

Morphological and molecular (SSRs) characterization of different diverse genotypes of blackgram (*Vigna mungo* L.)

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Abstract

Blackgram [*Vigna mungo* (L.) Hepper] is a leguminous crop and is an important source of plant-based protein. Thirty genotypes of Indian blackgram were analysed for genetic diversity using morphological, agronomical characters and SSR markers. Identifying elite lines is a major research priority for developing new varieties. The present investigation was carried out at the Advanced Centre for Rainfed Agriculture (ACRA) Dhiansar of SKUAST Jammu of UT of J & K during *kharif* 2021 to characterize all the eleven characters, *viz.*, plant height, days to fifty percent flowering, number of branches per plant, days to maturity, number of pods per plant, number of seeds per pod, pod length, number of clusters per plant, 1000 seed weight and seed yield per plant morphologically and molecularly. Present study aimed to appraise highly diverse genotypes of blackgram and their associations between molecular markers and yield-contributing traits. Based on dendrograms and PCA values, results from field data and molecular data exhibited two genetically different groups of genotypes namely PU-15-2, PU-13-05 and AZAD-2 were found in group-1, while five genotypes, namely, PU-UPU-97-1, OU-40, PU-KUG216 and PU-15-32 were found in group-2 and such screened genotypes can be further used in the introgressions of specific traits from one group to another group of genotypes. Based on morphological and molecular data, two genetically diverse groups were constituted, and which can be recommended for further utilization in hybridization programs. Three markers namely *viz.*, VR-102, CEDG-156 and CEDG-176 were identified and associated with seed yield per plant and could be useful in seed yield improvement programme of blackgram.

1 Introduction

Blackgram (*Vigna mungo* L.) is generally known as mash or urdbean and belongs to Leguminosae family (Naik et al., 2017). In accordance with Karpechenko (1925), blackgram is an autogamous diploid grain legume crop with a chromosomal number of $2n = 2x = 22$. It is the third most important pulse crop after gram and pea in our country. According to Jeberson et al. (2019), it is the richest source of numerous biomolecules, including proteins (25–26%), carbohydrates (60%), fat (1.5%), vitamins, minerals, and amino acids. India is a major global producer and consumers of blackgram (*Vigna mungo* L.). It was originally extensively utilized and grown in India, but it is now also grown in various tropical and subtropical regions, including the West Indies, Japan, and southern United States (Delic et al., 2009). According to Chauhan et al. (2020), Andhra Pradesh, Maharashtra, Madhya Pradesh, Tamil Nadu, and Uttar Pradesh are the states in India where blackgram are most often grown.

For any successful breeding programme a considerable amount of genetic diversity is required in any pulse crop (Kumar et al., 2021 and Kumawat et al., 2023). The evaluation of genetic diversity relies heavily on molecular characterisation. Polymerase chain reaction (PCR) based co-dominant SSR markers are widely used for the molecular characterization of any crop. Assessing the type and degree of genetic diversity in a population is essential before beginning any breeding program with a view to increasing yield and its component qualities (Singh et al., 2016). Marker-trait association (MTA) is highly useful for identifying the relationship between markers and traits of interest. With these observations in mind, the present study was carried out to evaluate molecular marker trait associations, variability, and diversity in selected blackgram genotypes.

2 Materials and methods

2.1 Plant Materials: Supplementary table-1 describes the selected plant material used in this research programme, which included thirty genetically distinct blackgram genotypes.

2.2 Isolation and purification of Plant Genomic DNA: DNA isolation and purification of thirty blackgram genotypes were carried out with the GenElute Plant Genomic DNA Miniprep Kit (Fig. 1).

2.3 DNA quality check: Following the isolation and purification of the plant genomic DNA, the quality of the DNA was assessed by using an 8% agarose gel and gel electrophoresis equipment.

2.4 Procedure of Agarose gel electrophoresis: A total of 150 mL of 1× TBE buffer and 1.2 gm of agarose powder were combined, and the mixture was then microwaved for 2.0 minutes. 6 µL of ethidium bromide (EtBr) was added, and the mixture was mixed after it had cooled for a few seconds. Two combs were placed in the casting tray, and the agarose solution was poured into the container. The container was left at room temperature for thirty to forty minutes for solidify. For each well, 3 µL of DNA mixed with 3 µL of DNA loading dye. The gel electrophoresis was performed at 100 V for 50 minutes. Then, 0.8 gel solution was viewed in the gel Documentation system.

2.5 Primers for PCR amplification: Based on previous studies (Tripathy and Das, 2021), a set of fifty SSR markers was selected for amplification of blackgram using genomic DNA. The lyophilized primers were suspended in millipore water at the mentioned volume to obtain a concentration of 100 µM each primer. The stock solution (stored at -80°C) was used, and working solution (stored at 4°C) at a concentration of 10 µM was obtained by adding 10 µL of stock solution and 90 µL of ddH₂O.

