemical and cooking quality traits of the rice grain. Nursery bed was sown in NIAB, Faisalabad, Pakistan in rice cropping season during 2020 and 30 days old seedling were transplanted in the field. Field trial was conducted in Randomized Complete Block Design (RCBD) with three replications (Plot size was $15m^2$ with sub-plot size $5m^2$ for each genotype in each replication). Recommended NPK fertilizers were applied to develop a healthy crop with implemented protection procedures. For quantitative analysis, agronomic data were collected at after harvesting i.e. Plant height (P.H), productive tiller/plant (P.T), panicle length (P.L), total spikelet/panicle (T.S), empty spikelet/panicle (E.S), days to 100% flowering, days to maturity, total number of grains/panicle, yields per plant (Y/P), 1000GW weight and panicle fertility. Foreign material and damaged seeds were removed and stored at room temperature for 45 days in cotton bags to attain equal levels of moisture content. De-hulled the grain to have brown rice. The grain size and shape of all rice samples were done by using the method of Ahmed *et al*., (2023) and Danbaba *et al*., (2011). For determination of cooking quality of rice genotypes, differences between before and after cooking were analyzed by given method of Danbaba *et al.*, (2011).

Experimental season and weather: The research was carried out at NIAB, Faisalabad, which is located at longitude 73ºE, latitude 31.43ºN, at an elevation of 184.5m above sea level. The climate of Faisalabad possesses the dry semiarid agro-climatic conditions but a well-organized irrigation system placed it in well productive agricultural zone. Summer monsoon season provides the most rainfall and air temperatures can exceed over 40°C. Faisalabad in 2020, June is the hottest month with a mean of 33.9ºC. The highest amount of the precipitation occurred in July with an average of 119mm followed by August, with an average of 115mm. The maximum humidity was recorded in August(65 %) with the highest sun rays were observed in May and June with an average of 12.3 hours (https://www.weather-atlas.com).

Basic soil properties of rice field: The methods for soil properties were adopted from the Manual of Soil, Plant, and Water Analysis by Devi *et al*., (2012). The experimental soil was loamy and exhibited low average organic matter content (0.65±0.31%), a medium alkaline soil pH (7.93±0.21), total nitrogen (0.07±0.002%), available phosphorus (10.06±0.38), extractable potassium (307±5.25 mg/kg), water soluble potassium (93.3±3.60mg/kg) and water-soluble sodium (283±15.90 mg/kg).

Physio-chemical and cooking quality of rice grain

Grain length (L) and width (W): The width and length in mm of the fully matured rice dehulled grains were calculated using a graphical tool after randomly selecting 10 grains from each genotype (Nadvornikova *et al*., 2018). Each grain was mounted horizontally on the graph sheet's X-axis. In a graph disc, one square grid is equal to 10 mm. afterwards, the length, width and length-width ratio was calculated by using the equations. Categorized rice grains into extra-long (more than 7.5 mm), long (6.6 to 7.5 mm), medium (5.51 to 6.6 mm), and short (less than 5.5 mm).

$$\text{Ratio of length to width} = \frac{\text{Average grain length (L)}}{\text{Average grain width (W)}}$$

Grain size & shape: The fraction of length to width dictated shape of brown rice genotypes. Size and shape of grains (Length to width ratio (L/W) is a highly stable varietal property that is used to measure the stability of different samples. Grain was classified using the IRRI classification system based on the length-width ratio; Slender over 3, Medium in 2-3, Bold in 1-2 and Round > 1.1.

Chalkiness: Chalky grains are rice grains that have a white chalky appearance due to either inadequate or incorrect starch crystallization, which can be detected visually. Chalky grains were isolated and weighed from a sample of twenty grains. In rice grain threshold rating scale of chalkiness is estimated by using the Standard Evaluation System (SES) scale, as small less than 10%, medium 11 to 20%, and large more than 20%. The mass of chalky grains expressed as a percentage in the equation below:

$$\text{Percent of chalky grain} = \frac{\text{Mass of chalky grain}}{\text{Mass of Sample}} \times 100$$

Water uptake ratio (WUR): Optimum cooking time, brown rice samples of 2 g in 20 ml water were cooked in a boiling water bath at 95°C. The leftover water was drained and the cooked rice was transferred to a filter paper for the absorbance of excess moisture. Water uptake ratio (WUR) was calculated by using following equation.

$$\text{WUR} = \frac{\text{Weight of kernel after cooking (W)}}{\text{Weight of kernel before cooking (W)}}$$

Elongation ratio (ER): The elongation ratio was computed by following formula:

$$\text{Elongation ratio} = \frac{\text{Average length before cooking}}{\text{Average length of uncooked rice}}$$

Cooked length-breadth ratio (CLBR): The following equation was used to estimate the length and breadth ratio of cooked rice.

$$\text{CLBR} = \frac{\text{Length of cooked rice (mm)}}{\text{Breadth of cooked rice (mm)}}$$

Gelatinization temperature by alkali spreading value: Lee (2015) approach was used to calculate the alkali spreading value (ASV) using triplicate samples of six (6) seeds of brown rice were soaked in 30ml of 1.7 % KOH and incubated at 30°C for 24 hours. It was calculated the scale of alkali spreading value by means of a scale position i.e. 1-7 (Intact and high= 1-2, Intermediate= 3-5, Low or considerably dispersed=6-7). ASV is related to the temperature of gelatinization (GT) of uncooked starch granules and relates to gelatinization temperature given as 1–2, high (74.5–80°C); 3, high moderate; 5, intermediate (70–74°C); and 6–7, low (less than 70 °C).

