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Morphological Characterization and Morphological Traits based Genetic Diversity Analysis of Farmer's Pea (*Pisum sativum* L.) Varieties of Uttar Pradesh using DUS Descriptors, as per PPV and FRA, 2001

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ABSTRACT

Background: The conditions of distinctness, uniformity and stability (DUS) must be met in order for a new variety to be registered or protected. DUS testing ensures that a novel variety is unique from other existing varieties and that the farmers will produce a variety with consistent traits that will remain intact in subsequent generations to follow. However, DUS testing does not provide information about genetic diversity as a result, cluster analysis was performed.

Methods: Twenty-two farmers' varieties (FVs) of pea (*Pisum sativum* L.) were collected from Vindhyan zone of eastern Uttar Pradesh and were grown in randomized block design with three replications during *rabi* 2019-20 and 2020-2021. FVs were characterized according to DUS guidelines of the PPV and FRA, 2001 and were subjected for Agglomerative cluster analysis by using Ward's method.

Result: All the characters were found uniform and stable in their expression in consecutive two seasons. PKKK-227 and PMKK-232 were similar to each other, while rest FVs were found distinct. The varieties were grouped into 5 clusters, among which maximum genotypes (8) were in cluster II and minimum genotypes (2) were in cluster V. Cluster I, III and IV had 4, 3 and 5 farmers' varieties respectively. FVs in cluster II are more or less similar to each other; hence they have a common ancestor. Ramaipur had maximum diversity among the villages of Vindhyan zone.

Key words: Cluster analysis, DUS descriptors (as per PPV and FRA, 2001), Pea (*Pisum sativum* L.).

INTRODUCTION

Pea (*Pisum sativum* L.) is a self-pollinated, diploid species (2n=2x=14) belonging to the family *Fabaceae* (*Leguminaceae*) and sub-family *Papilionaceae*. Being rich in vitamins (A, B and C), minerals, dietary fibres and anti-oxidant compounds, 22.5% protein, 4.8 mg/100 g iron and 62.1% carbohydrate, it is considered as a highly nutritive and beneficial crop (Urbano *et al*., 2003; Kesharwani *et al.* 2018).

The lack of genetic diversity has recently been discovered to be the cause of a yield plateau in the pea-crop. Consequently, either the wild relatives or the landraces must be spotlighted. Because crosses with wild relatives result in infertile or sterile offspring, landraces are the right approach (Singh *et al.,* 2014). However, little information on the farmers' varieties, traditional varieties, or landraces is accessible. Farmers' varieties are also disappearing at an alarming rate. Hence, the present investigation is carried out on farmers' varieties.

Varietal descriptors frequently refer to field characteristics rather than seed features. As a result, describing, distinguishing and characterizing the accessions is crucial for an effective seed quality control programme (Kanwar and Mehta, 2018; Manivannan *et al.,* 2013). The morphological markers are of great value in the study of crop (Gepts, 1993) and germplasm evaluation (Bretting and Wildrlechner, 1995), in varietal characterization and distinctiveness (Gilliland *et al.,* 2000; Mall, 2011) and can be used as reference varieties (Singh *et al.,* 2014).

Thus, DUS (Distinctness, Uniformity and Stability) testing principles, given by the Protection of Plant Varieties

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and Farmers' Rights (PPV and FR) Act, 2001 (Anonymous, 2007), are used for the characterization of 22 farmers' varieties of Vindhyan zone Uttar Pradesh. The DUS test will be used for their registration and protection (Singh *et al.,* 2014; Joshi *et al.,* 2018). In addition to this, the morphological traits based genetic diversity was studied by using Ward's method since assessment of genetic diversity may help in accomplishment of genetic enhancement for a specific character (s).

MATERIALS AND METHODS

The twenty two traditional varieties were collected from Vindhyan zone of Uttar Pradesh (Table 1) after extensive study and survey, as per the PPV and FRA project guidelines. The farmers' varieties were then grown under randomized block design (RBD) with three replications during *rabi* 2019-2020 and 2020-2021 at the Field Experimentation Centre of Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, India.

Twenty agro-morphological characters were assessed for the DUS test, as per the guideline of PPV and FR Act, 2001. The qualitative traits obtained were subjected for Agglomerative cluster analysis (Ward, 1963) in STAR 2.0.1 software (IRRI, 2013) to analyse the genetic diversity. The categorical variables recorded were transformed into binary form. Each descriptor was coded with 0 and 1, on the basis of presence and absence of the descriptor code for a specific character. Similar method of cluster analysis was used by Sinha and Mishra, 2013 during their research on rice.

