

Insight the quality parameters of diverse rice germplasm set of Chhattisgarh

Abstract: The present study focuses on the evaluation of quality parameters of 198 rice genotypes including 163 diverse germplasm, 29 mutants and 6 check varieties in augmented RCBD design. In ANOVA treatment ignoring blocks, heterogeneity among block was eliminated and for every new added treatment the mean sum of square for all the traits were found to be significant at 1% level of significance. Traits milling recovery, Iron content and Degree of milling were skewed, while rest were normally distributed. Highest paddy and grain length was found for accession Dokra dori (12.44mm). Highest milling recovery was noted for Luchai mutant (72.66%). Degree of milling found highest for Jeeraphool (93.95%). Apparent amylose content was highest in Ambemohar mutant-1(33.2%). Principal component analysis revealed that PC1 and PC2 explains variation for the trait, length and width of paddy, brown rice and milled rice transgressive segregants can be obtained for these traits by crossing genotype Chiko and Chaptigurmatiya parent, Angur gucha and Gobi buta, Chiko and Visnubhog mutant V-80, Khatia pati and Shri kamal as these were depicted as most diverse genotype.

Key words: Apparent amylose content, Augmented RCBD, Grain quality traits, Milling recovery, Principal components, Rice germplasm.

Introduction: Rice is not only the staple food for the people of south east Asian countries, but is the part of their life time journey. Being used in all the rituals from life to death, it is eaten widely in various forms viz; cooked rice, rice floors, puffed rice, flattened rice, rice crackers and in various flavorful desserts and sweets. Thus rice improvement programs should not only target yield, as the quality is game changers for any high yielding genotype. The occurrence and spread of rice is wide enough to cover all the topologies with evolution and domestication finger prints of rice explaining the reason behind its huge variability and adaptability (Gutaker *et al.* 2020). The germplasm is a treasure of traits which contribute to various aspects of rice improvement programme. The evaluation of rice quality starts from its appearance i.e. length, width, length-width ratio, chalkiness of endosperm, cooking parameters. Later it undergoes apparent amylose content, gelatinization temperature, gel consistency, grain hardness and grain cohesiveness checks (Emon *et.al.* 2020). Therefore in a breeding programme the choice of parents determines its success and the diversity analysis aids in the selection of parents which are used either in recombination breeding or in mutation breeding.

Variability in the quality can be observed in rice eating preferences around the globe. On cooking sticky, soft and tender, short *japonica* rice is predominantly found in north and eastern Asian countries and eaten in form of sushi, steamed rice balls, and sticky rice. While *Indicas* are fluffy and becomes flaky once they are cooled after cooking. People of middle east prefer long slender, flaky aromatic grains with significant length-wise expansion and restricted width wise expansion (Ahmed *et. al.* 2020). While in India the quality preference is soft, flaky, slender grains with intermediate amylose and soft gel consistency. Chhattisgarh state situated in south east part of India and has a huge agro-biodiversity of about 23,250 rice germplasm conserved and maintained at Indira Gandhi Agriculture University, Raipur (C.G) (Sahu *et. al* 2017). The present study emphasizes on the post harvest and major quality contributing factors of rice germplasm and their mutants. The intense diversity analysis performed on the selected core set cleaves the genotypes on the basis of dissimilarity index, into three major clusters which are further validated through various indices. Desirable genotypes will be selected and incorporated in the further breeding program through crossing and mutation breeding related activities.

2. Material and Methods:

Experimental materials:

The first experiment was conducted in *Kharif* 2019 in IGKV experimental fields, with 525 rice accessions (478 rice germplasm, 29 mutants in background of 16 parents and 6 released varieties as check). Experimental material was obtained from the medium term germplasm storage of IGKV, Raipur and Mutants as well as parents were acquired from BRNS-BARC-IGKV tri MoU project. Out of the 525 accessions a set of 198 accessions (163 diverse germplasm, 29 mutants and 6 check varieties) were selected on the basis of 100 seed weight, entries were selected from low, medium and high seed weight categories for the construction of core set. The core set was

evaluated in *Kharif* 2020 at IGKV research fields. The quality parameters of 198 accessions were evaluated in year 2021 at Rice quality laboratory, IGKV Raipur, Chhattisgarh.

Experimental detail:

The experimental design followed aforesaid trials was augmented block design while the experiment was conducted by following standard agronomic practices. The germplasm was planted in paired rows with 15 plants in each row. Plant to plant spacing was 15 cm while row to row was spaced by 20 cm each for both the year. Prior to post harvest & quality analysis all the samples were kept for two months, cleaned properly to ensure no dockage and dried in oven at 35°C for 48 hours to maintain optimum moisture content of 12% in all the samples and they were evaluated in duplicates. The characters under study are classified under three sub heads: Grain Physical Parameters, Grain physio-chemical and cooking parameters.

Observations recorded for Grain quality and nutritional traits:

Observation for paddy length & width, brown rice length width ratio, milled rice length width ratio, hulling percentage, milling recovery, head rice recovery, cooked rice length and width, cooked rice elongation ratio, gelatinization temperature were recorded as per the standard protocol developed by ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad (Ref 2014). While as apparent amylose content was done as per the standard protocol by Juliano. (1971). Elongation index was worked as per the methodology of Juliano et.al. (1984). The observations for Milling degree and Grain Iron Zinc content were as per the standard procedures of International Rice Research Institute, Manila, Philippines (Rice Knowledge Bank, IRRI,).

Statistical Analysis:

The analysis of augmented design was carried out using 'Augmented RCBD' R package (Aravind et al., 2020) in R studio version 4.2.0 (<https://aravind-j.github.io/augmentedRCBD>). Principal component analysis was done by R studio version 4.2.0 using the package FactoMineR (Le et. al., 2008). The percentage contribution of the variables (traits) to the Principal Components (PC) was worked out using the algorithm $(\text{var.cos}^2 * 100) / (\text{total cos}^2 \text{ of the component})$. Where as var.cos^2 represents the quality of representation of variable on factor map, i.e., lying of a particular variable (trait) on a coordinate and its contribution towards the total variance of PC. Cluster analysis was done using the Agglomerative hierarchical clustering also known as AGNES (Agglomerative Nesting), which works on similarity index, it groups the most similar genotypes and make larger groups of similar genotypes i.e. in bottom up manner (Kassambara, A., 2017). The Agglomerative hierarchical clustering was done by using the software R studio version 4.2.0 using the Euclidean distance which works on dissimilarity matrix and groups similar genotypes in a cluster and dissimilar in different clusters. Ideal number of clusters were selected with the help of package Nbclust (Charrad et al. 2014).

Result & Discussion:

I. ANOVA and descriptive statistics of genotypes.

The analysis of variance for the traits depicting the diversity for post-harvest and quality traits were studied on 192 rice genotypes with 6 checks, eliminating one way heterogeneity by taking the ANOVA of block adjusted. The mean sum of squares for all traits were highly significant at 5% and 1% level of significance. In ANOVA treatment ignoring blocks, heterogeneity among block was eliminated and for every new added treatment the mean sum of square for all the traits were found to be significant at 1% level of significance. The highly significant mean sum of squares for the checks ensures that all the controls under the study are significantly different from each other and their traits under study are significant at 5% and 1% level of significance. The MSS for test versus treatments are considered between two different groups, that is treatment group and check group, it is also found highly significant for all the traits under study signifies the difference between test genotypes and controls. The MSS for the test entries leaving checks are also highly significant which reflects that all the treatments are different from each other. The MSS for block eliminating treatments is non-significant for majority of the traits except, Apparent amylose content, Brown rice length, Elongation ratio, Iron-Zinc content of brown rice, Head rice recovery, Paddy length & width (Table 1).