Apparent amylose content (AAC): Rapid colorimetric method was used for approximating the amylose content of starches (Juliano *et al*., 1985). In this method iodine combines with amylose to create a blue color that was determined at 620nm wavelength for amylose content estimation. 100 mg of brown rice flour were used to add 1ml of ethanol (95%) with 9ml of NaOH. Incubate the samples in water bath at 80ºC for 10 minutes then cooled each samples and make up volume of 100 ml.

High-amylose rice has a value of >24% amylose, intermediate amylose rice has a value of 20–24%, low-amylose rice has 10–19% value, while waxy rice has less than 5% amylose content.

Statistical analyses

Physio-chemical and cooking features were statistically analyses using Statistics 8.1 software on the basis of Steel and Torrie 1980 to quantify the differences across average value (*p*=0.05) for all Physio-chemical and cooking traits. The tukey test (Tukey, 1945) was used to compare means. PAST (XL-STAT 238) software (version 2014) was used for principal component analysis (PCA), while the corrplot module in R 239 software was used for Pearson's correlation.

Results and Discussion

One way analysis of variance (ANOVA) examined among 21 rice genotypes for 16contributing traits indicated that mean square (MS) differences amongst the genotypes for different traits studied were significant at 5% level of probability (p). There were significant variances among the twenty-one rice genotypes. This variation among genotypes might be due to diverse rice genetic origins and genetic composition. Genetic variations in DNA sequences and environmental factors contribute to biological diversity for agronomic and qualitative parameters in all crops which is most significant in agricultural development programs, because this component is passed down to the next generations (Bibi & Arshad, 2020; Hawkes, 2013).

Morphological and cooking characteristics in rice genotypes: All attributes showed significant variation (*p*=0.05) in 21 rice genotypes that are presented in (Table 1). Plant height was ranged between 84.0 -180.6cm whereas CP-1 was tallest (180.6 cm) and shortest height was recorded in DM-1-20-3-06(84.0 cm). Roy & Shil 2020 also showed variability in plant height and other morphological features among rice genotypes. The most distinct short genotype who did not show resemblance to any other genotypes was DM-1-20-3-6 as shown in (Table1). One of the most important traits used in modern rice breeding is semi dwarfing due to its recessive nature, it produces a shorter culm with superior lodging resistance and a higher harvest index (Ferrero *et al*., 2019). Scientists reported the similar trend for peanuts seeds (Guo *et al*., 2020), wheat (Mondal *et al*., 2020), brassica napus (Fan *et al*., 2021) and banana (Shao *et al*., 2020). Highest productive tillers (27.3) were observed in EF-1-30-79 x ELD (34+33/10) and lowest (6.00 cm) was in PI.286178. Maximum was found in PI.317519 (38.1 cm) and minimum (28.6 cm) in DM 1-20-3-06. The highest total number of spikelets were found in cultivar CP-1 (297.6) and lowest in PI.636803 (79). Maximum empty spikelets (35.3) were recorded in DM-1-30-34-99 x ELD (33/1) and lowest (6.0) in EF-1-30-79 x ELD (34+33/10). Variations in yield characteristic might be due to different genetic makeup of yield related attributes of rice genotypes and response towards the environment throughout grain filling stage. Yield might be increased by selecting genotypes based on yield related attributes. Mean results showed variance in grain production across the rice genotypes, which might be attributed to differences in variety responsiveness to environmental circumstances or genotype function ELDx201001 exhibited the highest (33/7) yield per plant and DM-1-20-03-06 lowest (12.0g).The large variances across genotypes suggest that the expression of the phenotypic traits is influenced by the environment (Ahmed *et al*., 2023). The highest value was noted in PI.317519 (36.27g), and the lowest in DM-1-20-03-06 (10.6 g). Additionally, phenotypic and genotypic relationship of days to flowering 100% with days to maturity were shown to be considerably positive. Days to 100% flowering ranged 82-131 days with maximum in DM-1-30-34-99 x ELD 33/1 (125 days) and minimum in PS-2 Kainat. Early maturing attribute give a short life span to plant and it is a good sign for rice plant to avoid high temperature. Crop varieties that mature early can be used in areas with short rain-fall period and given the rapid pace of growth (Sarif *et al*., 2020). Highest panicle fertility per plant (95.2%) was found in Super Basmati and minimum was observed in PI.636803 (66%). Cheabu *et al*., (2019) reported that temperatures stress influenced on the panicle fertility. Spikelet fertility can be used to screen the germplasm in rice at the reproductive stages (Tenorio *et al*., 2013; Moungngam, 2016; Prasanth *et al*., 2016; Sukkeoa *et al*., 2017).

Table 1. Mean performance of agro-morphological, physio-chemical and cooking attributes among diverse rice genotypes.