RESULTS AND DISCUSSION

DUS characterization

Characterization of a variety is the foremost important step that should be done by breeders for the classification of a variety into different groups. A qualitative trait gains its own space in the world of breeders, since the qualitative traits are mostly genetically controlled (Sinha and Mishra, 2013). The descriptors used in DUS guidelines are generally qualitative in nature. The pooled data for DUS characterization of farmers' pea varieties is shown in Table 2. Polymorphisms were seen in 17 out of 20 qualitative characters of twenty two farmers' varieties. The non-polymorphic characters were normal stipule, more than 80 cm plant height and absence of seed parchment.

Visual assessment on stem anthocyanin revealed that out of twenty-two farmers' varieties, three varieties *viz.* PSLM-226, PARB-223 and PMRA-502 showed anthocyanin colouration at the base of stem. Farmers' varieties showed polymorphism in foliage colour with maximum genotypes in green foliage followed by light and dark green foliage colour, with five genotypes in each trait. Except genotypes PKKK-227 and PMKK-232, all other genotypes had waxy bloom foliage. PRRA-370, PRRA-370 (A) and PRBJ-229 showed afila type of leaflet while, others had leaflets.

PRRA-370 (A), PKKKK-228, PAKA-230, PARA-501 and PMRA-502 had purple axil colour, while others showed green coloured axil. Rabbit-eared shaped stipules were absent in the twelve varieties. All the genotypes are late flowering (>70 days) except PSRA-358, which flowered between 51-70 days.

Flower standard petal colour showed highest polymorphism among other traits. Fifteen genotypes had white coloured standard petal, five genotypes had pink and the two had blue flower. Thirteen farmers' varieties were having two pods per axil, which may contribute to higher yield. Also, the result revealed that none of the genotypes were having three or more than three pods per axil. Strong Pod curvature was observed for two genotypes; medium for five genotypes; weak for eleven genotypes and pod curvature was absent in four genotypes. Pointed distal part of pod were present in genotypes *viz.* FAIV-425, PATK- 278, PARA-308 (2), PRBJ-229, PARB-223, PAKA-230, PBCM-250, FSAB-428, PARB-223 A (3), PARA-501 and PMRA-502; rest eleven genotypes had blunt shape. Maximum genotypes, *i.e.*, eleven genotypes categorized in green pods.

On morphological assessment of seeds, maximum genotypes were grouped in dimpled seeds (11), followed by spherical seeds (8) and cylindrical seeds (3). Ten genotypes *viz.* FAIV-425, PARB-223, PATK-278, PRBJ-229, PKKKK-228, PAKA-230, PKKK-227, PMKK-232, PARA-501

Table 1: List of farmers' varieties used in the present investigation with their address.

Denomination number	Farmer's name and address
PLCM-225	Lalji Prasad, Chittvishram, Mirzapur
PSAB-309	Sunita Devi, Atanpur, Prayagraj
PRRA-370	Rajendra Pratap, Ramaipur, Prayagraj
PRRA-370 A	Rajendra Pratap, Ramaipur, Prayagraj
PBCM-250	Ballu Maurya, Chittvishram, Mirzapur
PSLM-226	L. Sandip, Lalthara, Prayagraj
FSAB-428	Susheela Devi, Atanpur, Prayagraj
FAIV 425	Ajit Kumar Verma, Itai, Varanasi
PARB-223	Ashutosh, Ramaipur, Prayagraj
PATK-278	Arvind Shukla, Tendui, Prayagraj
PARA-308 (2)	Ashutosh Kumar, Ramaipur, Prayagraj
PRBJ-229	Ramtol Patel, Banpurwa, Prayagraj
PKKKK-228	Kamlesh Kumar Kushwaha, Kulhariya, Prayagraj
PRAV-230	Rajnarayan Patel, Arak Pindi, Varanasi
PAKA-230	Amritlal Sahu, Karanpur, Prayagraj
PSAK-307	Santlal Kushwaha, Amba, Prayagraj
PSRA-358	Shivnath, Ramaipur, Prayagraj
PKKK-227	Kamlesh Kumar, Kulhariya, Prayagraj
PMKK-232	Mansingh Patel, Kulhariya, Prayagraj
PARB-223 A (3)	Ashutosh, Ramaipur, Prayagraj
PARA-501	Awdesh, Jabarapur, Praygraj
PMRA-502	Munish Chiaki, Ramaipur, Prayagraj

Table 2: Pooled data for DUS characterization of farmers' Pea varieties (as per guidelines of PPV and FRA, 2001).