Table 1: ANOVA table for block adjusted.

Source	Df	AA C	BRL	BR LB	BR W	CRL	CR W	DOM	EI	ERL	FE	GT	HRR	MR	MRL	MRLB	MRW	PL	PW	ZN
Treatment (ignoring Blocks)	197	21.45 **	1.11 **	0.31 **	0.08 **	1.69 **	0.1 **	69.34 **	0.04 **	0.06 **	17.55 **	1.49 **	40.9 **	104.54 **	0.7 **	0.25 **	0.07 **	1.82 **	0.13 **	11.91 **
Treatment: Check	5	20.84 **	1.75 **	1.12 **	0.17 **	6.45 **	0.76 **	159.52 **	0.13 **	0.14 **	15.11 **	8.2 **	119.42 **	372.98 **	1.15 **	0.85 **	0.18 **	0.49 **	0.38 **	120.37 **
Treatment: Test vs. Check	1	66.77 **	3.7 **	2.31 **	0.23 **	0.74 **	0.01 *	145.45 **	0.58 **	0.66 **	148.84 **	2.49 **	25.61 **	527.41 **	6.85 **	4.73 **	0.28 **	0.66 **	0.45 **	174.04 **
Treatment: Test	191	21.23 **	1.07 **	0.28 **	0.08 **	1.57 **	0.09 **	66.58 **	0.04 **	0.05 **	16.93 **	1.31 **	38.93 **	95.3 **	0.66 **	0.21 **	0.06 **	1.86 **	0.12 **	8.22 **
Block (eliminating Treatments)	5	1.3 *	0.02 *	0.01 ns	0.00094 ns	0.01 ns	0.0012 ns	0.75 ns	0.002 ns	0.02 *	0.54 *	1.3e-28 ns	4.08 **	0.95 ns	0.01 ns	0.0029 ns	0.01 ns	0.02 *	0.01 **	0.45 *
Residuals	25	0.4	0.01	0.02	0.01	0.0049	0.003	1.87	0.003	0.01	0.19	6.9e-29	0.82	1.35	0.01	0.03	0.01	0.01	9.1e-31	0.14

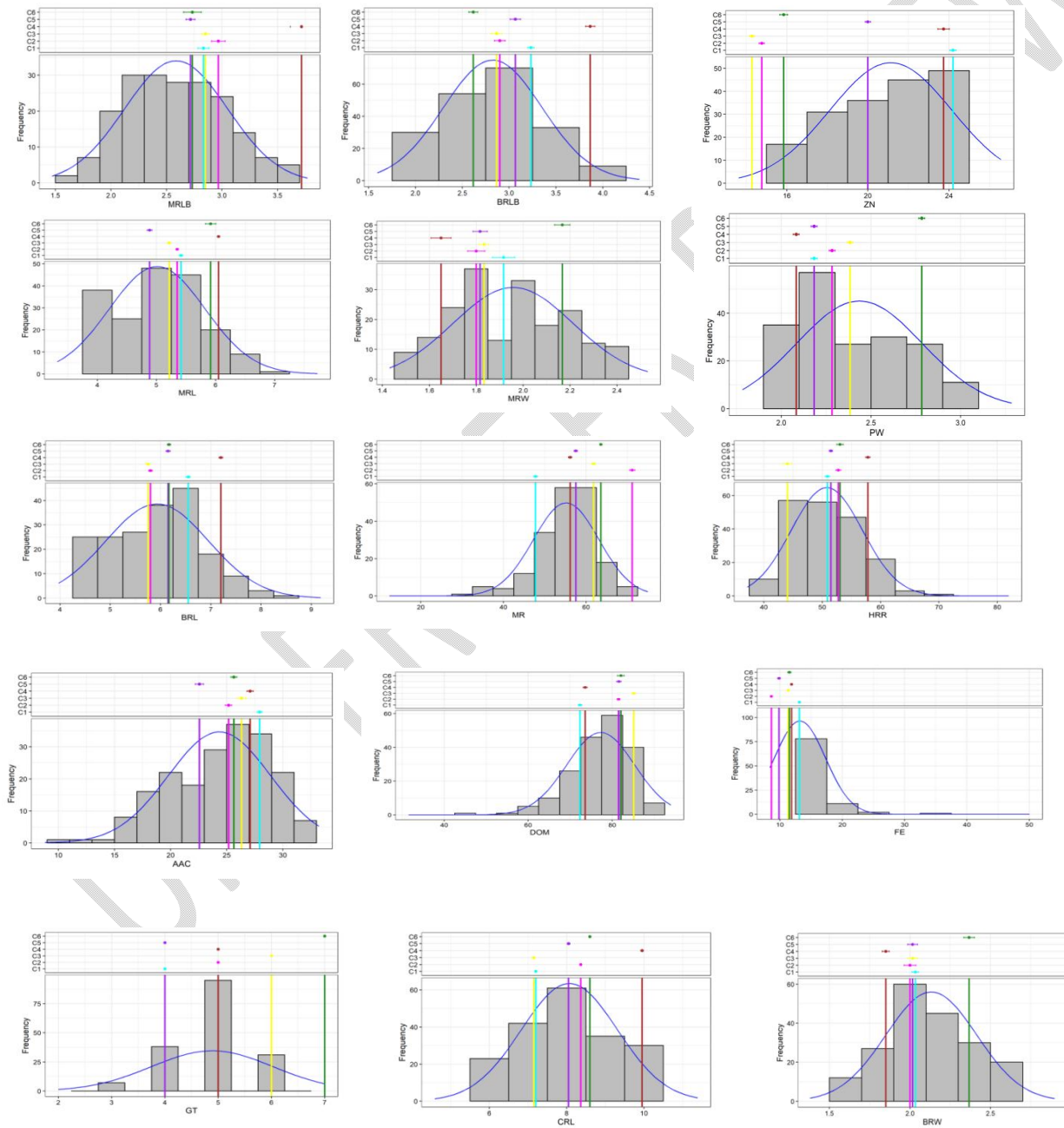
ns P > 0.05; * P <= 0.05; ** P <= 0.01

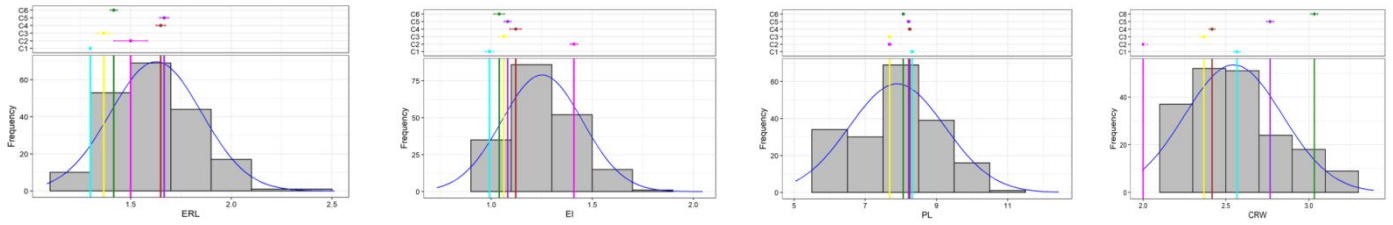
The traits BRW, BRLB, MRL, MRW, MRLB, CRL, CRW and ZN were normally distributed as their skewness is non-significant with value less than 0.5, while traits PL, PW, BRL, CRW, ERL, EI, HRR, AAC and GT were near normally distributed as the skewness values ranging from -1 to +1. The traits MR, DOM and FE are highly skewed, MR and FE are left handed skewed while DOM is right handed skewed (Table 2) and (Fig 1).