Genotype	P.H	P.T	P.L	T.S	E.S	Y/P	1000 GW	FD 100	MD	Fertility	Before L/W	After L/W	WUR	ER	CLBR	Amylose
Kisan Basmati	106.3^{K}	17.0^{CDE}	31.3^{CDEFG}	79.6^{F}	9.6^{A}	27.0^{ABC}	26.6^{CD}	90^{K}	120^{J}	87.7^{AB}	4.43^{BC}	3.78^{BE}	4.87^{GH}	1.27^{CDEF}	3.43^{DE}	24.2^{D}
NIAB Basmati 2016	115.6^{J}	18.0^{BCDE}	30.6^{EFG}	154.3^{BCDEF}	16.0^{A}	25.6^{CD}	20.3^{HI}	90^{K}	119^{J}	89.6^{A}	4.10^{B}	4.367^{C}	5.10^{G}	1.44^{ABC}	4.38^{C}	17.6^{E}
PS-2 (Kainat)	121.6^{GHI}	17.0^{CDE}	30.3^{FG}	85.3^{F}	8.3^{A}	29.0^{ABC}	27.4^{C}	82^{L}	112^{K}	90.7^{A}	4.53^{AB}	6.56^{A}	7.00^{AB}	1.58^{A}	6.60^{A}	4.6^{LM}
Super Basmati	130.0^{PF}	17.6^{DCDE}	30.8^{DEFG}	136.3^{BCDEF}	7.0^{A}	27.3^{ABC}	20.4^{GHI}	121^{C}	152^{C}	95.1^{A}	4.20^{CD}	5.26^{D}	6.13^{CD}	1.55^{AD}	5.26^{B}	23.4^{DE}
Noor Basmati	119.3^{HIJ}	12.0^{EFGHI}	30.0^{FG}	182.6^{BCD}	21.0^{A}	27.3^{ABC}	25.2^{DEF}	111^{GH}	141^{FG}	88.7^{AB}	4.76^{A}	5.21^{B}	5.23^{FG}	1.30^{CDE}	5.21^{B}	25.3^{C}
DM-1-20-03-06	84.00^{L}	12.6^{EFGHI}	28.6^{G}	200.3^{BC}	18.3^{A}	12.6^{E}	10.8^{J}	118^{D}	147^{D}	90.7^{A}	1.03^{L}	1.23^{I}	5.80^{DE}	1.40^{BC}	1.23^{H}	5.0^{JK}
DM-1-30.34-99xELD (33/1)	139.3^{BC}	14.3^{DEFG}	35.3^{ABCD}	207^{B}	35.3^{A}	26.0^{DCD}	24.6^{TF}	125^{BC}	154^{BC}	85.6^{AB}	3.76^{EFG}	3.03^{G}	7.33^{A}	1.14^{EF}	3.03^{EF}	6.4^{I}
DM-1-30-34-99xELD (34+33/2)	123.0^{GH}	22.6^{ABC}	31.1^{CDEFG}	132.3^{BCDEF}	15.6^{A}	26.3^{DCD}	21.1^{GHI}	126^{B}	155^{BC}	88.1^{AB}	3.76^{EFG}	3.11^{G}	6.30^{C}	1.14^{EF}	3.10^{EF}	10.5^{G}
Basmati 370(Mutant) (33/3)	128.3^{EF}	17.6^{DCDE}	30.9^{DEFG}	129^{BBCDEF}	12.3^{A}	30.3^{ABC}	19.8^{HI}	97^{J}	127^{I}	90.3^{A}	3.73^{EFG}	3.03^{G}	5.67^{EF}	1.16^{EF}	3.03^{EF}	7.2^{H}
EF 52x ELD-B (33/4)	117.3^{IJ}	22.3^{ABC}	31.6^{CDEFG}	111.6^{DEF}	9.00^{A}	29.6^{ABC}	20.7^{GHI}	108^{HI}	138^{GH}	92.2^{A}	4.20^{CD}	3.64^{EF}	5.10^{G}	1.12^{EF}	3.64^{D}	29.8^{B}
201001xELD (33/5)	126.0^{FG}	23.6^{AB}	32.0^{CDEFG}	84.3^{F}	13.3^{A}	29.3^{ABC}	25.2^{DEF}	114^{EFG}	144^{EF}	84.7^{AB}	3.37^{HI}	2.84^{GH}	5.20^{FG}	1.19^{BEF}	2.84^{FG}	7.1^{H}
EF-1-30-79xELD (33/6)	126.0^{FG}	20.6^{BCD}	31.9^{CDEFG}	113^{DDEF}	14.0^{A}	30.3^{ABC}	22.1^{G}	107^{I}	137^{H}	87.2^{AB}	4.00^{DEF}	3.29^{EFG}	6.80^{B}	1.18^{BEF}	3.29^{DEF}	10.4^{G}
ELDx201001(33/7)	133.0^{DE}	23.0^{ABC}	32.6^{BCDEFG}	147.6^{BCDEF}	26.3^{A}	31.3^{A}	20.6^{GHI}	126^{B}	156^{B}	82.4^{AB}	3.66^{GH}	2.98^{GH}	6.30^{C}	1.153^{EF}	2.97^{EFG}	5.7^{J}
ELDx201001(33/8)	136.0^{CD}	19.6^{BCD}	34.3^{ABCDEF}	150^{BBCDEF}	18.3^{A}	30.6^{AB}	19.4^{I}	113^{FG}	143^{EF}	87.4^{AB}	4.04^{DE}	3.16^{FG}	6.83^{B}	1.12^{EF}	3.16^{DEF}	5.4^{JK}
ELDx201001-3(33/9)	140.3^{BC}	20.6^{BCD}	35.6^{ABC}	178.6^{BCD}	13.3^{A}	29.3^{ABC}	19.8^{I}	131^{A}	161^{A}	92.6^{A}	2.6733^{K}	3.24^{EFG}	6.80^{B}	1.26^{CDEF}	3.24^{DEF}	4.1^{MN}
EF-1-30-79xELD (34+33/10)	109.00^{K}	27.3^{A}	30.4^{FG}	99^{EF}	6.0^{A}	29.0^{ABC}	21.53^{GH}	107^{I}	137^{H}	93.9^{A}	4.17^{CD}	4.18^{CD}	6.23^{CD}	1.10^{F}	4.18^{C}	3.9^{MN}
CP-1	180.6^{A}	8.3^{GHI}	34.3^{ABCDE}	297.6^{A}	33.3^{A}	31.6^{A}	23.9^{F}	112^{FG}	142^{F}	88.8^{AB}	3.33^{IJ}	2.83^{GH}	5.26^{FG}	1.36^{CD}	2.82^{FG}	6.31
PI286178	120.4^{HIJ}	6.0^{I}	36.6^{A}	120.3^{CDEF}	20.3^{A}	12.6^{E}	25.7^{CDE}	85^{L}	115^{K}	82.9^{AB}	3.31^{IJ}	2.52^{H}	4.90^{GH}	1.17^{EF}	2.51^{G}	12.7^{F}
PI389864	135.3^{CD}	9.3^{FGHI}	35.0^{ABCDE}	96.3^{EF}	7.0^{A}	14.0^{E}	33.0^{B}	117DE	146DE	93.2^{AB}	3.70^{FGH}	3.16^{FG}	5.20^{FG}	1.18^{BEF}	3.16^{DEF}	1.2^{O}
PI636803	121.0^{DHI}	6.3^{HI}	30.7^{EFG}	79^{F}	26.3^{A}	12.0^{E}	33.2^{B}	73^{M}	103^{L}	66.7^{B}	3.03^{J}	3.13^{G}	5.26^{FG}	1.16^{EF}	3.13^{DEF}	23.8^{D}
PI317519	143.0^{B}	15.6^{DE}	38.1^{AB}	92^{F}	19.6^{A}	22.0^{D}	36.2^{A}	115^{DEF}	145DE	78.8A^{B}	4.26^{CD}	5.43^{B}	4.60^{H}	1.39^{BC}	5.43^{B}	30.5^{A}
Grand mean	126.46	16.762	32.513	136.98	16.69	25.41	23.724	108.11	138.0	87.55	3.719	3.615	5.806	1.257	3.605	11.735
CV	1.31	12.76	4.48	19.63	7.66	6.31	2.31	1.02	0.78	8.35	2.86	4.38	2.6	4.77	5.5	2.52
Tukey test	5.144	6.637	4.526	83.483	37.15	4.986	1.704	3.43	3.36	22.68	0.133	0.212	0.4693	0.1862	0.5038	0.4867