Sr. no.	Characteristics	States	Note	Farmers' varieties
1. (*)	Stem: Anthocyanin colouration	Absent	1	PLCM-225, PSAB-309, PRRA-370, PRRA-370 (A), PBCM-250, FSAB-428, FAIV-425, PATK- 278, PARA-308 (2), PRBJ-229, PKKKK-228, PRAV-230, PAKA-230, PSAK-307, PSRA-358, PKKK-227, PMKK-232, PARB-223 A (3), PARA-501
		Present	9	PSLM-226, PARB-223, PMRA-502
2. (*)	Foliage: Colour	Light green	3	PSAB-309, PBCM-250, PSLM-226, PARA-308 (2), PKKKK-228
		Green	5	PLCM-225, FSAB-428, FAIV-425, PARB-223, PRAV-230, PAKA-230, PSAK-307, PSRA-358, PKKK-227, PMKK-232, PARA-501, PMRA-502
		Dark green	7	PRRA-370, PRRA-370 (A), PATK- 278, PRBJ-229, PARB-223 A (3)
3. (*)	Foliage: Waxy bloom	Absent	1	PKKK-227, PMKK-232
		Present	9	PLCM-225, PSAB-309, PRRA-370, PRRA-370 (A), PBCM-250, FSAB-428, FAIV-425, PATK- 278, PARA-308 (2), PRBJ-229, PKKKK-228, PRAV-230, PAKA-230, PSAK-307, PSRA-358, PARB-223 A (3), PARA-501, PSLM-226, PARB-223, PMRA-502
4. (*)	Leaf: Leaflets	Absent (afila type)	1	PRRA-370, PRRA-370 (A), PRBJ-229
		Present	9	PSAB-309, PBCM-250, PSLM-226, PARA-308 (2), PKKKK-228, PLCM-225, FSAB-428, FAIV-425, PARB-223, PRAV-230, PAKA-230, PSAK-307, PSRA-358, PKKK-227, PMKK-232, PARA-501, PMRA-502, PATK- 278, PARB-223 A (3)
5.	Leaf: Axil colour	Green	1	PSAB-309, PBCM-250, PSLM-226, PARA-308 (2), PLCM-225, FSAB-428, FAIV-425, PARB-223, PRAV-230, PSAK-307, PSRA-358, PKKK-227, PMKK-232, PATK-278, PARB-223 A (3), PRRA-370, PRBJ-229
		Purple	2	PRRA-370 (A), PKKKK-228, PAKA-230, PARA-501, PMRA-502
6.(*)	Stipule: Rabbit-eared stipules	Absent	1	PBCM-250, FSAB-428, FAIV-425, PATK- 278, PARA-308 (2), PSLM-226, PARB-223, PRAV-230, PSRA-358, PKKK-227, PMKK-232, PARB-223 A (3)
		Present	9	PLCM-225, PSAB-309, PRRA-370, PRRA-370 (A), PRBJ-229, PKKKK-228, PAKA-230, PSAK-307, PARA-501, PMRA-502
7.	Stipule: Type	Normal	1	PLCM-225, PSAB-309, PRRA-370, PRRA-370 (A), PBCM-250, FSAB-428, FAIV-425, PATK- 278, PARA-308 (2), PRBJ-229, PKKKK-228, PRAV-230, PAKA-230, PSAK-307, PSRA-358, PARB-223 A (3), PARA-501, PSLM-226, PARB-223, PMRA-502, PKKK-227, PMKK-232
		Vestigial	3	-
8.(*)	Flower: Opening (days)	Extra early (<40)	1	-
		Early (40-50)	2	-
		Medium (51-70)	3	PSRA-358
		Late (>70)	4	PSAB-309, PBCM-250, PSLM-226, PARA-308 (2), PKKKK-228, PLCM-225, FSAB-428, FAIV-425, PARB-223, PRAV-230, PAKA-230, PSAK-307, PKKK-227, PMKK-232, PARA-501, PMRA-502, PATK- 278, PARB-223 A (3), PRRA-370, PRRA-370 (A), PRBJ-229
9.	Flower: Standard petal colour	White	1	PLCM-225, PSAB-309, PRRA-370, FSAB-428, FAIV-425, PATK- 278, PARA-308 (2), PRBJ-229, PSLM-226, PARB-223, PRAV-230, PSAK-307, PSRA-358, PARB-223 A (3), PARA-501
		Blue	2	PKKK-227, PMKK-232
		Pink	3	PRRA-370 (A), PBCM-250, PKKKK-228, PAKA-230, PMRA-502
		Red	4	-
		Purple	5	-
10.(*)	Pod: Number/Axil	Single	1	PBCM-250, PSLM-226, FSAB-428, PKKKK-228, PARB-223 A (3), PARA-501, PKKK-227, PMKK-232, PMRA-502
		Double	2	PLCM-225, PSAB-309, PRRA-370, FAIV-425, PATK- 278, PARA-308 (2), PRBJ-229, PARB-223, PRAV-230, PSAK-307, PSRA-358, PRRA-370 (A), PAKA-230
		Multiple	3	-