2.6 PCR amplification profile: PCR amplification was performed in a 0.2 mL PCR tube with a total reaction volume of 15.0 µL. PCR tubes containing all eight reagents along with template DNA (Table-1) were thoroughly mixed and subjected to thermal profiling. Amplification was carried out in a nexus gradient master cycler. Initial denaturation was performed at 94°C for 4 minutes, followed by a loop of 37 cycles of denaturation (at 94°C for 30 seconds), annealing (at 50°C to 62°C for 30 seconds), and extension (at 72°C for 30 seconds) followed by a final extension at 72°C for 5 minutes. The amplified PCR products were stored at 4°C.

2.7 Procedure of PAGE gel casting: The two glass plates were carefully cleaned with tap water, distilled water and detergent after they were allowed to air dry. After that, the dried plates were air-dried after being cleaned with ethanol. Clamps were used to help assemble the plates with spacers. Brown ceiling tape or a tiny volume of acrylamide solution (containing 1% APS) and TEMED were used to seal the bottoms of the plates. The necessary amount of TEMED was added, and then the PAGE solution was rapidly poured between the glass plates (care was taken to prevent the entrance of air bubbles). The comb was then placed in, the gel, after which the gel was polymerized for nearly twenty-five minutes. Following polymerization, the comb was gently removed and added, and distilled water was carefully added to the wells of the gel. The gel was separated on a vertical gel electrophoresis instrument, the gel plates were installed. After mixing the amplified product with loading buffer and dye, 15 µl was loaded into the gel and electrophoresed for 4 hours at a constant voltage of 100 volts. Silver staining was applied to the gels following electrophoresis (Tagelstrom, 1992).

2.8 PAGE gels stained with silver: After various alterations were made, silver staining was performed in accordance with Tagelstromm (1992) to resolve the SSR results. The steps involved were as follows: after electrophoresis, the gel was carefully removed from one of the glass plates, transported to a tray with distilled water, and shaken gently for five minutes. Subsequently, fixing solution was added to the tray above, and the mixture was gently shaken for seven minutes. The fixing solution mentioned above was removed from the tray and kept for later use. After that, the gels were placed in a tray to be stained with silver solution (0.3 g AgNO₃ powder in 150 ml 10% methanol solution with 750 µl glacial acetic acid). The gels were shaken gently for 7 minutes. After removing the silver solution from the tray, distilled water was used to rinse the gel. The developing solution (made by dissolving 4 g of NaOH pellets in 150 ml of distilled water with 450 µl of 40% formaldehyde) was added to the gel after it was transferred. For five to ten minutes, the solution in the tray was gently shaken, resulting in the appearance of DNA bands. By raising the gel in the fixing solution for an additional five minutes, staining was prevented.

2.9 Statistical analysis: A statistical analysis of the field and laboratory recorded data was performed on the pooled mean values. Analysis was performed using the R studio version (2021), and the statistical techniques that were used are mentioned below.

2.10 Polymorphic information content (PIC): PIC value provides insight into the level of genetic polymorphism in a population. This approach is helpful in the study of genetic diversity. According to (Botstein et al., 1980), the PIC value was calculated using the following formula:
$$\text{PIC} = 1 - \sum_{i=1}^n p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^n 2p_i p_j$$

2.11 Marker trait association studies: Marker trait association studies are useful for assessing association between a molecular marker and specific traits of interest. Marker trait association studies were performed by Student's t-test with the given formula: = TTEST (array1, array2,1,2)

3 Results

3.1 Assessment of variance: Assessment of variance was accomplished for eleven agro-morphological characters. The results (Table-2) suggested that, for 11 agro-morphological parameters, the variance attributed by genotypes was highly significant at 1% level of significance. This indicates that the genotypes associated with the agro-morphological traits in this study exhibited a considerable degree of genetic variability.

3.2 Analysis of the correlation coefficient: A statistically significant and positive association was found between the seed yield per plant and other yield-related characteristics like number of days to 50% flowering (0.509**) and number of days to maturity (0.495**), as presented in Table-3. Number of days to 50% flowering and the number of days to maturity are the traits that are positively correlated with the yield of seed. This relationship between DTF and DTM with SYP helps in the development of early-maturing varieties. Early maturing varieties can sometimes escape disease and increase seed yield. Sridhar et al., (2020) showed similar results with respect to days to maturity.