PH= Plant height; PT= Productive tillers; PL= Penicle length; TS= Total number of spikelets; ES=; YP = Yield/plant; FD= Days to flowering; MD= Days to maturity; L/W= Length to width ratio; WUR= Water uptake ratio; ER= Elongation ratio; CLBR= Cooked length/breath ratio

It was observed that all the pure basmati rice genotypes had the longest cooking time (Malabadi *et al*., 2022). The dimensions and forms as long/ short rice grains were precise and findings are presented in (Table 2). Noor Basmati variety of Pakistan had the longest de-hulled grain length (9.4 mm) followed by PI. 317519 (9.1 mm). DM-1-30-20 had shortest (2.9 mm) length of rice genotype in Pakistan. According to Rice Knowledge Bank, IRRI Phillipine give the standard for long type had an average length of 6.7 to 7.0mm, whereas short type had an average length of 5.2 to 5.4mm. The results are reported ranges of 6.6-7.7 mm for long type and 5.5mm for short type. The highest L/W ratio (4.76) was exhibited in Noor Basmati while minimum (1.02) was in DM-1-20-3-6. After cooking (L/W) ratio was highest in PS2 (6.56 cm) and minimum in DM-1-20-3-06 (1.23cm). Fang *et al*., (2016) reported that grain form depending on length/ width ratio was classed as slim (3.00) or bold (1.01-2.00). Water uptake ratio (WUR) varied from 4.60-7.33. The highest water absorption was observed in DM-1-30-34-99xELD (34+33/2), while the lowest was recorded in accession PI.317519. Devi *et al*., (2012) evaluated twenty-three milled rice varieties for physicochemical, cooking and textural properties. Results revealed that water uptake ratio and elongation ratio were varied from 2.37-4.45 and 1.29-1.74 mm respectively. Fang *et al*., (2016) reported that endosperm, cytoplasm and maternal genes all work together to determine grain form. Highest value of rice elongation ratio (E.R) was observed in genotype PS2 (1.58 cm) and minimum was observed in EF-1-30-79xELD (34+33/10) (1.10 cm). Elongation ratio is one of the significant traits of basmati rice genotypes and highly demanded in the consumer market. Fang *et al*., (2016) evaluated the cooking quality of fifteen traditional aromatic basmati rice varieties and found elongation ratio between the ranges of 1.25 to 1.91 which is coincide with our results. Kumari *et al*., (2013) showed the highest elongation ratio in samba mahsuri (1.84), followed by bhava purisannalu (1.78)..The cooked length bridth ratio (CLBR) ranged between 1.2 to 6.60 cm with CV was 5.50%. Among all genotypes CLBR was observed highest in Kaynat (6.60 cm) and DM-1-20-3-06 was noted lowest(1.23 cm) ratio (Kumari *et al*., 2013).Genetic diversity for agronomic and qualitative parameters in practically all crops is significant that parents identified based on divergence would be more promising for rice breeding program (Bibi *et al*., 2020). Study of genetic divergence across plant materials is critical tool for plant breeders in selection of parents to use for plant improvement. In the early 1970s, public authorities recognized need to gather, manage, and protect genetic resources, with particular focus on vital food crops such as wheat rice and barley (Bibi *et al*., 2012; Hawkes, 2013). Different traits revealed substantial coefficients of variation at the phenotypic basis and variability in agro-morphological traits were also reported in many previous studies (Mamun *et al*., 2022).