Table 2: Continue.....

Table 2: Continue.....

11.(*)	Pod: Curvature	Absent	1	PLCM-225, PSAK-307, PMKK-232, PKKK-227
		Weak	3	PRRA-370, PSAB-309, PRBJ-229, PRAV-230, PSRA-358, PARB-223 A (3), PARA-501, PRRA-370 (A), PBCM-250, PSLM-226, FSAB-428
		Medium	5	FAIV-425, PARB-223, PARA-308 (2), PAKA-230, PKKKK-228
		Strong	7	PATK- 278, PMRA-502
12.(*)	Pod: Shape of distal part	Pointed	1	FAIV-425, PATK- 278, PARA-308 (2), PRBJ-229, PARB-223, PAKA-230, PBCM-250, FSAB-428, PARB-223 A (3), PARA-501, PMRA-502
		Blunt	9	PLCM-225, PSAB-309, PRRA-370, PRRA-370 (A), PSLM-226, PRAV-230, PKKKK-228, PKKK-227, PMKK-232, PSAK-307, PSRA-358
13.	Pod: Intensity of green colour	Light green	3	PLCM-225, PKKKK-228, PSAK-307, PSRA-358
		Green	5	PSAB-309, PRRA-370, PRRA-370 (A), PSLM-226, PRAV-230, PKKK-227, PMKK-232, FAIV-425, PARA-308 (2), PRBJ-229, PARB-223, PAKA-230, PBCM-250, FSAB-428, PARB-223 A (3), PARA-501, PMRA-502
		Dark green	7	PATK- 278
14.(*)	Plant: Height	Short (<60 cm)	3	-
		Medium (60-80 cm)	5	-
		Long (>80 cm)	7	PLCM-225, PKKKK-228, PSAK-307, PSRA-358, PSAB-309, PRRA-370, PRRA-370 (A), PSLM-226, PRAV-230, PKKK-227, PMKK-232, FAIV-425, PARA-308 (2), PRBJ-229, PARB-223, PAKA-230, PBCM-250, FSAB-428, PARB-223 A (3), PARA-501, PMRA-502, PATK- 278
15.(*)	Seed: Shape	Spherical	1	PRRA-370, PRRA-370 (A), PATK- 278, PRBJ-229, PSAK-307, PSRA-358, PARB-223 A (3), PMRA-502
		Cylindrical	2	PLCM-225, PBCM-250, FAIV-425
		Dimpled	3	PSAB-309, PSLM-226, FSAB-428, PARB-223, PARA-308 (2), PKKKK-228, PRAV-230, PKKK-227, PMKK-232, PARA-501, PAKA-230
16.	Seed: Surface	Smooth	1	FAIV-425, PARB-223, PATK-278, PRBJ-229, PKKKK-228, PAKA-230, PKKK-227, PMKK-232, PARA-501, PMRA-502
		Wrinkled	2	PLCM-225, PSAB-309, PRRA-370, PRRA-370 (A), PBCM-250, PSLM-226, FSAB-428, PARA-308 (2), PRAV-230, PSAK-307, PSRA-358, PARB-223 A (3)
17.(*)	Seed: Cotyledon colour	Creamy	3	PLCM-225, PRRA-370, PRRA-370 (A), PBCM-250, FSAB-428, PARB-223, PARA-308 (2), PRAV-230, PSAK-307, PARB-223 A (3)
		Green	5	PSLM-226, FAIV-425, PATK-278, PRBJ-229, PKKKK-228, PAKA-230, PSRA-358, PARA-501, PMRA-502
		Yellow	7	PSAB-309
18.	Seed: Weight of 1000 seeds	Small (<150 g)	3	PLCM-225, PRRA-370, PRRA-370 (A), PBCM-250, FAIV-425, PATK- 278, PARA-308 (2), PRBJ-229, PKKKK-228, PRAV-230, PAKA-230, PSAK-307, PSRA-358, PKKK-227, PMKK-232, PARB-223 A (3), PARA-501, PMRA-502
		Medium (150-200 g)	5	PSAB-309, PSLM-226
		Large (>200 g)	7	FSAB-428, PARB-223
19.	Seed: Testa mottling	Absent	1	PLCM-225, PRRA-370, PRRA-370 (A), PBCM-250, PARA-308 (2), PRBJ-229, PKKKK-228, PRAV-230, PAKA-230, PSRA-358, PKKK-227, PMKK-232, PARB-223 A (3), PARA-501, PMRA-502, PSAB-309, PSLM-226, FSAB-428, PARB-223
		Present	9	FAIV-425, PATK- 278, PSAK-307
20.	Seed parchment	Absent	1	PLCM-225, PKKKK-228, PSAK-307, PSRA-358, PSAB-309, PRRA-370, PRRA-370 (A), PSLM-226, PRAV-230, PKKK-227, PMKK-232, FAIV-425, PARA-308 (2), PRBJ-229, PARB-223, PAKA-230, PBCM-250, FSAB-428, PARB-223 A (3), PARA-501, PMRA-502, PATK- 278
		Present	9	-