3.3 PCA assessment using phenotypic data: The mean values of the morphological data were subjected to principal component analysis (PCA). In the present study, 30 genotypes were characterized into 2 different groups by principal component analysis (Fig. 4). Group-1 having 19 genotypes namely AZAD-2, PU-13-05, PU-15-2, PU-15-23, PU-15-26, PU-15-28, PU-15-29, PU-15-30, PU-15-31, PU-15-35, PU-15-40, PU-19, PU-31, PU-35, PU-7, PU-8, PU-9, PU-IPU-2-43 and PU-KU-99-21, while group-2 had 11 genotypes namely MASH-114, NU-1, PU-07-7, PU-10, PU-15-21, PU-15-32, PU-15-34, PU-17-4, PU-40, PU-KUG216 and PU-UPU-97-1. The initial two principal components (PCs), which collectively explained 47.60% of the total variations were chosen. The eigenvalues, variability %, and cumulative contributions are presented in Table-5. The highest eigen values were shown by PC1 (2.71), followed by PC-2 (2.45), PC3 (1.23) and PC4 (1.09). In PC1, the

maximum contributions were shared by three characters, namely seed yield per plant (SYP), number of pods per cluster (NPC) and number of pods per plant (NPP), which are represented by red colour. The largest contribution in the second cluster was divided among the number of branches per plant, the number of seeds per plant, and the pod length (Fig. 5).

3.4 Molecular characterization of blackgram genotypes: Molecular marker-based (DNA-based) assessment of genetic diversity is an efficient method for determining the genetic diversity present of blackgram (*Vigna mungo* L.) genotypes. In the present investigation, thirty different blackgram genotypes were selected for molecular characterization. The blackgram plant genomic DNA was extracted with a GENELUTE PLANT GENOMIC DNA KIT. The quality of the plant genomic DNA was examined on a 0.8% agarose gel. Thirty blackgram genotypes were molecularly characterized *via* polyacrylamide gel electrophoresis (PAGE), and fifteen PCR-based SSR primers were used.

3.5 Primer-based amplification

The genetic diversity of blackgram was assessed among 30 blackgram (*Vigna mungo* L.) genotypes by using PCR based SSR markers (Table-4). Fifteen SSR primers were used in this investigation out of fifteen, fourteen SSR primers, namely, CEDG176, CEDG156, CEDG128, CEDG199, VR303, VR102, VR216, LR738A, DMBSSR217, DMBSSR182 (Fig. 2), CEDG198, CEDG092, CEDG245 and VR-9, exhibited genetic polymorphism among the blackgram (*Vigna mungo* L.) genotypes.

3.6 Polymorphism information content (PIC): The ability of a marker's power to identify polymorphisms among individuals in a population is measured by its polymorphism information content (PIC), and the greater this capacity is, the greater the marker's usefulness. In genetic investigations, it is one of the flags of marker quality indicators. For codominant markers, the PIC values vary from 0 (monomorphic) to 1 (very informative, containing many alleles with similar frequencies). Polymorphic information content values provide an estimate of a locus's discriminatory power by considering both the relative frequency and the number of expressed alleles. PIC value of each SSR primer is presented in Table-4. In the present study, the polymorphic information content (PIC) values ranged from 0.18 to 0.70. The highest polymorphic information content (PIC) value was exhibited by the CEDG245 primer (0.70) followed by CEDG198 (0.65) and VR-9 (0.58).

3.7 Similarity coefficients: The similarity coefficient among thirty blackgram (*Vigna mungo* L.) genotypes based on SSR markers amplification was analyzed *via* Jaccard's coefficient of similarity; thus, the similarity matrix is presented in Table-6. Estimates of genetic similarity derived from the binomial data generated through these markers utilizing Jaccard's coefficient ranged from 0.13 (PU-15-30/ PU-07-7) to 0.93 (PU-KUG216 between PU-9/ PU-IPU-243) exhibiting significant diversity among the thirty blackgram genotypes and suggesting their further use as potential parents in blackgram improvement breeding programs. Based on the molecular data, a similarity score of 0.93 was found between the PU-9 and PU-IPU-2-43 genotypes indicating greater genetic similarity. The results revealed significant molecular diversity among the thirty blackgram (*Vigna mungo* L.) genotypes.

3.8 Cluster analysis using SSR markers: The molecular data obtained from all thirty blackgram (*Vigna mungo* L.) genotypes *via* SSR markers were analyzed with unweighted paired group method on arithmetic averages (UPGMA) method *via* NTSYS software, and a dendrogram was constructed from the genetic similarity coefficients to explain the genetic relationships among the blackgram genotypes (Fig. 3). Thirty blackgram (*Vigna mungo* L.) genotypes were grouped into two major clusters. Cluster I was further divided into two subgroups comprising twelve genotypes (Azad-2, MASH-114, PU-15-23, NU-1, PU-15-26, PU-15-28, PU-15-29, PU-07-7, PU-10, PU-13-05, PU-15-21 and PU-15-2), while the other eighteen genotypes belonged to cluster II, and were further divided into two subgroups. The eighteen genotypes in cluster II, namely, PU-15-30, PU-40, PU-15-31, PU-8, PU-9, PU-IPU-2-43, PU-KUG216, PU-UPU-97-1, PU-KU-99-21, PU-31, PU-35, PU-7, PU-12-32, PU-15-34, PU-15-35, PU-15-40, PU-17-4 and PU-19, were distantly linked to one another and there seemed to be considerable amount of genetic variation among them. The findings of this study clearly showed that the fifteen SSR primers used in the present analysis revealed a significant amount of genetic variation in the genotypes of blackgram.