Table 2: Descriptive Statistics for the post harvest grain quality and nutritional characters.

Trait	Mean	Min	Max	Std.Error	Std.Deviation	CV%	Skewness	Kurtosis
PL	7.89	5.04	12.44	0.1	1.35	17.11	0.34 *	3.52 ns
PW	2.44	1.78	3.28	0.02	0.35	14.34	0.5 **	2.41 *
BRL	5.93	3.97	9.19	0.07	1.02	17.20	0.36 *	3.31 ns
BRW	2.13	1.38	2.9	0.02	0.28	13.15	0.2 ns	2.74 ns
BRLB	2.82	1.59	4.39	0.04	0.53	18.79	0.2 ns	2.49 ns
MRL	5.01	3.32	7.72	0.06	0.81	16.17	0.21 ns	2.87 ns
MRW	1.96	1.43	2.53	0.02	0.26	13.27	0.16 ns	2.18 **
MRLB	2.59	1.47	3.77	0.03	0.46	17.76	0.24 ns	2.53 ns
CRL	8.07	4.59	11.39	0.09	1.24	15.37	0.12 ns	2.64 ns
CRW	2.55	1.99	3.39	0.02	0.3	11.76	0.47 **	2.71 ns
ERL	1.63	1.08	2.51	0.02	0.23	14.11	0.45 **	3.8 *
EI	1.25	0.73	2.05	0.01	0.2	16.00	0.57 **	4.55 **
MR	55.17	12.44	74.9	0.56	7.93	14.37	-1.18 **	7.18 **
DOM	77.25	31.52	93.95	0.58	8.09	10.47	-1.47 **	8.24 **
HRR	50.79	37.15	81.94	0.43	6.1	12.01	0.91 **	5.62 **
AAC	24.35	8.8	33.2	0.32	4.56	18.73	-0.56 **	3.06 ns
GT	4.89	2	7	0.08	1.14	23.31	-0.5 **	3.71 ns
FE	13.2	8.38	50.05	0.29	4.09	30.98	4.93 **	39.69 **
ZN	21.13	13.62	26.56	0.21	3.01	14.25	-0.3 ns	2.2 **

Figure 1: Histogram representation of grain quality and nutritional characters





The variability in the studied germplasm set and their mutants was very well reflected in Table 3 which shows top five and bottom five genotypes contributing to each character. The range for Paddy length (PL) was from 12.44 mm (Dokra dokri) to 5.04 mm (Vishnubhog fine parent), for Paddy width (PW) it was from 3.28 mm (Bagri) to 1.78 mm (Nagri Dubraj mutant). Brown rice length (BRL) was 9.19 mm (Dokra dokri) to 3.97 mm (Vishnubhog mutant V-80), highest brown rice width was 2.9 mm (Jalpan) to 1.38 mm (Gobi buta). Brown rice length width ratio was 4.39 (Lanji) to 1.59 (Paltu). The range for milled rice length (MRL) varied from 7.72 mm (Chiko) to 3.32 mm (Lokti musu). MRW for milled rice width was 2.53 mm (Beda kabro) and 1.43 (Red jawaphool mutant). Range for MRLB milled rice length breath ratio was 3.77 (Beda kabro) and 1.47 (Angur guchcha). Cooked rice length CRL ranges from 11.39 mm (Chiko) to 4.59 mm (Red jawaphool mutant), accession GP-145-43 has highest cooked rice width (3.39 mm) among all 198 genotypes while Vishnubhog mutant V-80 shows narrowest cooked grain (1.99 mm). ERL is the elongation ratio is length wise expansion of rice grain of cooking it was highest for CG Jawaphool Trombay (2.51), and lowest for Siyar dhan (1.08). Elongation Index is the ratio of total change in length and width of cooked and uncooked rice it ranges from 2.05 (Dongar Goyandi) to 0.73 (Red jawaphool mutant). The range for the milling recovery was 72.66% (Luchai mutant) to 12.44 % (Grassy Dubraj), while the Degree of Milling highest with 93.35% (Jeeraphool) to 31.52% (Grassy Dubraj). Head rice recovery has been highest with 81.94% (Bahal binjo) to 37.15% (Vishnubhog mutant V-80). The range for Apparent amylose content was 33.2% (Ambemohar mutant-1) to Chiko (8.8%). Gelatinization temperature score varied from 7 (Chiko) to 2 (TCDM-1). The Iron content of brown rice ranged from 50.05 PPM (Red jawaphool mutant) to 8.38 PPM (Unknown) while the brown rice Zinc content ranged from 26.56 PPM (Satha dhan) to 13.62 PPM (Jaldubi).

Table 3: Top five genotypes contributing to maximum and minimum values for various grain quality and nutritional traits.

S.No	Trait	Top Five Contributors	Bottom five contributors
1.	PL-Paddy Length (mm)	Dokra dokri(12.44), Dokra mechha (12.24), Chiko (11.97), Khatia pati (11.54), Chiko (10.74).	Samundchini mutant S-48 (5.44), Loktijhool (5.35), Red jawaphool mutant (5.35), Lokti Musi (5.14), Vishnubhog fine parent (5.04)
2.	PW-Paddy Width (mm)	Bagri (3.28),Hardi chudi (3.28), Poni (3.28) Chiko (3.28) , Jalpan (3.18)	Chaptigurmatiya Parent (1.98), Samundchini mutant (1.98) Gobi buta (1.88), Red jawaphool mutant (1.78), Nagri Dubraj Parent (1.78)
3.	BRL-Brown Rice Length (mm)	Dokra dokri (9.19), Dokra mechha (9.19),Chiko (9.14), Khatia pati (8.69)Chiko (8.07).	Samundchini mutant S-48 (4.27), Angur Guchcha (4.17),Lokti Musi (4.17), Vishnubhog fine parent (4.07), Vishnubhog mutant V-80 (3.97)
4.	BRW-Brown Rice Width (mm)	Jalpan (2.9), Paltu (2.9), Kumda phool (2.71), Dongar goyandi(2.7), Kating (2.7).	Jalsinga(1.6), Nagri Dubraj mutant-1 (1.6), Vishnubhog mutant V-80 (1.6), Bhawara 1-I(1.58),Gobi buta (1.38),
5.	BRLB-Brown Rice	Lanji (4.39), Gobi buta (4.06),	Shri kamal (1.94), Angur Guchcha (1.86),