and PMRA-502 showed smooth seeds whereas, other twelve genotypes showed wrinkled seeds.

Cotyledon colour revealed that maximum genotypes were having creamy coloured cotyledon, followed by green coloured cotyledon and yellow coloured cotyledon. Eighteen farmers' varieties were having small seed with less than 150 g weight. Genotypes PSAB-309 and PSLM-226 were grouped into medium seeds category. Genotypes FSAB-428 and PARB-223 exhibited more than 200 g for 1000-seeds. Only three Genotypes showed testa mottling. Morphological assessment of seeds revealed that all the genotypes under study were lacking seed parchment.

Farmer's varieties are generally niche specific and dispersed through informal system of seed exchange. This implies that the main basis of the farmers' varieties may not have plant types with spectacular morphological variation. Yet, careful observations reveals perceivable differences for flower colour, stipule type, anthocyanin colouration, pod shape, seed shape and cotyledon colour *etc.*

It is estimated that all the 20 characters under study were found uniform and stable in their expression in consecutive two seasons. These characteristics were found to be sufficient to assess distinctiveness, which means that no statistical method was needed for the interpretation of these visually assessed characteristics. The farmers' varieties PKKK-227 and PMKK-232 were similar to each other, rest other farmers' varieties of pea were distinct from each other.

Similar studies of varietal characterization using DUS guideline have also been made in Pea by Singh *et al.* (2014), Ardhani *et al.* (2019), Kalapchieva *et al.* (2020) and Rosero-Lombana and Checa-Coral (2021). Katiyar *et al., (*2010) in urdbean, Mall, (2011) in french-bean, Mounika *et al.* (2020) in mung-bean, Durga *et al.* (2015) in horse gram and Janghel *et al.,* (2020) in chickpea, also evaluated their crops according to DUS guideline and they came to similar findings.

Pre-breeding or genetic enhancement needs emphasis for transfer or introgression of genes and gene combinations from un-adapted source into more usable breeding material (Singh *et al*., 2014). Further, these varieties can be used in varietal improvement programme depending upon the desired characteristics.