3.9 PCA assessment using molecular data: The molecular data were subjected to principal component analysis (PCA) in these studies. Thirty blackgram genotypes were fall in dim-1 and dim-2. Dim-1 contributed 19.1%, while dim-2 contributed 12.7% to the total diversity (Fig. 6). Based on molecular data, two different genetic groups were identified in this study i.e. group-1 had eleven genotypes, namely, PU-19, PU-15-2, PU-13-05, PU-15-21, NU-1, PU-15-34, PU-15-35, PU-07-07, PU-10, PU-17-4 and AZAD-2, which indicated by reflected by red dots and group-2 had nineteen genotypes, namely, PU-15-32, PU-KUG216, PU-IPU-2-43, PU-8, PU-40, PU-9, PU-15-30, PU-15-23, PU-UPU-97-1, PU-KU-99-21, PU-7, PU-15-31, PU-31, PU-35, PU-15-26, MASH-114, PU-15-28, PU-15-29 and PU-15-40.

3.10 Associations of SSR markers with the traits determined using Student's t-test: For under studied eleven quantitative traits, marker traits association was studied using Student's t-test. In the present investigation, out of fifteen markers three markers namely, VR-102 (0.0055), CEDG-156 (0.0508) and CEDG-176 (0.0505) were associated with seed yield per plant. *P* values from one-tailed t tests are given in brackets. Three markers *viz.*, VR-303 (0.0070), VR-216 (0.0258), and CEDG-156 (0.0158), displayed association with the number of branches per plant. One marker, DMBSSR-182 (0.0028), exhibited an association with the number of pods per plant. The marker namely VR-102 (0.0162) associated with the number of seeds per pod. Pod length was significantly associated with DMBSSR-182 (0.0377), CEDG-156 (0.0311) and CEDG-199 (0.0408). Three markers, namely, CEDG-092 (0.0412), CEDG-176 (0.0463) and CEDG-245 (0.0227) were associated with the number of clusters per plant. The Marker, CEDG-156 (0.0262) associated with the number of pods per cluster. Among

the 15 SSRs, five, CEDG-156 (0.0078), CEDG-154 (0.0047), CEDG-092 (0.0013), VR-216 (0.0337) and CEDG-176 (0.0122), were associated with 1000 seed weight. Four markers, DMBSSR-182 (0.0008), CEDG-156 (0.0069), VR-303 (0.0487) and VR-216 (0.0211), were strongly associated with plant height. Marker, VR-303 (0.0067) was strongly associated with days to fifty percent flowering.

4 Discussion

The determination of the morpho molecular diversity of blackgram genotypes *via* SSR markers is useful to identifying the potential of different parents for determining seed yield and other yield related traits. Among the most commonly used DNA markers, microsatellites are employed for a variety of applications including genetic diversity, genome mapping and varietal identification. The present investigation utilized 15 SSR markers to reveal genetic polymorphism and confirmed unambiguous identifications. Fourteen SSR markers were polymorphic in nature, indicated sufficient genetic diversity among the 30 blackgram genotypes. Genotypes were highly significant at 1% level. This indicated that there is a considerable amount of genetic variability among the genotypes for agro-morphological variables examined in this investigation. The number of days to 50% flowering and number of days to maturity are the traits that showed positive correlation with seed yield/plant. This relationship between DTF and DTM with SYP helps in the development of early maturing varieties. Early maturing varieties can sometimes escape disease and increase seed yield. Sridhar et al., 2020 showed similar results with respect to days to maturity. In the present investigation, the polymorphic information content (PIC) values were ranged from 0.18 to 0.70. The highest PIC was exhibited by the CEDG245 primer followed by CEDG198 and VR-9 (0.58). A higher polymorphic information content (PIC) accelerates genetic diversity studies. A higher PIC value helpful in identifying more polymorphic populations. Botstein et al. (1980) described markers as very informative if their PIC value was greater than 0.5, fairly informative if it was between 0.25 and 0.50, and not very useful if it was less than 0.25. Among the 15 markers, seven with PIC value greater than 0.5 and were selected due to highly informative and polymorphic in nature, which indicated that their usefulness in studying the genetic diversity of blackgram. Fourteen markers exhibited a polymorphic nature, which is useful for identifying diverse parents in blackgram breeding programs. Baisakh et al. (2021) reported that molecular marker-based genotyping revealed a tremendously greater percentage of polymorphism (97.05%) with a high average polymorphic information content (PIC) value (0.75). Mogali et al. (2021) reported 24 SSR markers used for detecting polymorphisms in mungbean genotypes.