	Length Breath (ratio)	Poornima (3.87),Abhaya (3.86), Shymala (3.86).	Bhaniya (1.86),Gajai (1.86),Paltu (1.59),
6.	MRL-Milled Rice Length (mm).	Chiko (7.72), Dokra mechha (7.26), Khatia pati (6.96), Barangi (6.74), Tedesi (6.56).	Loktijhool (3.64), Samundchini mutant S-48 (3.62), Angur Guchcha (3.54), Goverdhan kali kamod (3.34), Lokti Musi (3.32).
7.	MRW-Milled Rice Width (mm)	Beda kabro(2.53), Kating(2.53) Jodhari (2.43), Banko Dhan (2.43),Gathuwan (2.43)	Chinor parent (1.53), Nagri Dubraj mutant-1 (1.5),Vishnubhog mutant V-80 (1.5), Kanakgopala (1.43), Red jawaphool mutant (1.43)
8.	MRLB: Milled Rice Length Breath (Ratio)	Gobi buta(3.77), Poornima(3.72), Shymala(3.62), Chandrahasahni (3.62),Lanji (3.59).	Bhaniya (1.77), Gajai (1.77), Gathuwan (1.69),Paltu (1.69), Angur Guchcha (1.47).
9.	CRL: Cooked Rice Length (mm)	Chiko (11.39), Shymala (10.99), Maheshwari (10.79), Dongar goyandi(10.79), Poni(10.47).	Samundchini mutant S-48 (5.99), Lokti Musi (5.99), Siyar dhan (5.39), Ambemohar Parent (5.39), Red jawaphool mutant (4.59).
10.	CRW: Cooked Rice Width (mm)	GP-145-43 (3.39), Dokra dokri (3.23),Dagad Deshi(3.19), E-1702 (3.19),Maheshwari(3.19).	Maharaji (1.99), Rongobati (1.99), TRR-4 (1.99), TCDM-1 (1.99), Vishnubhog mutant V-80 (1.99).
11.	ERL: Elongation Ratio Length	CG Jawaphool Trombay (2.51),Dongar goyandi(2.37), Tulsi manjar (2.11), Bisni (2.09),Poni (2.08).	Khatia pati (1.2), Dokra mechha (1.12), Red jawaphool mutant (1.1), Lahagati (1.09), Siyar dhan (1.08).
12.	EI: Elongation Index (Ratio)	Dongar goyandi(2.05), CG Jawaphool Trombay (2.04),Shri kamal (1.74), Bhaniya (1.67), Tulsi manjar(1.66).	Dokra mechha (0.87), Siyar dhan (0.87),Kanakgopala (0.86), Geeta (0.81), Red jawaphool mutant (0.73).
13.	MR: Milling Recovery (%)	Luchai mutant (72.66), Nawabbhog(72.66), Bisni (71.46), Karma Mahsuri (71.17),Dhaiya Phool (69.56)	Samundchini mutant (35.08), Jaldubi (33.89), Sanchuriya Parent (32.74), Kadamphool mutant-2 (30.48), Grassy Dubraj (12.44)
14.	DOM: Degree Of Milling (%)	Jeera phool (93.95), Bisni (93.73), Raja bhog (91.79), Chaptigurmatiya mutant-1 (91.2), Red Jawaphool parent (89.38).	Alsakar Parent (58.04), Nagri Dubraj mutant-1 (57.62), Kadamphool mutant-2 (53.52), Sanchuriya Parent (46.06), Grassy Dubraj (31.52).
15.	HRR: Head Rice Recovery (%)	Bahal binjo (81.94),Dhaniya phool (69.23), Chiko (66.96), Loktijhool (64.14), Indira sugndhit dhan-1 (63.9).	Bagri (41.12), WR99 (40.81), Kanakgopala (40.44), Adanga Dhan (40.21), Vishnubhog mutant V-80 (37.15).
16.	AAC: Apparent Amylose Content (%)	Ambemohar mutant1 (33.2), Barangi (32.3), Kumda phool (32.19),Alsakar Parent (31.79), Samleshwari(31.7).	Red Jawaphool parent (15.89),Jalpan (13.55), Jaldubi (12.4), Kating (10.05), Chiko (8.8).
17.	GT: Gelatinization Temperature (Score)	Chiko (7), Shymala (7), Indira sugndhit dhan-1 (7), Moroberekan (7), IR55419 (7).	WR116 (2),R-RF-75 (2), Kalanamak (2), Layacha (2), TCDM-1 (2),
18.	Fe: Iron content in brown rice (PPM)	Red jawaphool mutant (50.05), Shri kamal (36.23), Pinwari ajan (24.03), Indira Sughandit Dhan-1 (23.58),Alsakar mutant-8 (21.48).	Loktijhool (9.15), Bhataphool (8.98), Indira sugndhit dhan-1 (8.88), Karma Mahsuri (8.62), Unknown (8.38).
19.	Zn: Zinc content	Satha dhan (26.56),Chhatri – 1	Barma tripal (15.36), Karma Mahsuri (14.77), Swarna

in brown rice (PPM)	(26.29), Karhani(26.26), Nagri Dubraj mutant-1 (26.26), Bisni (25.99).	(14.28), Banda (14.06), Jaldubi (13.62).
----------------------------	--	---

I. Principal Component analysis for the quality contributing characters:

The eigenvalues for the corresponding Principal component describe the proportion of variance carried by it. The Principal components having eigen score >1 are considered as main contributors of variance and first five Principal components of the given data set contributes for 74.37% of total variance. The PC1 has the highest eigen value and contributes to maximum percentage of variance i.e 26.39% followed by PC2 (21.62%), PC3 (12.17%), PC4 (7.71%) and PC5(6.46%) (Table 4) .The Scree plot for the eigenvalues of the data set confirms the maximum variance contributing Principal components as per the data set (Figure 2)

Table 4: Principal Components with respective eigen scores, percentage of variance and cumulative percentage of variance

Principal Components	Eigenvalue	Percentage of Variance	Cumulative percentage of Variance
1	5.01	26.39	26.39
2	4.11	21.63	48.02
3	2.31	12.18	60.20
4	1.47	7.71	67.91
5	1.23	6.46	74.37
6	0.99	5.18	79.56
7	0.83	4.35	83.90
8	0.79	4.13	88.04
9	0.70	3.66	91.70
10	0.58	3.05	94.75
11	0.34	1.78	96.53
12	0.22	1.16	97.70
13	0.20	1.07	98.77
14	0.14	0.74	99.51
15	0.05	0.24	99.75
16	0.02	0.10	99.85
17	0.02	0.09	99.94
18	0.01	0.04	99.98
19	0.00	0.02	100.00