Multivariate analysis of rice genotypes: Principal component analysis (PCA) biplot was used to summarize the data in this investigation of 21 diverse rice genotypes and 16 attributes. The Eigen values which were found to measure the heterogeneity kept by PCA exhibited 0.26-100 % of genetic variation among the quality of grain yield related attributes. From the PCA, first seven principal component with Eigen value >1 were selected and these components accounted for 94% of cumulative variance and remaining were excluded and or less significant. The cumulative indicated that all the attributes had been described to an acceptable level as communalities ranged from 0.971-0.998. The PCA biplot revealed substantial genetic variability among the studied genotypes, with PC1 (28.2%) and PC2 (24.0%) together explaining 52.2% of the total variation (Fig. 1B). Grain yield–related traits, particularly 1000-grain weight, yield per plant, and water uptake ratio, were major contributors to variability and showed positive associations (Fig. 1C).

Bi-plot of genotypes and variables in PC1 and PC2 in (Fig. 1A) showed that majority of traits closely found in reference lines and in Basmati varieties. The 1st two principle components account for about 52.2 % variations from total variation which were plotted on PC1 on X-axis & PC2 plotted on Y-axis to show the relationship of rice genotypes between diverse clusters. Positive components on both axes (B) comprising of high yielding rice genotypes such as PI636803, PI317519, Kisan Basmati, NIAB Basmati 2016, and PS-2 (Kainat). Genotypes such as were positioned close to these vectors, indicating superior performance for yield and physiological traits. In contrast, some genotypes located opposite to these traits may possess comparatively lower yield potential, reflecting trait trade-offs. Overall, the PCA effectively differentiated high-performing genotypes and highlighted key traits contributing to genetic diversity.

A small number of genotypes from component A and C could be employed in a crossover breeding program to improve desirable traits. The accessible genetic diversity also serves as a hedge against uncertain future demands and situations, contributing to the local, national, and global stability of farming systems (Azuka *et al.*, 2022). Study of genetic divergence across plant materials is a critical tool for plant breeders in making informed decisions about which parents to use for plant improvement. Parents with wide genetic range are more likely to produce good segregants and/or high heterotic crosses (Roy & Shil *et al.*, 2020). Hawkes *et al*., (2013) said that parents identified based on divergence would be more promising for rice breeding program.

Pearson correlation: Phenotypic correlation analysis was used to assess trait relationship and the results are shown in Table 3. In general, the results revealed a high order of association between the majority of the qualities studied plant height was highly and significantly correlated with all agronomical traits except productive tiller (-0.18), amylose (-0.17) and fertility (-0.08). Productive tillers reveal negative correlation with panicle length (-0.25), total number of spikelet (-0.26), empty spikelet (-0.45), 1000GW (-0.40), Elongation ratio (-0.20) and amylose (-0.15). Yield/ plant was observed the positive correlation with days to flowering (0.30), maturity (0.31), fertility (0.40), length /width ratio before cooking (0.50), length /width ratio after cooking (0.30), WUR (0.39). CLBR (0.30) accounted negative correlation with 1000GW (-0.30), ER (-0.01), amylose (-0.13) and ASV (-0.02). Fertility was found negatively correlated with amylose (-0.44) and ASV (-0.10). Elongation Ratio showed negative and non-significant relation with amylose (-0.01). Cooked length and bridth ratio determined was highly and significantly correlated with amylose (0.26) and with ASV (0.22).