Genetic similarity analysis using Jaccard's coefficient revealed that 0.13 (PU-15-30

/ PU-07-7) to 0.93 (PU-KUG216 between PU-9/ PU-IPU-243) exhibited significant diversity among the thirty blackgram genotypes, suggesting their further use as potential parents in blackgram improvement breeding programmes. However, a similarity coefficient of 0.93 was shown between genotypes PU-9 and PU-IPU-2-43, indicating a significant degree of genetic resemblance. The results revealed significant molecular diversity among the thirty blackgram genotypes. Kanavi et al., (2019) used a binary method to score the DNA bands produced by SSR-PCR amplification, and NTSYS-pc version 2.1 was utilized to construct a Jaccard's similarity matrix. The similarity index helps in the identification of genetically diverse genotypes. Thirty genotypes of blackgram were grouped into two major clusters. Cluster I was further divided into two subgroups comprising twelve genotypes, while eighteen genotypes belonged to cluster II. Cluster II was further divided in two subgroups. The different clusters were distantly linked to one another, and there seemed to be considerable variation among them. Present findings clearly showed that the fifteen SSR primers revealed a significant amount of genetic variation in the genotypes blackgram. With respect to the dendrogram, a similar type of study was performed by Souframanien and Gopalakrishna, (2009). On the basis of dendrograms, PCA of field data and molecular data of three genotypes, namely, PU-15-2, PU-13-05 and AZAD-2 were performed for group-1; these three genotypes were used for crossing with five other genotypes, namely, PU-UPU-97-1, PU-40, PU-KUG216, MASH-114, and PU-15-32. Taken together, these finding indicates that according to these studies, all the genotypes belong to different genetic backgrounds. This approach is useful for improving blackgram through hybridization programs. With respect to cluster analysis, similar results were obtained *via* PIC value and PCA studies by Nikhil et. al., (2023). In the present investigation, out of fifteen markers, five SSR markers, CEDG-156, CEDG-154, CEDG-092, VR-216 and CEDG-176, were associated with 1000 seed weight. Three markers, namely, VR-102, CEDG-156 and CEDG-176, were associated with the seed yield per plant. These trait-specific markers were selected for further utilization in improving the specific traits associated with blackgram. Morphological and molecular data were subjected to principal component analysis (PCA) in the present study. Two different groups were formed by PCA of field and molecular data. These finding indicates that the morphological and molecular data are related to each other and produce similar results. Thirty blackgram genotypes exhibited enough genetic diversity to be further used in blackgram crossing programs for the genetic improvement of blackgram.

Abbreviations

d.f = Degrees of freedom; PH= Plant height (cm); DTF= Number of days to 50 % flowering; NBP= Number of branches per plant; DTM= Number of days to maturity; NPP= Number of pods per plant; NSP= Number of seeds per pod; PL= Pod length; NCP= Number of clusters

per plant; NPC= Number of pods per clusters; 1000 SW (g)= 1000 seed weight (g); SYP= seed yield per plant (g); PCR Buffer- Polymerase Chain Reaction Buffer; dNTPs- Deoxynucleotide triphosphates; MgCl₂- Magnesium Chloride; F- primer- Forward primer; R-primer- Reverse primer; Taq DNA Polymerase - Thermus aquaticus DNA Polymerase (thermostable) and PC- Principal component.

Declarations

Authors Contributions: RSK and SK conceptualized the experiments and designed the methodology. RSK, SK, SCK and AS performed the field trials and recorded data. RK and SK performed molecular study. RK, SK and SK analyse the data, software implementation and visualization and wrote the original manuscript. SK contributed to the interpretation of results and revision of the manuscript. All the authors have read and approved the final manuscript.

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Conflicts of interest. The authors declare that there are no conflicts of interest.

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Tables

Table 1 List of PCR components

S. No.	Reagents	Concentration	Quantity (μL)
1.	PCR Buffer	1 X	3.0
2.	dNTPS	0.25 mM	0.38
3.	MgCl ₂	2.00 Mm	1.2
4.	F- primer	10 μM	0.75
5.	R- primer	10 μM	0.75
6.	Taq DNA Polymerase	0.75 U	0.15
7.	Template DNA	50 ng/ μl	2.0
8.	PCR grade sterile water	-	6.77
Total			15.0

Table 2 Analysis of variance for different agro-morphological traits under rainfed conditions in blackgram.

Where level of significance: 1% = **, 5% = *

Source	d.f.	PH	DTF	NBP	DTM	NPP	NSP	PL	NCP	NPC	1000 SW	SYP
Replications	2	229.94	10.07	0.40	0.81	107.44	0.24	1.02	.1.80	0.69	4.59	1.54
Genotypes	29	114.70**	19.94**	4.94**	18.95**	91.92**	5.10**	1.69**	11.29**	2.67**	71.97**	5.67**
Error	58	21.62	4.87	0.41	7.44	11.48	0.43	0.33	0.74	0.33	1.68	0.55

Table 3 Phenotypic (r_p) and genotypic (r_g) correlation coefficient analyses for seed yield with ten agromorphological traits.