Figure 2 Contribution of Variables to the Principal Components

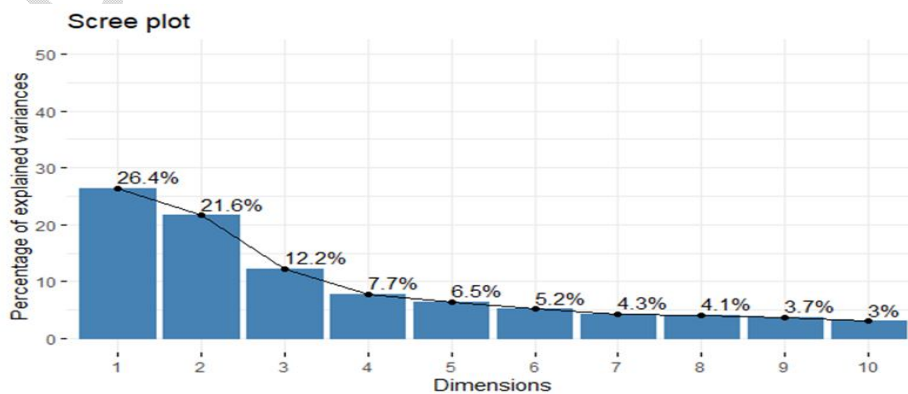




Table 5: Contribution of the variables to Principal Components

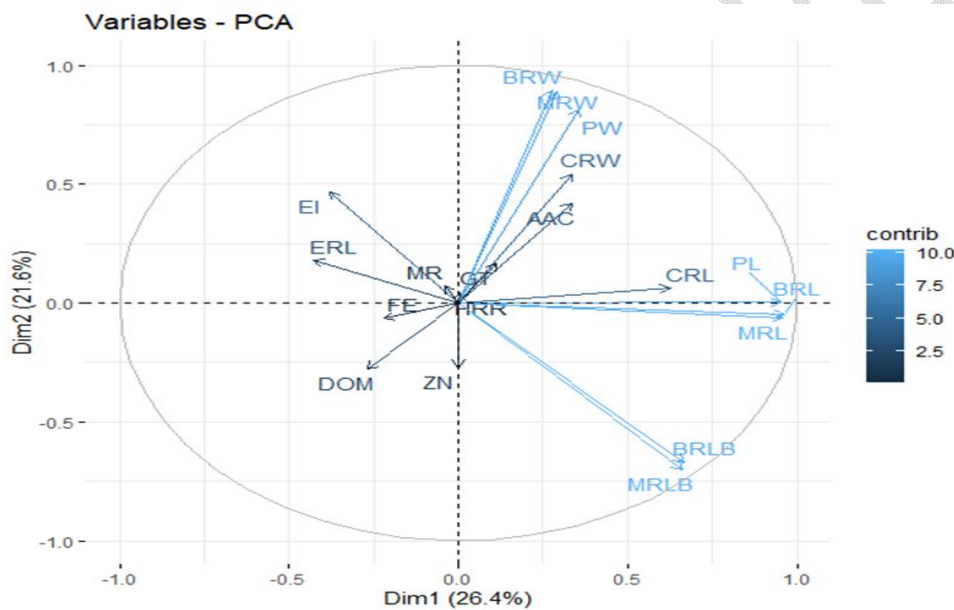
Variable	Dim.1	Dim.2	Dim.3	Dim.4	Dim.5	Dim.6	Dim.7	Dim.8	Dim.9	Dim.10	Dim.11	Dim.12	Dim.13	Dim.14	Dim.15	Dim.16	Dim.17	Dim.18	Dim.19
AAC	2.25	4.31	0.40	2.01	2.75	11.61	5.95	0.24	65.83	0.50	1.77	1.97	0.27	0.05	0.00	0.02	0.03	0.03	0.00
BRL	18.54	0.05	0.18	0.02	0.06	0.03	0.70	0.06	0.73	0.02	2.82	8.36	1.59	5.31	11.79	1.50	32.56	11.16	4.52
BRLB	8.91	10.91	0.95	0.83	0.00	0.05	0.36	0.04	0.57	0.00	1.29	14.59	1.32	6.71	22.44	2.83	16.23	8.12	3.85
BRW	1.53	19.47	0.59	0.88	0.01	0.18	0.07	0.13	2.40	0.01	0.09	3.64	0.00	43.04	12.03	0.00	9.99	4.72	1.22
CRL	7.84	0.10	13.97	15.71	0.42	0.03	0.17	0.07	0.07	0.70	1.35	8.69	3.42	0.05	0.48	18.53	8.63	17.19	2.58
CRW	2.24	7.14	0.08	4.03	4.24	2.60	49.99	0.56	4.12	0.18	0.43	1.58	0.63	0.23	0.01	3.61	0.71	12.64	4.98
DOM	1.45	1.87	12.83	7.66	6.22	4.22	4.33	17.37	1.94	0.00	38.55	2.69	0.48	0.03	0.11	0.23	0.01	0.00	0.00
EI	2.87	5.34	12.35	6.97	3.31	2.60	19.45	0.04	1.34	0.08	0.63	1.07	1.65	0.00	0.00	7.42	1.52	23.78	9.57
ERL	3.63	0.78	15.82	24.29	0.32	0.16	1.87	0.07	2.12	0.25	0.41	0.01	8.61	0.12	0.42	37.39	1.47	0.51	1.77
FE	0.97	0.10	2.67	6.21	18.30	25.54	12.69	10.92	0.21	21.39	0.53	0.00	0.32	0.01	0.09	0.03	0.03	0.00	0.00
GT	0.26	0.67	8.09	5.47	5.46	31.54	1.15	12.90	7.72	25.18	0.87	0.00	0.45	0.06	0.12	0.04	0.00	0.00	0.00
HRR	0.00	0.04	8.45	2.03	16.59	12.62	0.19	53.13	0.11	1.69	3.88	0.77	0.20	0.20	0.01	0.04	0.02	0.00	0.00
MR	0.03	0.13	18.22	11.02	15.13	3.83	0.15	1.58	0.66	3.48	40.67	3.49	1.37	0.14	0.00	0.09	0.00	0.00	0.00
MRL	18.37	0.09	0.07	0.01	0.06	0.14	0.54	0.27	1.49	0.01	0.46	7.35	12.27	3.73	0.28	24.86	0.79	0.01	29.22
MRLB	8.70	11.89	0.26	0.00	0.40	0.08	0.02	0.00	0.00	0.62	2.03	21.32	0.01	0.10	0.32	0.02	18.19	21.13	14.93
MRW	1.72	19.24	0.10	0.00	0.68	0.64	0.50	0.55	1.73	1.48	0.27	6.18	22.11	10.56	0.01	2.06	4.57	0.31	27.29
PL	18.19	0.00	0.17	0.02	0.06	0.00	0.31	0.10	0.50	0.35	2.57	9.85	5.08	5.76	51.54	0.00	5.09	0.38	0.01
PW	2.50	16.10	0.77	0.18	0.10	0.00	0.31	0.35	7.88	0.08	0.34	8.05	39.30	22.55	0.11	1.19	0.15	0.00	0.05
ZN	0.00	1.77	4.03	12.63	25.90	4.12	1.26	1.63	0.56	43.98	1.06	0.40	0.91	1.35	0.23	0.13	0.00	0.02	0.00

 Colourfills indicating highest percentage of variability contributed by a trait in major PC.

 Colourfills indicating highest percentage of variability contributed by a trait in particular PC.

The percentage contribution of the variables (traits) to the Principal Components (PCs) is elaborated in table 5, while the quality of representation of variable on factor map, i.e. lying of a particular variable (trait) on a coordinate and its contribution towards the total variance of PC (Figure No. 3). The variables (traits) under study accounts for percentage of variability in a given Principal Components (dimensions). Variables that are not correlated with any major PC or correlated with last PC i.e dimensions show less contribution towards variance of Principal Components having eigenvalue more then one. In table 4, Principal components 1 to 5 are the major PCs (eigenvalue >1) which accounts for 74.37% of total variability of the data set. While the major traits which accounts for the same in major PCs are, BRL (Brown Rice Length) 18.54% in PC1, BRW (Brown Rice Width) 19.473% in PC2, milling recovery (18.22%) in PC3, ERL (Elongation Ratio Length) 24.29% in PC4, Zn(zinc) 25.90% in PC5, HRR (Head Rice Recovery) 16.59% in PC5, MR (Milling Ratio) 18.21% in PC3, MRL(Milled Rice Length) 18.37% in PC1, PL (Paddy Length) 18.19% in PC1, PW (Paddy Width) 16.10% in PC2 and ZN (Zinc) 25.89% in PC.