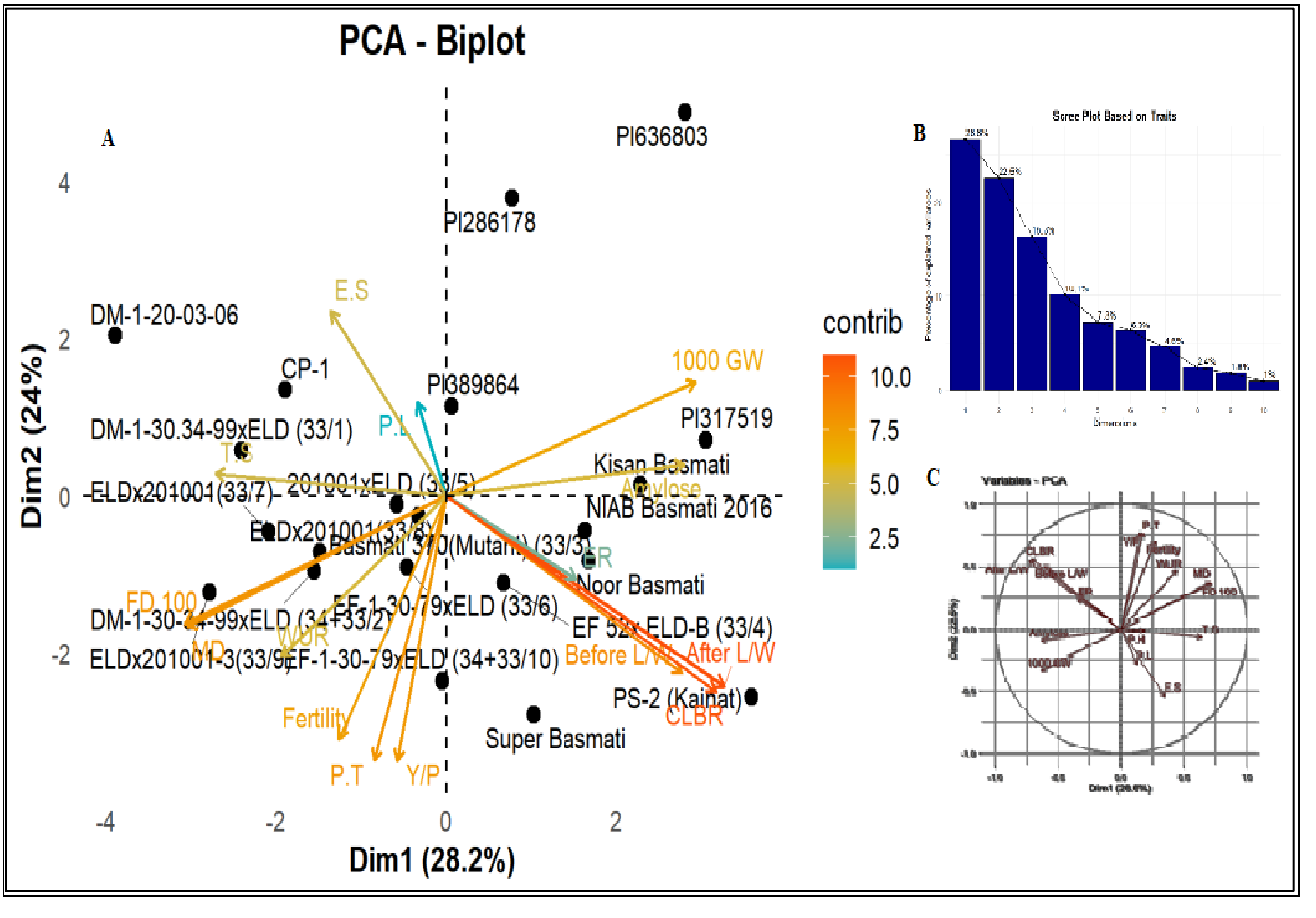

Fig. 1A & B. Principal component analysis distribution of the 21 rice genotypes by using agro-morphological, physio-chemical and cooking attributes.

Table 2. Physio-chemical seed profiling of twenty one rice genotypes.

Genotypes	Amylose %	Alkali spreading value (ASV)	Gelatinization temperature (G.T)	Chalkiness grade	Size classification length (mm)	Shape classification
Kisan Basmati	24.2	6	High	1	Extra long	slender
NIAB Basmati	17.6	4	Intermediate	1	Long	slender
PS-2 (Kainat)	4.6	6	High	1	Extra long	slender
Super Basmati	23.4	2	Low	1	Long	slender
Noor Basmati	25.3	3	High Intermediate	1	Extra long	slender
DM-1-30-20	5.0	2	Low	2	Short	Bold/Round
DM-1- 30.34xELD(33/1)	6.4	2	Low	1	Extra long	slender
DM-1-30-.34xELD(34+33/2)	10.5	3	High Intermediate	2	Extra long	slender
Basmati Mutant370(33/3)	7.2	2	Low	3	Extra long	slender
EF 52xELD.B(33/4)	29.8	6	High	1	Extra long	slender
201001xELD(33/5)	7.1	6	High	3	Medium	slender
EF-1-30-79xELD(33/6)	10.4	3	High Intermediate	2	Extra long	slender
ELDx201001(33/7)	5.7	4	Intermediate	1	Long	slender
ELDx201001(33/8)	5.4	4	Intermediate	1	Extra long	slender
ELDx201001-3(33/9)	4.1	3	High Intermediate	2	Extra Long	Medium
EF-1-30-79xELD(34+33/10)	3.9	2	Low	2	Extra long	slender
CP-1	6.3	2	Low	1	Medium	slender
PI.286178	12.7	3	High Intermediate	3	Medium	Medium
PI.389864	1.2	6	High	3	Extra long	slender
PI.636803	23.8	4	Intermediate	2	Extra long	Medium
PI.317519	30.5	4	Intermediate	1	Extra long	Slender

Table 3.Pearson correlation of agro-morphological, physio-chemical and cooking attributes among diverse rice genotypes.

Attributes	P.H	P.T	P.L	T.S	E.S	Y/P	1000 GW	FD 100	MD	Fertility	Before L/W	After L/W	WUR	ER	CLBR
P.T	-0.183														
P.L	0.626	-0.248													
T.S	0.449	-0.257	0.101												
E.S	0.451	-0.450	0.320	0.627											
Y/P	0.357	0.690	-0.104	0.186	-0.091										
1000 GW	0.333	-0.401	0.464	-0.442	0.080	-0.295									
FD 100	0.284	0.380	0.253	0.426	0.106	0.304	-0.334								
MD	0.291	0.385	0.254	0.421	0.099	0.316	-0.332	0.999							
Fertility	-0.079	0.385	-0.206	0.231	-0.555	0.398	-0.526	0.393	0.393						
Before L/W	0.193	0.260	0.038	-0.341	-0.251	0.498	0.410	-0.186	-0.176	0.117					
After L/W	0.075	0.149	-0.071	-0.317	-0.339	0.307	0.406	-0.217	-0.205	0.135	0.756				
WUR	0.077	0.385	-0.073	0.189	0.059	0.393	-0.391	0.354	0.354	0.254	-0.030	0.020			
ER	-0.003	-0.200	-0.194	0.145	-0.143	-0.012	-0.001	-0.134	-0.128	0.228	0.039	0.561	-0.060		
CLBR	0.088	0.148	-0.066	-0.302	-0.328	0.303	0.397	-0.203	-0.191	0.135	0.741	0.998	0.039	0.562	
Amylose	-0.167	-0.153	0.015	-0.271	0.073	-0.128	0.400	-0.363	-0.361	-0.440	0.335	0.285	-0.619	-0.007	0.263

