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IMAGE ANALYSIS AS AN EMERGING TOOL FOR VARIETAL IDENTIFICATION IN CHICKPEA (*CICER ARIETINUM L*) VARIETIES

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ABSTRACT

24 desi chickpea genotypes were evaluated for distinctiveness based on 13 qualitative and 7 quantitative morphological DUS descriptors. Eight traits each were observed to be monomorphic and dimorphic in nature; and only four traits were observed to be polymorphic in nature. Identification profiles were generated on the basis of grouping and essential characters prescribed by DUS Guidelines of PPV & FR Authority. Out of twenty four desi genotypes, distinct profiles could not be established for all the genotypes owing to overlapping of state of expression for various traits. Twenty-four genotypes of desi group were grouped into four clusters a critical appraisal of the observations suggested that none of the clusters contained genotypes with all the desirable traits, which could be directly selected and utilized. Interestingly, all the minimum and maximum cluster mean values were distributed in relatively distant clusters. For image analysis studies, a flatbed scanner was used for image acquisition and all the images were grabbed at resolution of 600 dpi. The database of images generated for characterization was essential to display variability of the material and also provided a supplementary description of the descriptors by visual parameters. Out of various images examined differentiation on the basis of flower traits viz. colour intensity of petals and the petal venation pattern; were found to be most useful. Thus, the identity of all the genotypes could be established on the basis of visual differences observed on the basis of these traits. Hence, image analysis technique can be used to successfully complement the existing DUS morphological descriptors for establishing distinctiveness of closely related varieties.

Keywords: Chickpea, Distinctiveness, Image Analysis, Machine Vision, Morphological Descriptors.

I. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important and earliest cultivated legumes, approximately 7500 years old [1] and ranks second among the world's food legumes in terms of area. India is the largest producer of chickpea (Bengal gram) which is highly rich in protein content and other essential nutrients. As per the Protection of Plant Varieties and Farmer's Rights Act, 2001, 20 morphological DUS descriptors are used as a taxonomic tool for distinguishing chickpea at varietal level [2]. Plant morphological characters have been recognized as the universally undisputed descriptors for DUS testing and varietal characterization of crop varieties. Keeping this in view, the study was taken up with the objective to determine the relative extent of distinctiveness of different morphological DUS descriptors for 24 *desi* genotypes. Since manual identification of varieties by specialized technicians is slow, has low reproducibility, and possesses a degree of subjectivity [3],

image analysis studies were carried out to establish distinctness of the varieties. The diversity analysis was also worked out using 7 agro-morphological traits so as to ascertain the variability level of the present material.

II. MATERIALS AND METHODS

The research material consisted of 24 genotypes of *desi* chickpea. The genotypes were raised during *rabi* season 2014-15 and 2015-16 at the research farm of Division of Seed Science and Technology, ICAR-IARI, New Delhi. A plot size of 5 rows with each row of 5 meter length was maintained. Row to row and plant to plant spacing was maintained at 45cm x 20cm. The material was replicated thrice and all the agronomic practices were followed to raise a good crop. Ten competitive plants were randomly selected from each genotype in each replication to record the data. National DUS Test Guidelines (2007) were followed beginning from the trial layout to recording of the last field-related observation.

Since the major objective of cultivar characterization is to establish distinctness among varieties, hence an attempt was made to study the utility of the observed characters in establishing distinctness. The characters were grouped on the basis of their ability to differentiate varieties viz. monomorphic, dimorphic and polymorphic traits. The DUS guidelines recognises four traits as grouping characters viz., time of flowering, flower colour, seed colour and seed size. An identification key involving grouping and essential characters was developed for generating distinct varietal signatures for these *desi* genotypes. The 7 agro-morphological traits viz., stem height at initiation of first flower, time of flowering, leaflet size, peduncle length, plant height, pod size, and seed size were also used to assess the variability of these varieties using Ward's Minimum Variance Cluster analysis which helps in assessment of pattern and extent of variation in the germplasm.

The image analysis studies were carried out on the flowers of *desi* chickpea varieties. Observations were recorded on ten flowers per variety and were replicated thrice. A flatbed scanner (Canon LiDE 110 version 1.2.00) was used for image acquisition. Images of all the flowers were grabbed at resolution of 600 dpi. All images were grabbed using identical settings. The images were stored in *.tif* format for further analysis.

III. RESULTS AND DISCUSSION

3.1. Morphological Characterization

The accurate description and identification of chickpea genotypes is crucial for DUS Testing. Hence, an attempt was made to establish the distinctive profiles of chickpea varieties by using a set of morphological characteristics prescribed in DUS Test Guidelines. The characters were grouped on the basis of their ability to differentiate varieties viz., monomorphic, dimorphic and polymorphic traits. In d*esi* type chickpea, out of 13 qualitative traits, 7 traits viz. stem anthocyanin colouration, leaf pattern, flower colour, stripes on standard of flower, number of seeds/pod, seed ribbing and seed type were observed to be monomorphic, 2 traits viz. green colour intensity of foliage, and number of flower/peduncle were dimorphic and the rest 4 viz. growth habit, seed colour, seed shape and seed testa texture were polymorphic in nature. With respect to 7 quantitative traits, 6 traits (stem height at the initiation of first flower, leaflet size, peduncle length, plant height, pod size and seed size) were observed to be dimorphic in nature and only 1 (days to 50% of flowering) was monomorphic in nature.