Characters		DTF	NBP	DTM	NPP	NSP	PL	NCP	NPC	1000 SW	SYP
PH	r_g	-0.161	0.522**	-0.073	0.181	0.153	0.467*	0.477**	0.303*	0.402	0.531**
	r_p	0.261	-0.422**	0.173*	-0.081	-0.053	-0.367**	-0.377	-0.203	-0.302	-0.431*
DTF	r_g	-	-0.176	0.395*	-0.457**	-0.258	-0.380*	-0.217	-0.268	0.370	-0.409**
	r_p		0.276	-0.295	0.557**	0.358	0.480*	0.317**	0.368**	-0.270	0.509**
NBP	r_g		-	0.199	0.062	0.201	0.210	0.453**	0.307*	0.230	0.507*
	r_p			-0.099	0.038	-0.101	-0.110	-0.353*	-0.207	-0.130	-0.407**
DTM	r_g			-	-0.251	-0.197	-0.246	-0.187	-0.324**	-0.241	-0.395**
	r_p				0.351*	0.297	0.346*	0.287	0.424**	0.341	0.495**
NPP	r_g				-	0.377**	0.175	0.402**	0.531**	-0.325	0.674**
	r_p					-0.277	-0.075	-0.302*	-0.431**	0.425**	-0.574**
NSP	r_g					-	0.594*	0.229	0.36**	0.022	0.429*
	r_p						-0.494**	-0.129	-0.26	0.078	-0.329**
PL	r_g						-	0.367	0.292	0.319	0.5**
	r_p							-0.267*	-0.192	-0.219	-0.4*
NCP	r_g							-	0.006	0.110	0.734**
	r_p								0.094	-0.001	-0.634**
NPC	r_g								-	0.211	0.752**
	r_p									-0.111	-0.652**
1000 SW	r_g									-	0.195
	r_p										-0.095

** and * indicate significance at the 1% and 5%, respectively.

Table 4 Details of information about 15 SSR markers used in the study

S. No.	Primer Name	Primer Sequence	Annealing temperature (°c)	Amplicon Size (bp)	PIC
1.	CEDG176	F- GGTAACACGGGTTTCAGATGCC R- CAAGGTGGAGGACAAGATCGG	60°	150-160	0.465
2.	CEDG156	F- CGCGTATTGGTGACTAGGTATG R- CTTAGTGTTGGGTTGGTCGTAAGG	60°	190-200	0.180
3.	CEDG128	F- CTGCCAAAGATGGACAACCTTGGAC R- GCCAACCATCATCACAGTGC	58°	200-210	0.180
4.	CEDG199	F- CCTTGGTTGGAGCAGCAGC R- CACAGACACCCTCGCGATG	56°	163-166	0.422
5.	CEDG154	F - GTCCTTGTTTTCTCTCCATGG R - CATCAGCTGTTCAACACCCTGTG	62°	200	-
6.	VR303	F - GTCCTTGTTTTCTCTCCATGG R - CATCAGCTGTTCAACACCCTGTG	54°	230-240	0.532
7.	VR102	F-CATGTGAGCTACCTTTCAACA R-CAAGGACTGCTATATCCAAGGC	54°	250-260	0.398
8.	VR216	F – TTCCCTGTGTCCTTATATGTCC R- GAGGATAGTGAATTTTGAAGGC	54°	100-160	0.555
9.	LR738A	F- CGCAAAGAGAGAGAGAGAG R- CCCCCATCTGAAAGAAAGAG	57°	206-210	0.518
10.	DMBSSR217	F- TCCTTGCCTTATGATTCTGTGA R- TTTGGCCACTTCCAACTTTA	55°	207-300	0.185
11.	DMBSSR182	F- TAGAGCCTTCTGGTTTTTCACA R- AGGAGGAGGATTTTGATGATGA	54°	150-368	0.298
12.	CEDG198	F CAAGGAAGATGGAGAGAATC R- CCTTCTAAGAACAGTGACATG	50°	206-236	0.652
13.	CEDG092	F- TCTTTTGGTTGTAGCAGGATGAAC R- TACAAGTGATATGCAACGGTTAGG	59°	110-123	0.544
14.	CEDG245	F- GATAGAGCTTAAACCCTC R- CTTTTGATGACAAATGCCC	52°	78-89	0.705
15.	VR-9	F – GGTAGTTCATTTTCGGCCACTT R –GGTAGTTCATTTTCGGCCACTT	61°	257-282	0.586

Table 5 Eigenvalues of the correlation matrix.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
Eigenvalues	2.781	2.458	1.238	1.091	0.792	0.744	0.642	0.490	0.337	0.276	0.149
Proportion	0.253	0.223	0.113	0.099	0.072	0.068	0.058	0.045	0.031	0.025	0.014
Cumulative Proportion	0.253	0.476	0.589	0.688	0.760	0.828	0.886	0.931	0.961	0.986	1.000

Table 6 is available in the Supplementary Files section.