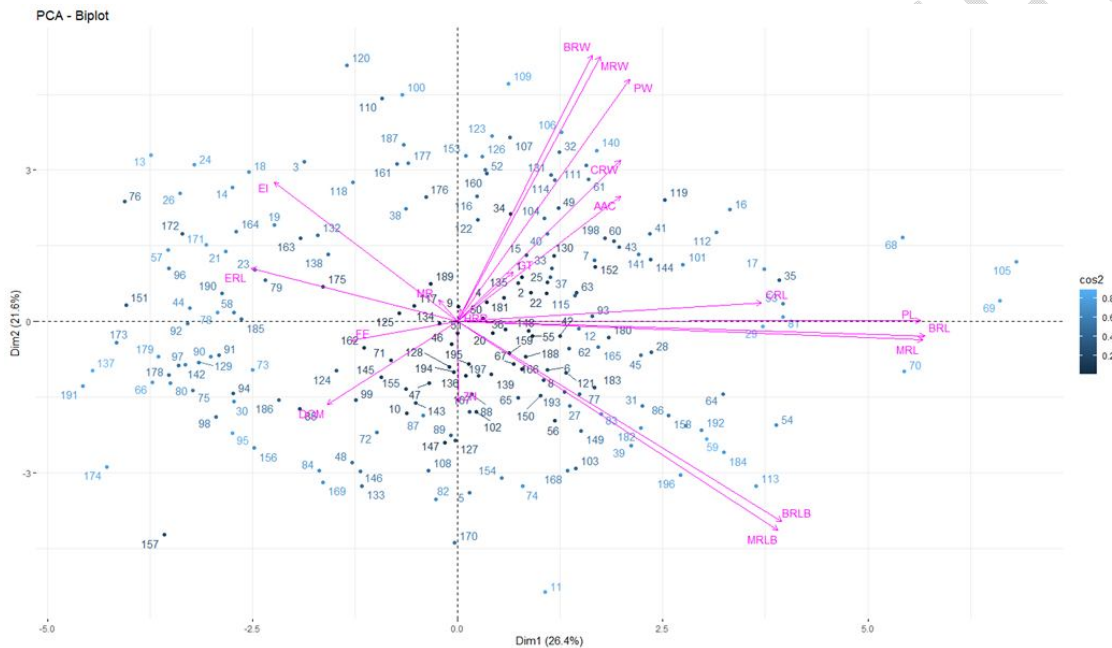
Figure 3: Contribution of variables (traits) to the variance accounted by PC1 and PC2



The 74.37% of variance is covered by the starting five Principal Components and the individuals contributing towards the variance in PC1 ranges from 0.000145 (Alsakar Mutant-8) to 4.674(Maharaji) and there are 37 genotypes ranging from 1 to 4.67. In PC2 the range of variance varies from 0.000182 (Kheerasar) to 3.530 (Gobi-buta) while in this PC there are 39 genotypes which range from 1 to 3.53. The PC3 variance ranges from 0.000156 (Grassy Dubraj) to 7.59 (Swarna) and the genotypes from variance 1 to 7.5 are 22. PC4 the minima to maxima range of variance is from 0.000207 (Paltu) to 4.20 (Balki) and total of 31 genotypes the variance is ranging from 1 to 4.20. In PC5 the minima to maxima for variance is from 0.00358 (BPT 5204 improved) to 12.164 (Vikram TCR) and in PC5 for the range 1 to 12.164 there are 24 genotypes.

Cluster analysis for the quality contributing characters: The Agglomerative hierarchical clustering also known as AGNES (Agglomerative Nesting), which works on similarity index, it groups the most similar genotypes and make larger groups of similar genotypes i.e in bottom up manner (Kassambara, A. ,2017). Ideal number of clusters for the data set are three. The size of derived three clusters are C1: 71 genotypes,C2:29 genotypes and C3:98 genotypes (Figure 5 , Table 6).For the cluster validation total three intracuster and six. intercluster linkage diameters are worked out for the data set. The intra cluster diameter tells about the divergence between two genotypes lying in same cluster on the basis of similarity while as the inter cluster diameters comes out with the distances between two dissimilar clusters on the basis of similarity Interpretation of various diameters (Bolshakova, Nadia & Azuaje, F.. 2003) are as *Intracuster Diameters* viz., *Intracuster Complete Diameter* manifesting the highest within cluster distance between two genotypes, belonging to the same cluster.

Figure : 4 Contribution of variables and genotypes to the variance accounted by PC1 and PC2.



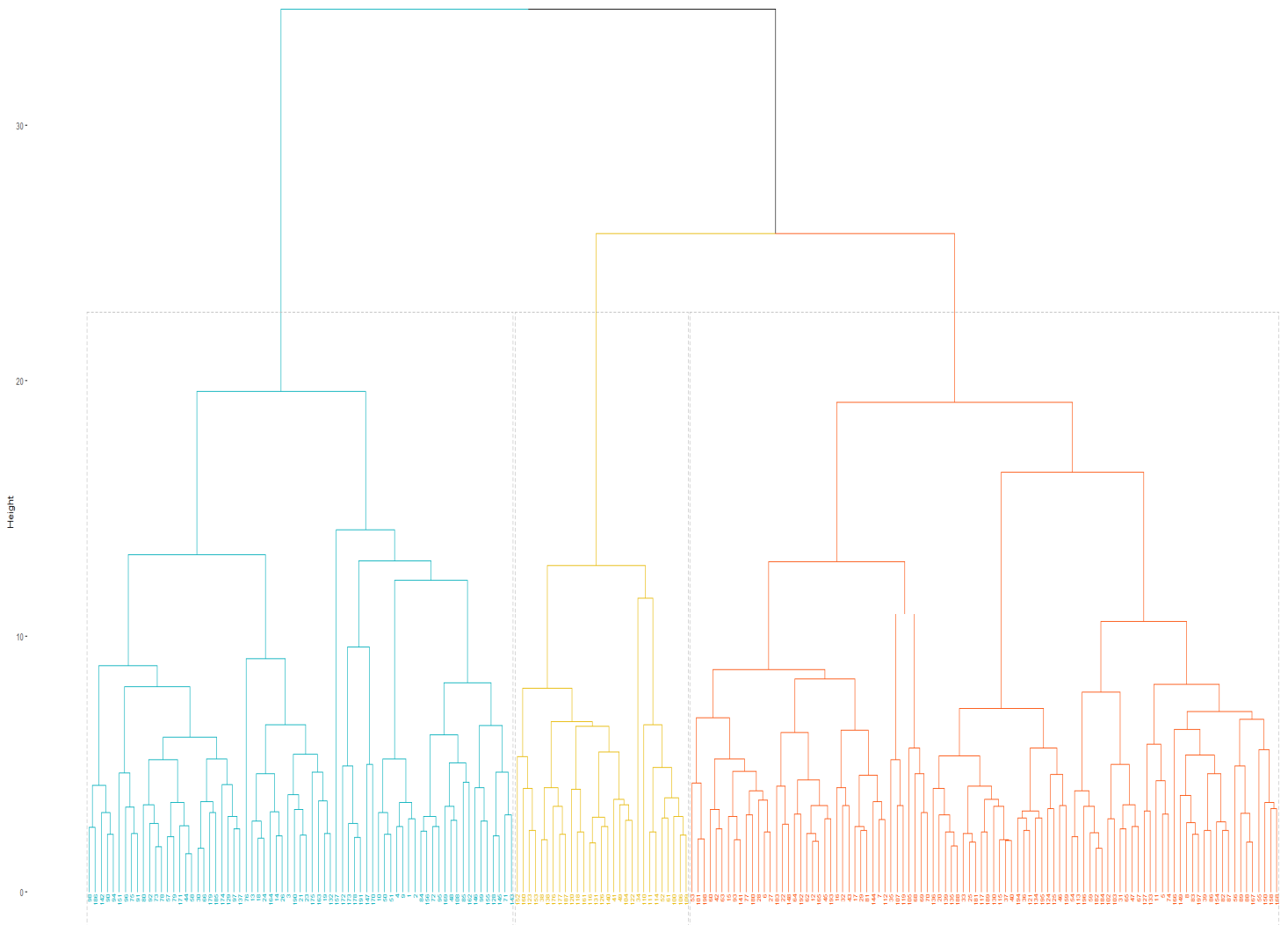
The intracuster complete distance is highest in C3 (98.53) > C1(86.62) > C2(68.70). *Intracuster Average Diameter* depicts the average distance between the genotypes of a particular cluster, the lesser the average distance the high is the cluster compactness. Cluster C1 is the least compact with average distance of 21.20 followed by C3 (20.53) and C2 is the most compact cluster with average distance of 16.10. *Intracuster Centriod* reflects the double of average distance between all genotypes of a cluster and the cluster centre. It is highest in C1 (14.63) > C3(13.52) and C2(11.12). *Intercluster Single Distance* is the nearest distance between the two genotypes of two different clusters. The closest observed distance is between C1:C2 (3.31). *Intercluster Complete Distance* is the largest distance between the two genotypes belonging to two different clusters. It is between cluster C1:C3 (125.13) *Intercluster Average Distance* is average distance between all the genotypes of two clusters. Highest intercluster average distance is observed between C1:C3 (23.93) while shortest is between C1:C2 (19.52). *Intercluster Centroid Linkage* is centroid linkage is the distance between the centers of two clusters. It is highest between C1:C3 (11.08) and shortest between C1:C2 (5.53). *Intercluster Average Centroid Linkage*: The distance between all the genotypes of one cluster to the center of another cluster. The average centroid distance between the center of cluster C3 and C1 is the highest (18.48) while it shorest between C1 and C2 (13.86). *Intercluster Hausdorff Matrics* is the maximum distance from genotypes of one cluster to the nearest genotypes of another cluster. It is maximum between the C3:C1 cluster (55.62).