Since the major objective of cultivar characterization is to establish distinctness among varieties, hence, an attempt was made to study the utility of the observed characters in establishing distinctness. The DUS guidelines recognises four traits as grouping characters viz., time of flowering, flower colour, seed colour and seed size. The two plant morphological traits viz., time of flowering and flower colour were monomorphic in nature since all were observed to flower late (> 80 days) and all *desi* genotypes had pink flowers. Hence, distinctive profiles could not be generated on the basis of grouping characters alone. Therefore, essential characters were used to establish the individual identity by preparing an identification key for all the genotypes in both the groups. However, no single trait could identify the genotypes individually, but was used in combinations. Similar results were also reported earlier in chickpea [4]. The expression of the qualitative traits in all the genotypes was similar for both the seasons, confirming the stability of genotypes.

Anthocyanin colouration of the stem was present in all the ***desi*** genotypes (Fig.1). Four genotypes viz. Pusa 372, JG 315, C 235 and Radhey had medium stem height (*first group*) whereas the remaining twenty genotypes had high height of stem at initiation of first flower (*second group*). The four genotypes of the first group with shorter height of stem could be individually identified on the basis of number of flower per peduncle, peduncle length and plant height. In the second group, JG 11 could be identified from the spreading nature of the plant. Genotypes RSG 143-1, BG 1103 and IPC 2009-198 were erect in nature and could be singled out on the basis of peduncle length for RSG 143-1 (medium) and plant height (medium for BG 1103 and tall for IPC 2009-198). The remaining genotypes were semi-erect in nature and traits like leaflet size, peduncle length, plant height, number of flowers per peduncle and pod size were used to establish identity. However, genotypes Pusa 256 and JG 14 could not be differentiated on this basis. Similarly, H 00-108 BG 362 were found to be similar. PDG 84-16, PG 96006, KGD 1168 AND BGD 72 also fell in the same group and could not be individually differentiated.

3.2. Diversity Analysis

The analysis of variance revealed the presence of significant variability among the genotypes for all the characters studied. Twenty-four genotypes of *desi* group were grouped into four clusters. Maximum number of genotypes (8) was included in cluster I (TABLE 1). Cluster II and III had 6 genotypes each whereas cluster IV had 4 genotypes. A perusal of results on cluster means (TABLE 2) revealed that the cluster I could be characterized by shorter height of stem at initiation of first flower (14.73), early flowering (100.63), small leaflet size (0.96), medium plant height (49.11), small pod size (2.03) and lowest seed size (11.42) as compared to genotypes of Cluster IV with tall height of stem at initiation of first flower (16.48), late flowering (111.75), large leaflet size (1.23), taller plant height (65.94) and higher pod size (2.37).

Thus, a critical appraisal of the observations suggested that none of the clusters contained genotypes with all the desirable traits, which could be directly selected and utilized. Interestingly, all the minimum and maximum cluster mean values were distributed in relatively distant clusters. The results thus emphasise that hybridization between genotypes of different clusters is necessary for the development of desirable genotypes. Ahmad *et al.* (2012) [5] studied the genetic diversity of 70 accessions of *Cicer arietinum* using morphological traits, seed protein and molecular markers and reported that the clustering pattern did not show any grouping that could be attributed to either the geographic distribution or the field performance. Similar findings were also reported by

other researchers [6 and 7] while studying the genetic divergence of rice varieties. Hence, depending on the *per se* performance of the best genotypes within the clusters, they may be directly used for adaptation or may be used as parents in future hybridization programmes. The grouping of genotypes is of practical value to chickpea breeders in identifying the genotype with desired trait for utilization in breeding program for genetic improvement [8].

3.3 Image Analysis Studies

In *desi* chickpea genotypes, it was observed that flower colour for all the varieties was pink. Since flower colour is listed as a grouping character in the DUS guidelines, hence an attempt was made to distinguish the *desi* varieties on the basis of visual differences observed in the petal colour intensity and venation pattern. The various floral images generated through machine vision i.e. scanner are depicted in Fig.2. These pictures for characterization are essential to display variability. They also provide a supplementary description of the descriptors by visual images. The morphological characterization revealed that out of twenty four desi genotypes, distinct profiles could be created only for sixteen varieties. Genotypes Pusa 256 and JG 14 were difficult to differentiate from each other. Similarly, H 00-108 and BG 362 were also identical for all observed traits. Genotypes PDG 84-16, PG 96006, KGD 1168 and BGD 72 could also not be individually differentiated.