Cluster analysis

According to the agglomerative cluster analysis, twenty-two farmers' pea varieties were grouped into five clusters (Table 3, Fig 1). Maximum genotypes (8) were grouped into cluster II, whereas minimum genotypes (2) were grouped into cluster V. Cluster I, III and IV included 4, 3 and 5 farmers' varieties respectively (Table 3). Varieties of each cluster were collected from the different parts of Vindhyan Zone of Uttar Pradesh which indicated that the clustering pattern did not follow the geographical distribution. The observation is in conformity with the observation of Sinha and Mishra, 2013. When materials of the same region are grouped into different clusters, they indicate the broad genetic base of the

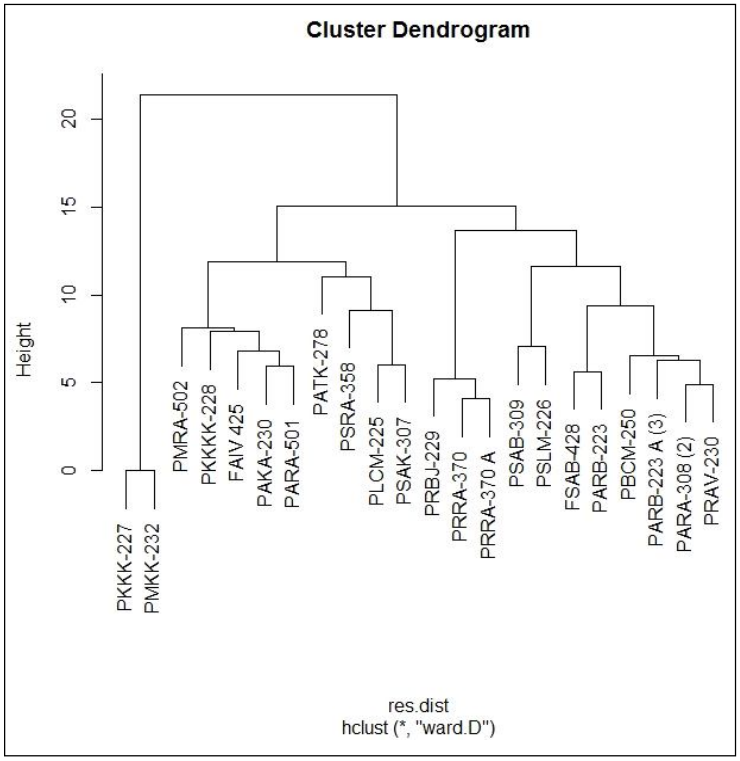

Fig 1: Dendrogram obtained by agglomerative cluster analysis by ward's method estimated from 20 agro-morphological traits analyzed in 22 farmers' varieties of pea.

Table 3: Distribution of 22 FVs into different clusters.

Clusters	Number of FVs	Farmer varieties (FVs)
I	4	PLCM-225, PATK-278, PSAK-307, PSRA-358
II	8	PSAB-309, PBCM-250, PSLM-226, FSAB-428, PARB-223, PARA-308 (2), PRAV-230, PARB-223 A (3)
III	3	PRRA-370, PRRA-370 A, PRBJ-229
IV	5	FAIV 425, PKKKK-228, PAKA-230, PARA-501, PMRA-502
V	2	PKKK-227, PMKK-232

genotypes belonging to that region (Shanmugam and Rangasamy, 1982). Therefore, Ramaipur village can be said to have maximum diversity among these villages (Table 1). The genotypes in cluster II are more or less similar to each other, as a result, it may be safely said that these genotypes have a common ancestor. Since they had a common origin, the potential variability is less among these eight genotypes. So breeders should focus on selecting genotypes from any two different clusters to develop potential variability in their progenies.

CONCLUSION

It can be concluded that the morphological DUS descriptors can be effectively used for identification and grouping of varieties. Farmer's varieties satisfying the DUS criteria for these morphological descriptors could be registered under PPV and FR Act. A wide range of variation was assessed during DUS characterization for all the characters except type of stipule, plant height and seed parchment, which was normal, long and absent, respectively in all farmers' varieties. Out of twenty-two farmers' varieties, twenty farmers' varieties were found distinct from each other. Since the DUS field test is not able to establish that the variety PKKK-227 and PMKK-232 is distinct, therefore special laboratory tests can be conducted (physiological/chemical/molecular tests) for them to establish that the varieties are distinct (Section 29, PPV and FR rules). Cluster analysis revealed that genotypes can be grouped together based on their differences in qualitative traits. Breeders will benefit from this categorization of varieties to different clusters since, cluster analysis is a valuable method for determining variability and identifying potential varieties which can be used in future breeding programmes.

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Conflict of interest: None.

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