PH= Plant height; PT= Productive tillers; PL= Penicle length; TS= Total number of spikelets; ES=; YP= Yield/plant; FD= Days to flowering; MD= Days to maturity; L/W= Length to width ratio; WUR= Water uptake ratio; ER= Elongation ratio; CLBR= Cooked length/breath ratio

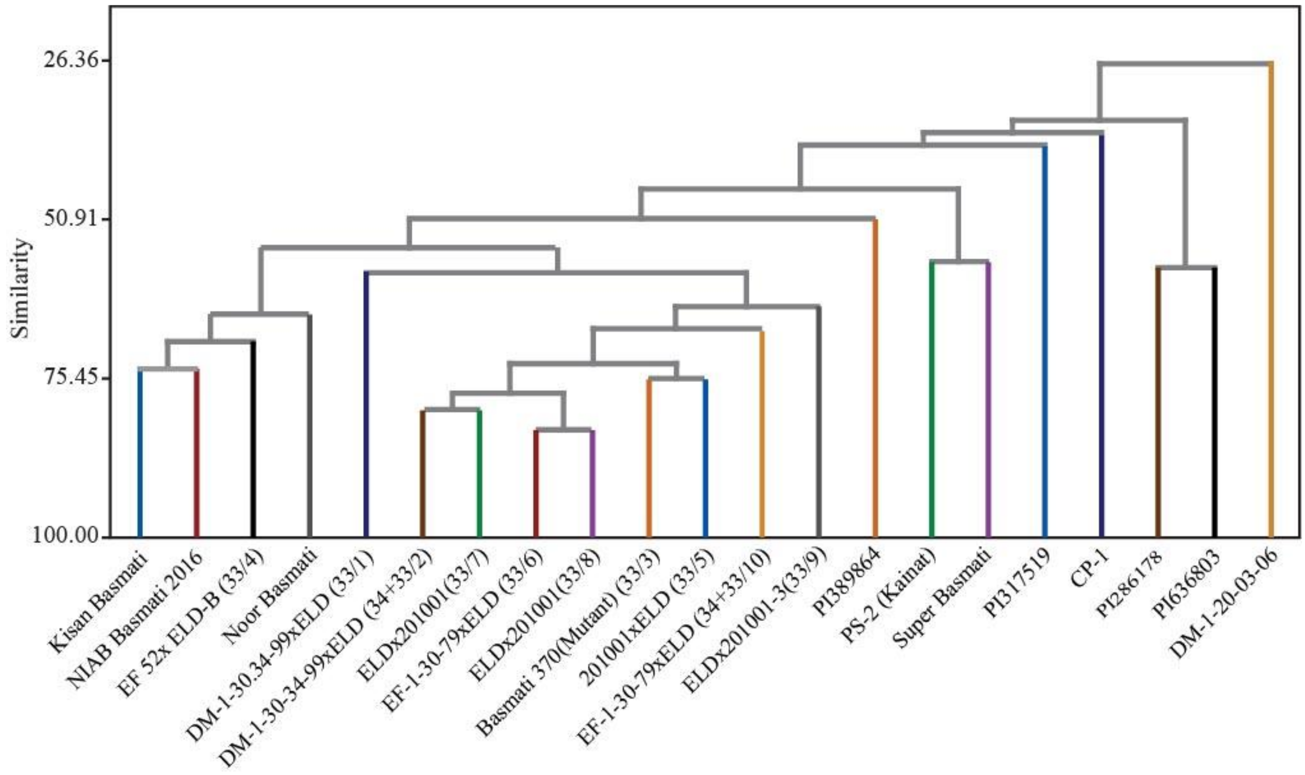

Fig. 2. Dendrogram of diverse rice genotypes based on agro-morphological, physio-chemical and cooking attributes constructed by means of paired group algorithm and Euclidean distance.

Hierarchical cluster analysis: Distance matrix was used to generate the dendogram showing similarity, distance among all the varieties as shown in Figure 2 (Bibi *et al*., 2012). In this matrix, the distance coefficients ranged from 1.7to 7.6 with similarity level varied from 26.4 to 83.2 %. Sixteen agronomical and cooking attributes clustered twenty-one diverse rice genotypes into six clusters and four groups as shown in (Fig. 2). It was observed that cluster one (group one) contained kisan basmati, NIAB basmati-2016 and EF-52xELD-B (33/4) and Noor basmati. Clusters II comprised of two genotypesDM-1-30-34-99xELD (34+33/2) and ELDx201001 (33/7). Cluster III again consist of two genotypes EF-1-30-79xELD (33/6) and ELDx201001 (33/8). Cluster IV exhibited four genotypes named Basmati-370 (mutant) (33/3), 201001 x ELD (33/5), EF-1-30-79xELD (34+33/10) and ELDx201001-3(33/9). Clusters II, III and IV comprised of group two along with two distinct genotypes as DM-1030-34-99xELD (33/7) and PI.389864. Group three formed by cluster V and distinct genotypes i.e. PI.317519 and CP-1. DM-1-20-03-06 is highly distinct among rest of the genotypes and included in group four with cluster VI. Khare *et al*., 2014 found the similar results that traditional Basmati varieties were closely related and were found in the same group. Among the several rice species/varieties, Basmati has a stronger aroma than the traditionally farmed scented rice variants and is commonly farmed throughout Asia (Lahkar *et al*., 2020; Oladosu *et al*., 2014).