Based on visual differences between floral images, it was observed that Pusa 256 had bright pink petals compared to dull pink petals of JG 14. Further, the venation pattern was more prominent in the latter as compared to the former. In Pusa 256, petal width was equal from top to bottom whereas for JG 14, the petals were broader at the middle. Similarly, H 00-108 and BG 362 could also be distinguished on the basis of floral morphology. H00-108 had light pink, elliptical petals with veins prominent only near the centre, whereas BG 362 had dark pink, round petals with prominent venation pattern observed throughout the petal width. Genotypes PDG 84-16, PG 96006, KGD 1168 and BGD 72 could also be distinguished easily on the basis of visual differences between petal colour intensity and venation pattern. Hence, image analysis techniques can be used to complement the existing DUS morphological descriptors, since it is also a cost-effective technology wherein a scanner can also detect the visual differences. Lootens *et al.* (2007) reported that image analysis is helpful to evaluate all colours present in given material and is stable as it is referenced against standard colour charts. Besides the use of this method for DUS testing, the derived quantitative colour data can be applied to quantitative genetics for inheritance studies. Many researchers also suggested machine vision as an alternative for an automated, non-destructive and cost-effective technique to quantify the qualities of various rice varieties [9,10 and 11].

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Table 1: Clustering pattern for 24 desi Chickpea Genotypes

Cluster	No. Of Genotypes	Varieties
I	8	JG 315, GNG 663, Pusa 372,C235, SAKI 9516, Radhey , GPF-2, PDG 84-16
II	6	IPC 92-1, BG 362, JG 11, JG 14, BGD 72, IG 72933
III	6	FG 711, PG 96006, RSG 143-1, Chaffa , KGD 1168, CSG 9807
IV	4	IPC 2009-198, H 00-108, BG 1103, PUSA 256

Table 2. Cluster mean values for agro-morphological traits and their relative contribution to genetic divergence for *desi* type

Character	Cluster I	Cluster II	Cluster III	Cluster IV
Stem: Height at initiation of first flower	14.73	16.29	15.07	16.48

Time of Flowering	100.63	110.50	106.50	111.75
Leaflet: Size	0.96	1.19	1.16	1.23
Peduncle: Length	1.07	1.29	1.02	1.13
Plant: Height	49.11	53.33	49.73	65.94
Pod Size (Length)	2.03	2.21	2.05	2.37
Seed Size:(Wt. of 100 seeds at 10% moisture content)	11.42	12.71	20.56	17.63

Figure 1. Identification key for *desi* chickpea varieties based on essential characters

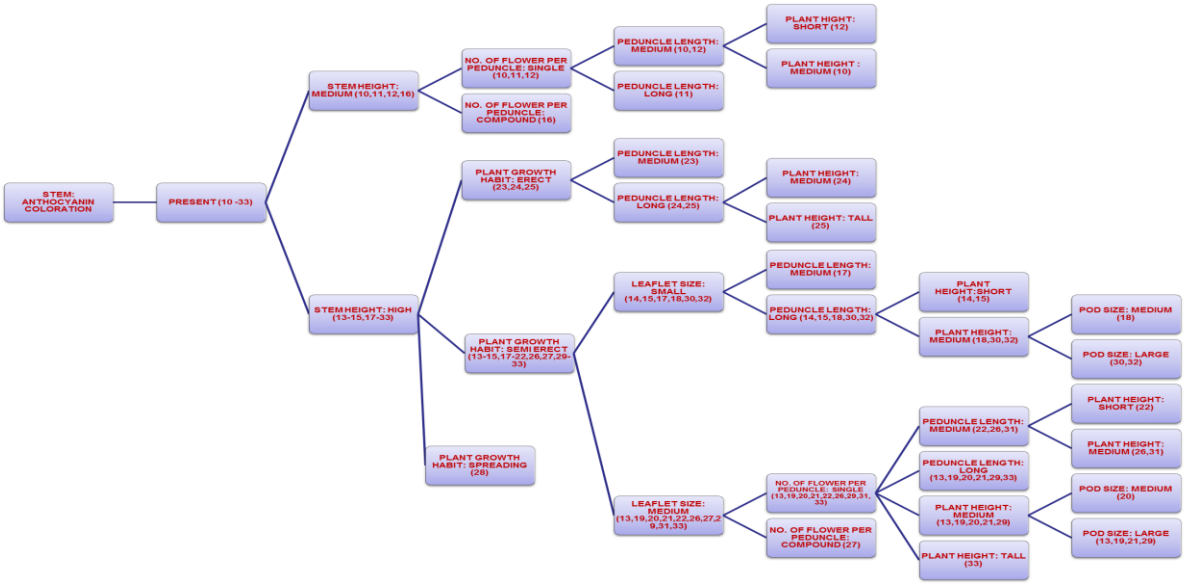

Figure2. Image of *Desi* type Flower by Scanner