Table 6: Genotypes contribution to each cluster

UNDER PEER REVIEW

S.No	Cluster	Number of Genotypes	Name of Genotypes
1.	Cluster 1	71	Jeeraphool, Bhadshahbhog selection-1, Loktijhool,Samudra-fan, Dhaniya phool,CG Jawaphool trombay, Tulsi manjar , Badshah bhog – 2, Gopal bhog, Raja bhog, Nawabbhog, Tulsi manjari, Amrit bhog, Tarunbhog, Thakur bhog, Kadamphool parent , Til- kasturi-1,Bisni-I, Lalloo-14, Kadam phool, Samundchini mutant s-48, Vishubhog selection -1, Vishnubhog mutant V-80, Jeeraphool , Jou phool – 2, Goverdhan kali kamod ,Shri kamal, Angur guchcha, Bhejari, Bhaniya, Ambemohar mutant-1, Anjaniya 1, Gajai ,Jodhari, Til kasturi , Ama dhool, Bans bod,Chaptigurmatiya parent ,Ambemohar parent , Nagabel, Kheerasar, Red jawaphool mutant, Kadamphool mutant-2, Vishnubhog fine parent , Samundchini mutant, Lokti musi , Grassy dubraj , Nagri dubraj mutant-1, Luchai(a),WR99, WR116, Satha dhan, Karhani, Bagri, Hardi chudi, Chhatri – 1, Red jawaphool parent ,Anterved, Safed jira, Nagri dubraj parent,BPT 5204 (improved), Jalsinga, Bisni,Alsakar mutant-8,TCDM1, Kalanamak ,Chinor mutant-1,Balki,Mai dubraj,Kubri mohar,TRR-4,
2.	Cluster 2	29	Sanchuriya parent, Alsakar parent,Puse banda,Sanchuriya mutant -6, Annada,Mainpat-2,Chaptigurmatiya mutant-1,Chaptigurmatiya mutant-2,Adanga dhan,Paltu,Kohikari,Alsakar mutant-3,Kal mai, Shivganga, Puse banda,Kumda phool,Bakal,Jitpiti,Mehar dhan,Kari khuji,Bahal binjo,Dongar goyandi, Banda,Barma tripal,E-1702,GP-145-43, Gathuwan,Hardi ganthi,Beda kabro.
3.	Cluster 3	98	Maheshwari,Bhejari,Bamleshwari, Moroberekan, Bhataphool,IR55419, Baikoni,Bodela,Sukala phool,Gathuwan,Kanak,Faram, Hiranakhi, Indira sughandit dhan-1 ,Pinwari ajan,Safri 17 parent, R-RF-75, MTU-1010, Shenong, No.:25, Luchai parent, Vandana, Safri 17, Banko dhan, Poni, Dagad deshi, Barangi, Khatia pati, Tedesi, IGKV R1, Gurmattia. Urai butta. Chiko, Jalpan, Kating, Chiko, Dokra dokri, Dokra mechha, Khatia pati, Siyar dhan, Safri(II), Turyagada khuta, Dhal kabri, Dawardhan, Unknown, Duggi, Bhejari, Korma, Makdo dhan, Lahagati , Jalkeshar, Gene marker no. 73 II, Buddha, Karma Mahsuri, Sonapan, Dudh malai, Rongobati, Swarna, Pandri luchai, Safed luchai, Samund chini-1, Tilkormal mutant -8, Shymala, Lanji,Poornima,Ramjiyan, Danteshwari , Chandrahasahni, Layacha, Maharaji, Padari dhan iv, DxD (124), Dubraj, Khajur jhopa, Geeta,Kanakgopala, Gobi buta, Bhawara 1-I, Lokti machhi, Luchai mutant, Jitpiti, Hardi gudi, Chini Kapoor, Indira Aerobic, Abhaya,IR-64,Chinor parent, Katarni bhog, Atma shital,Indira sugndhit dhan-1,Tulsi Prasad, Dhawara sawa, Jhilli parent, Jaldubi, Safri 17-2-48, Tilkormal parent, Jhilli mutant 13-5.

Figure 5: Agglomerative Hierarchical Cluster



Discussion: The accessions under study were taken from the one of the largest rice germplasm collection of Indira Gandhi Krishi Vishwavidyala along with their derived 29 mutants in order to sight better understand the behavior of the rice quality characters, the high level of significance in analysis of variance confirms that all germplasm were different from the checks and there were no significant effect of blocking on the experiment. From coefficient of variation, range and mean in table 2 gives detail about the amount of variability present in the various quality contributing traits in the germplasm used for the study . The traits MR,FE and DOM depicting significant skewness, rest in the data set are normally or near normally distributed. In the histogram coloured lines shows the distribution of control genotypes which lies in the first quadrante or near the mean is due to the reason that they are released varieties (i.e. they are improved varieties).Leptokurtic curves were observed for the trait PW,MRW,ERL,EI,MR,DOM,HRR,FE and ZN similar study was done by Kanavi et.al.(2020).The observed variability for all the traits helped to recognize donors for specific traits like genotype Dokra dokri could contribute for paddy and grain length, for slender grains Red jawaphool mutant can be used while to improve milling recovery Luchai mutant could be used in the breeding programmes, and in order to study the genetic architecture of these characters Genome Wide Association Mapping can be done using these mutants as suggested by Viana et.al (2019).While to study the trait at genetic level, mutants of dubraj viz., TCDM1, Grassy dubraj and its wild type can be used as they shows wide range of

variation for the trait similar results obtained by Adjah *et al.* (2020). The removal of bran from brown rice is the degree of milling, which is highest in Jeeraphool, hence less time is needed for milling while the intact and hardest to remove were of Grassy dubraj, Sanchuriya parent similar study was carried out by Wang *et al.* (2021). Head rice recovery is the most important trait and decides the success of a variety, in order to seek the donors for the same Bahal binjo is the landrace with least broken and highest head rice recovery Laips *et al.* (2019). Now a days resistance starch is the emerging trait studied widely, and is associated with high apparent amylose content, the Ambemohar mutant-1, Barangi, Kumdaphool, Alsakar parent and Samleshwari are screened for higher AAC, using standard protocols these genotypes can be further evaluated as similar study was carried out by Naseer B *et al.* (2021). Iron and Zinc were evaluated for the brown rice of all the genotypes, and found highest for Red jawaphool mutant, Satha dhan respectively further studies can be done for the translocation of Fe-Zn into endosperm as well as their retention in rice bran oil Miah *et al.* (2014). In the given dataset five major clusters were derived but highest variation is explained by PC1 and PC2 therefore improvement of highest variation contributing traits *viz.* length and width of paddy, brown rice and milled rice could be done by crossing genotype 105 (Chiko) and 157 (Chaptigurmatiya parent), 13 (Angur gucha) and 11 (Gobi buta), 35 (Chiko) and 174 (Visnubhog mutant V-80), 70 (Khatia pati) and 76 (Shri kamal) as were depicted as most diverse and genotype.