Physio-chemical seed profiling of rice genotypes: Physio-chemical properties of the rice grain among all selected genotypes were examined and also presented in Table 2. Rice genotypes were divided into two categories high and intermediate on the basis of alkali spreading value (ASV) and gelatinization temperature (GT) which depends on the coarseness of the grain. NIAB Basmati-2016, Noor Basmati, ELD x 201001(33/7), ELD x 201001(33/8), PI.636803 and PI.317519 showed intermediate gelatinization temperature and same varieties presented in grade 4 for alkali spreading value which is not suitable for cooking. Kemashalini *et al*., (2018) reported that during the process of gelatinization temperature, intermolecular deposition of starch particles exposed to water and heat, it decomposes. Lower alkali spreading value and gelatinization temperature were observed in Super Basmati, DM-1-30-20, DM-1-30-34 x ELD (33/1), Basmati Mutant 370 (33/3), EF-1-30-79 x ELD (34+33/10) and CP-1which are suitable for cooking. High alkali spreading value and gelatinization temperature were contained in Kisan Basmati, Kainat (PS-2), EF 52 x ELD. B (33/4), 201001 x ELD (33/5) and PI.389864. Intermediate gelatinization temperature and grade 4 of alkali spreading value were recorded in NIAB Basmati, ELDx201001 (33/7), ELDx201001 (33/8), PI. 636803 and PI.317519. High intermediate gelatinization temperature and grade 3 alkali spreading value was recorded in Noor Basmati, DM-130.34 x ELD (34+33/2), EF-1-30-79 x ELD (33/6), ELD x 201001-3 (33/9) and PI. 286178. Long grain, with an intermediate to high alkali spreading once heated, value tends to have a solid firmness, whereas short and medium grain rice has a low alkali spreading value as resulting in softer and stickier cooked rice (Kemashalini *et al*., 2018; Tuano *et al*., 2021).

Starch is ready up of the linear construction of amylose and the chain assembly of amylopectin. Many scientists assessed the amylose and amylopectin are crucial factors in determining rice grain quality (Haluszka *et al*., 2022; Jenkins *et al*., 2021; Kemashalini *et al*., 2018; Meera *et al*., 2019; Kaur *et al*., 2016; Tuano *et al*., 2021). These scientists reported the hardness and stickiness of rice after cooking was found to be highly associated with amylose content and the chain structure of amylopectin. Result revealed that the amylose content of twenty-one rice genotypes ranged from 1.2 to 30.5 percent (Table 2). Low amylose content detected in NIAB Basmati 2016(17.6%), DM-1-30-.34 x ELD (34+33/2) (10.5%), EF-1-30-79 x ELD (33/6) (10.4%), PI.388273 (12.7%). The amylose content was under intermediate range in Kisan Basmati (24.2%), Super Basmati (23.4%), and PI.636803 (23.8%). High amylose content was recorded in Noor Basmati (25.3%), EF 52 x ELD.B (33/4) (29.8%) and PI.317519 (30.5%). Most of the advanced lines and traditional varieties showed very low amylose content which were contained in PS-2 (Kainat) (4.6%), DM-1-30-20 (5.0%), DM-1- 30-.34 x ELD (33/1) (6.4%), Basmati Mutant 370KR (33/3) (7.2%), 201001 x ELD(33/5) (7.1%), ELD x201001(33/7) (5.7%), ELD x 201001(33/8) (5.4%), ELD x 201001-3 (33/9) (4.1%), EF-1-30-79 x ELD (34+33/10) (3.9%), CP-1(6.3%). Only a single international standard i.e. accession number PI.389864 comprised of 1.2% amylose denoted as glutinous and waxy rice. Kemashalini *et al*., 2018 reported that the low amylose content rice becomes moist and sticky after cooking. High AC varieties Noor Basmati (25.3%), EF 52 x ELD.B (33/4) (29.8%) and PI.317519 (30.5%) are good selection for the diabetic patients and along with medically view these varieties also have good extra-long appearance along with good chalkiness grade, alkali spreading value and Gelatinization Temperature. Hence, these germplasm have potential for consumer preferences and it might be there used in future breeding programs for the enhancement of valuable grain quality traits.

Conclusion

In conclusion, high amylose containing varieties named Noor Basmati, EF 52 x ELD.B (33/4) and PI. 317519 are the good selection for the diabetic patients as these varieties has high amylose content results in good consumption in diabetes type II. Along with medically view these varieties also have good extra-long appearance along with good chalkiness grade, alkali spreading value ad gelatinization temperature. Cultivar CP-1 and EF-1-30-79xELD (34+33/10) exhibited high alkali spreading value corresponding to low gelatinization temperature having good cooking quality required for consumer preference, while accession number PI.317519 and Super basmati are highly nutritive genotypes Accession number PI. 317519 was observed highest in 1000GW and super basmati found higher in fertility.

This finding provides that genotypes which have been ranked high in respective grain quality and nutritive attributes can be employed in future rice cross breeding program to enhance the improvement of the traits of interest.

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Author's Contribution: Sajida Bibi: Conceptualization, Methodology, Software & Data curation, Investigation, Writing – review & editing. Jawaria Ishfaq: Methodology, Investigation, Data analysis, Writing – original draft. Zia-ul-Qamar: Supervising, Review & editing. Abdul Rusul Awan: Investigation, Review & editing. Irem Waqar: Formal analysis. Sadaf Shamim: Grain quality analysis

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