Figures

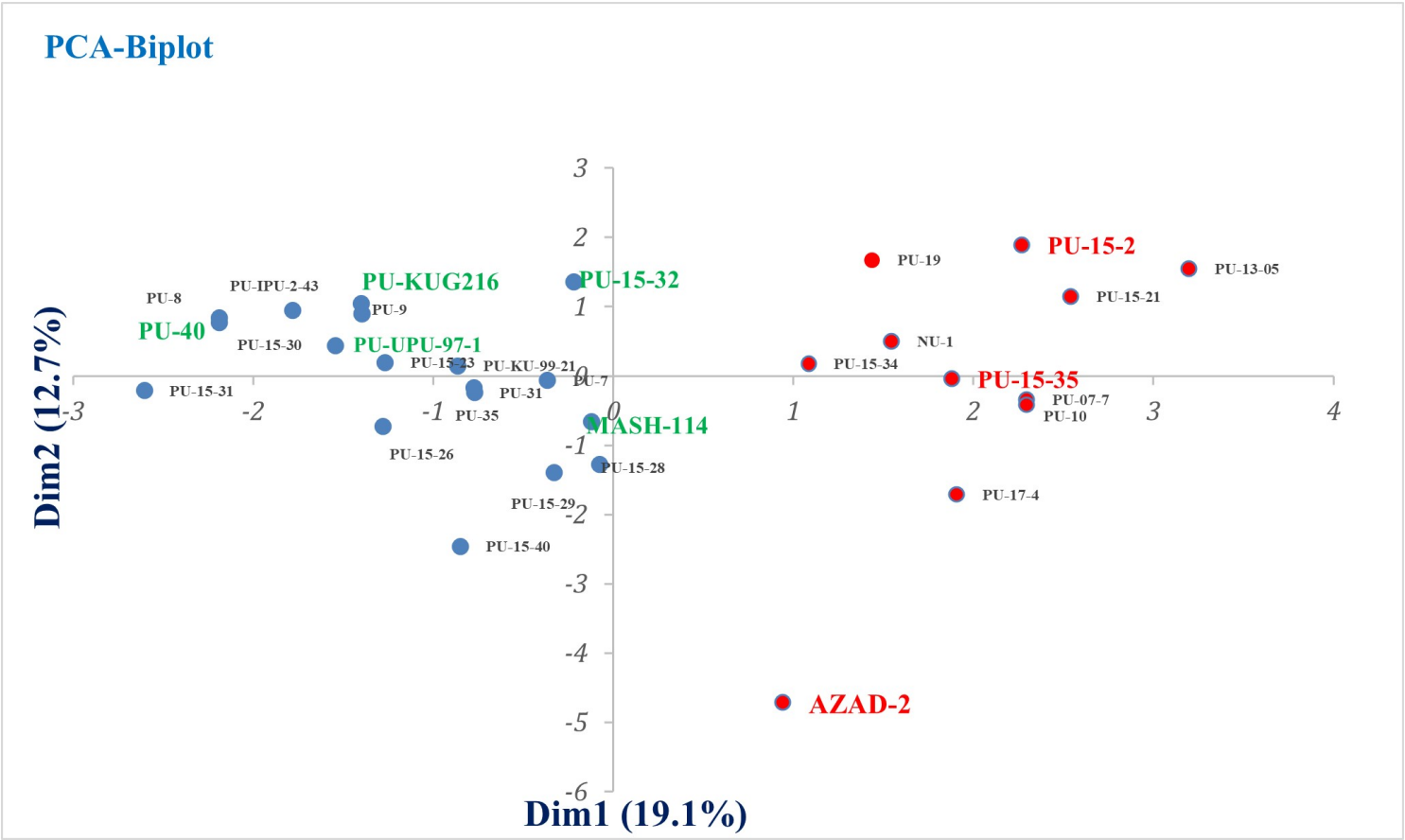


Figure 4
 Distribution of thirty genotypes using phenotypic data on biplot axes

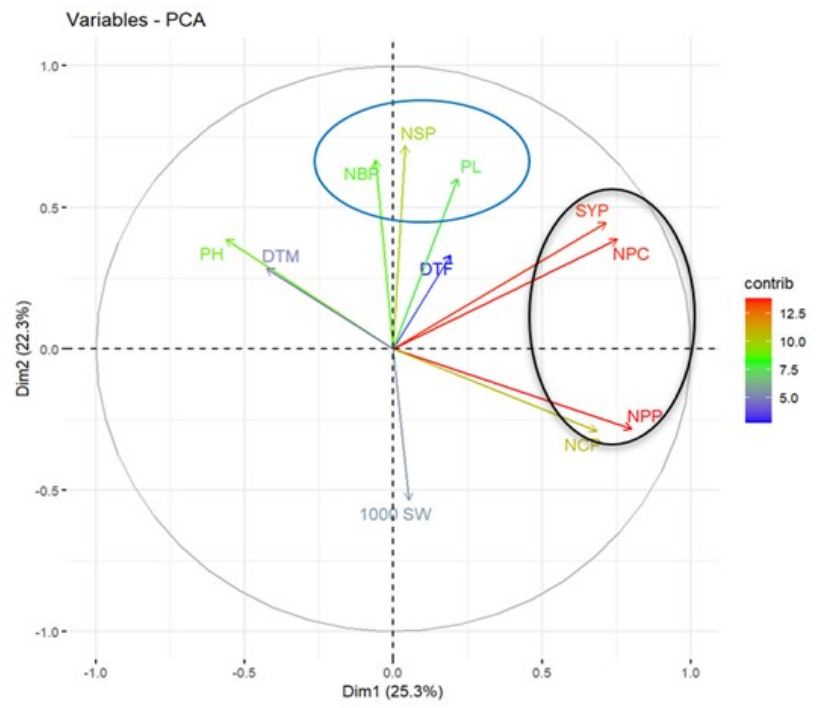


Figure 5
 Distribution of 30 genotypes on the biplot axes from phenotypic data