Conclusion : The present study focuses on the diversity for the post harvest and quality traits of germplasm and there derived mutants, the initial screening aids to identify the range as well potential donors for the improvement of the traits, while further studies are needed to study diversity at genotypic level. The study of variation in mutants and their parents for the studied traits will help to study the genetic architecture of trait of interest. Further studies using more advance protocols can be done to confirm the obtained diversity for the genotypes.

References:

1. Costa de Oliveira *et al.* (eds.), The Future of Rice Demand: Quality Beyond Productivity, https://doi.org/10.1007/978-3-030-37510-2_1.
2. Adjah, K.L., Abe, A., Adetimirin, V.O. *et al.* Genetic variability, heritability and correlations for milling and grain appearance qualities in some accessions of rice (*Oryza sativa* L.). *Physiol Mol Biol Plants* 26, 1309–1317 (2020). <https://doi.org/10.1007/s12298-020-00826-x>.
3. Ahmed, Fahim & Tanweer, Fatah & Kabir, Md & A., Latif. (2020). Rice Quality: Biochemical Composition, Eating Quality, and Cooking Quality. 10.1007/978-3-030-37510-2_1.
4. Aravind J, Sankar SS, Wankhede DP and Kaur V (2020) AugmentedRCBD: analysis of augmented randomised complete block designs. R package version 0.1.3. Available at <https://aravindj.github.io/augmentedRCBD/https://cran.rproject.org/package=augmentedRCBD>.
5. Bolshakova, Nadia & Azuaje, F.. (2003). Cluster validation techniques for genome expression data. *Signal Processing*. 83. 825-833. 10.1016/S0165-1684(02)00475-9.
6. Charrad M., Ghazzali N., Boiteau V., Niknafs A. (2014). "NbClust: An R Package for Determining the Relevant Number of Clusters in a Data Set.", "Journal of Statistical Software, 61(6), 1-36.", "URL <http://www.jstatsoft.org/v61/i06/>".
7. Deepika, K., Lavuri, K., Rathod, S., Yeshala, C., Jukanti, A., Narender Reddy, S., Badri, J. (2021). Multivariate analysis of geographically diverse rice germplasm for genetic improvement of yield, dormancy and shattering-related traits. *Plant Genetic Resources: Characterization and Utilization*, 19(2), 144-152. doi:10.1017/S1479262121000186.
8. Emon, R.M., Ahammed, G.J. (2020). Germplasm and Genetic Diversity Studies in Rice for Stress Response and Quality Traits. In: Roychoudhury, A. (eds) *Rice Research for Quality Improvement: Genomics and Genetic Engineering*. Springer, Singapore. https://doi.org/10.1007/978-981-15-4120-9_3

9. Gutaker, R.M., Groen, S.C., Bellis, E.S. *et al.* Genomic history and ecology of the geographic spread of rice. *Nat. Plants* 6, 492–502 (2020). <https://doi.org/10.1038/s41477-020-0659-6.10>.
10. Juliano BO (1971) A simplified assay for milled rice amylose. *Cereal Science Today* 16: 334-340.
11. Juliano, Bienvenido & Perez, C.. (1984). Results of a Collaborative Test on the Measurement of Grain Elongation of Milled Rice During Cooking. *Journal of Cereal Science*. 2. 281-292. 10.1016/S0733-5210(84)80016-8.
12. Kanavi, M. & Koler, Prakash & Somu, G. & N., Marappa. (2020). Genetic Diversity Study through K-means Clustering in Germplasm Accessions of Green gram [*Vigna radiata* (L.)] Under Drought Condition. *International Journal of Bio-resource and Stress Management*. 11. 138-147. 10.23910/IJBSM/2020.11.2.2078.
13. Kassambara, A. (2017). *Practical Guide to Cluster Analysis in R: Unsupervised Machine Learning* (Vol. 1). STHDA.
14. Knowledgebank.irri.org. Rice Knowledge Bank, International Rice Research Institute.
15. Lapis, Jennine & Cuevas, Rosa & Sreenivasulu, Nese & Molina, Lilia. (2019). Measuring Head Rice Recovery in Rice: Methods and Protocols. 10.1007/978-1-4939-8914-0_5.
16. Le, S., Josse, J. & Husson, F. (2008). FactoMineR: An R Package for Multivariate Analysis. *Journal of Statistical Software*. 25(1). pp. 18<https://www.jstatsoft.org/v25/i01/>.
17. Manual on Grain Quality of Rice, September-2014, Directorate of Rice Research, Rajendernagar, Hyderabad.
18. Miah, Mohammed & Ara, Hamid & Jabin, Syeda & Abedin, Nusrat & Azad, A. & Hossain, Abul & Ara, Umme. (2014). Nutritional Composition and Stabilization of Local Variety Rice Bran BRRI-28. *International Journal of Science and Technology*. 3. 306-313..
19. Naseer B, Naik HR, Hussain SZ, Shikari AB, Noor N. Variability in waxy (Wx) allele, in-vitro starch digestibility, glycemic response and textural behaviour of popular Northern Himalayan rice varieties. *Sci Rep*. 2021 Jun 8;11(1):12047. doi: 10.1038/s41598-021-91537-0. PMID: 34103609; PMCID: PMC8187641.
20. Sahu, Parmeshwar & Sharma, Deepak & Mondal, Suvendu & Kumar, Vikash & Singh, Satyapal & Baghel, Samarth & Tiwari, Ashish & Vishwakarma, Gautam & Das, B.K.. (2017). Genetic variability for grain quality traits in indigenous rice landraces of Chhattisgarh India. *Journal of Experimental Biology and Agricultural Sciences*. 5. 439-455. 10.18006/2017.5(4).439.455.
21. Viana VE, Pegoraro C, Busanello C and Costa de Oliveira A (2019) Mutagenesis in Rice: The Basis for Breeding a New Super Plant. *Front. Plant Sci*. 10:1326. doi: 10.3389/fpls.2019.01326.
22. Zhiming Wang, Mingwei Zhang, Guang Liu, Yuanyuan Deng, Yan Zhang, Xiaojun Tang, Ping Li, Zhencheng Wei, Effect of the degree of milling on the physicochemical properties, pasting properties and in vitro digestibility of Simiao rice, *Grain & Oil Science and Technology*, Volume 4, Issue 2, 2021, Pages 45-53, ISSN 2590-2598, <https://doi.org/10.1016/j.gaost.2021.04.002>.

UNDER PEER REVIEW