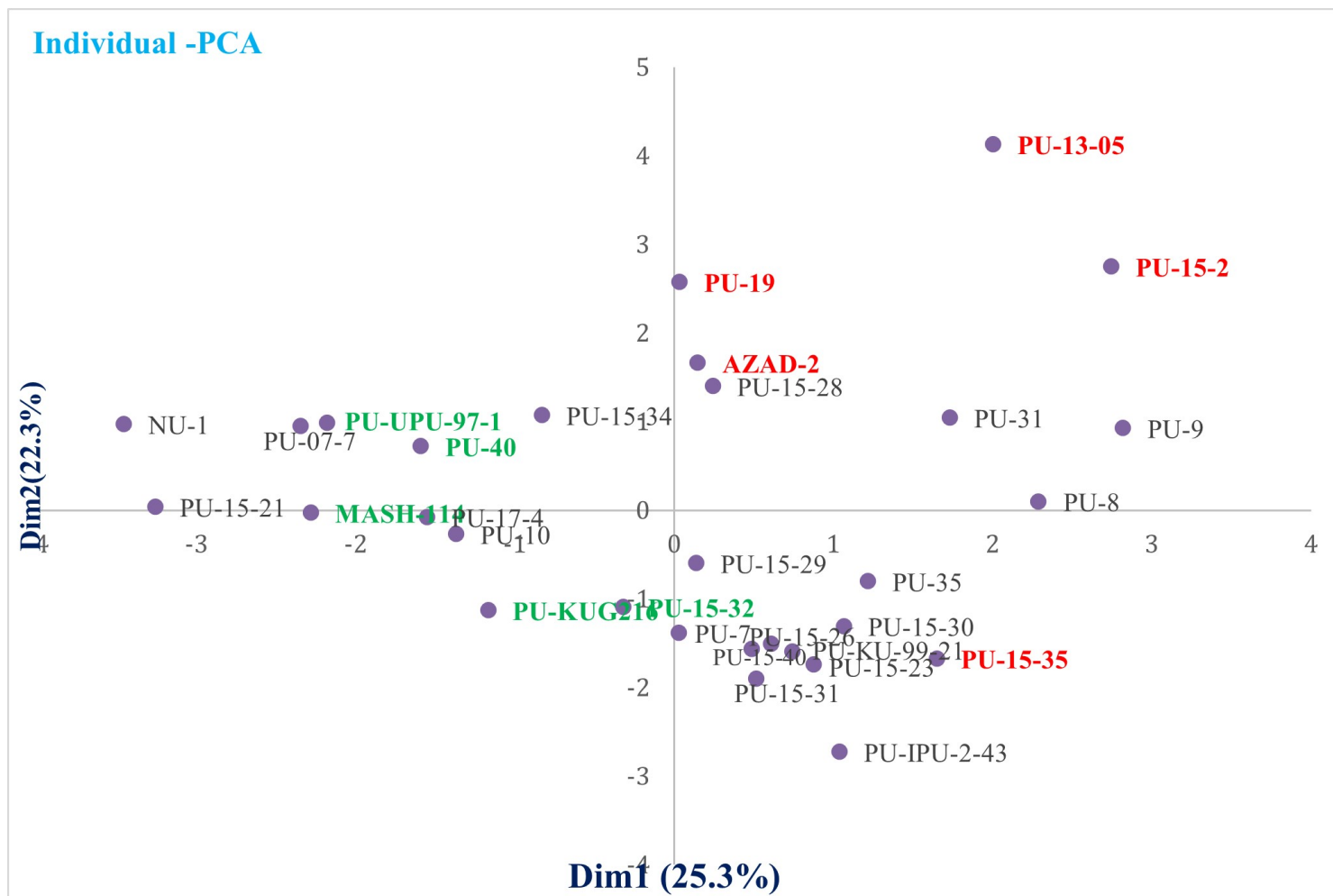


Figure 6

Scatterplot of 30 genotypes on the biplot axis on the basis of molecular data

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table6.docx](#)
- [Supplementaryfile.docx](#)