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Cultivar identification and alternative varietal distinction techniques in selected marigold (*Tagetes* spp. L.)

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ABSTRACT

Marigold is mainly cultivated and propagated through seeds and the cultivar identification is the key issue in crop improvement programme because of less variation available in the genotypes. Hence, a study was undertaken to identify the genotypes at various stages of seed crop growth. The experimental results showed that, genotypes can be identify/distinguish at various growth stages with available descriptors and also using additional descriptors as well. Based on seed geometric properties, seed phenotyping was made using image-based machine vision software showed that these seed traits and can be successfully used to distinguish the cultivar seeds and in an attempt made to identify/distinguish two species of marigold (African and French) resulted as a successful approach in establishing alternative varietal identification system.

***Keywords* :** Cultivar identification, seed phenotyping, venation pattern and marigold seeds

Marigold (*Tagetes species* Linn.), a member of family Asteraceae, is an annual flower crop native to Central America (Neher, 1968). Genetic diversity in marigold (within and between a species) is important for its plant improvement programme (Janakiram and Rao, 1991). There are nearly 36 species in *Tagetes* most widely known are *T. erecta* and *T. patula* which are commonly called as African and French types respectively. The African marigold is noted for their large flower heads which are aromatic and plants have pinnate leaves on glabrous and angular stems. Whereas, the French marigold is a compact annual and possess small aromatic flowers, plants have pinnate leaves with toothed and lance-shaped leaflets, which are also aromatic. Besides its significance in ornamental horticulture, it is the oldest medicinal plant (Krol, 2011). The marigold flowers are widely used in folk medicine, particularly for curing inflammatory disorders (Nahak and Sahu, 2017); finds applications in cosmetic industries, colouring industries and therapeutic industry (Gupta and Vasudeva, 2012). Apart from these, its extract can be used as (pre-sowing) seed priming treatments (Mavi, 2014). To maintain the quality of seed, a careful attention is needed at every stage of seed production. The proper identification of a variety itself serves the important goals such as maintenance of genetic purity, mitigating legal claims and confirming intellectual property rights; thereby studies are need to be undertaken to identify the plant varieties. Marigold cultivars are highly cross pollinated and having lack of homogeneity in traits such as flower colour, floret type, shape and size of the flower. Further, presence of large proportions of off-types in seed lots resulting impact on economic yield and quality of the marketable produce. Currently, the varietal identification in marigold is generally done using morphological descriptors and to some extent with the help of biochemical or molecular markers. The phenotypic evaluation of field-based morphological characteristics time consuming and it is seems to be inadequate because of less genetic variability, and biochemical and molecular markers are costly and they require high technical skills. Except for the flower size/colour, these types (African/French) and cultivars (within the type) are very difficult to identify at various growth stages. The established descriptors are not enough to characterize all the available cultivars (Chennem, 2016; Monika *et al*, 2017). Hence, some additional descriptors are needed to be developed besides to the DUS/PPVandFRA descriptors. There are number of marigold verities are currently in cultivation whose identity and distinctness needed to be established by various approaches like machine vision and using simple alternative techniques.

MATERIALS AND METHODS

The genotypes are evaluated for various morphological characters as described by the UPOV and PPV and FRA. The experiment was laid out in a Randomized Block Design (RBD) with five replications; 30 days old seedlings were transplanted during Rabi 2015-16 and 2016-17 in 60 × 45 cm spacing and the crop was raised as per the standard agronomic practices. The observations were made on five randomly selected plants in each replication throughout the growing period. Besides, an attempt was made to distinguish these genotypes using leaf venation pattern and leaflet traits. The obtained results were analyzed using SAS 9.4 available at ICAR-IASRI, New Delhi.

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Machine vision analysis was done using leaf, flower (florets) and seeds. The observations (for seed traits) were recorded on ten seeds/variety using three replications in each genotype. a. Image acquisition: A flat-bed scanner (Canon LiDE 110 version 1.2.00) at 600 dpi resolution was used to capture high quality image of leaf, flower (florets) and seeds. b. Data processing and analysis: Leaf and flower venation patterns were computed using scanned images, and seed size and shape differences were observed and computed using software designed and developed by CIAE, Bhopal. Total eleven seed geometric properties were studied and measured from the software.

RESULTS AND DISCUSSION

The genotypes evaluated for various morphological and machine vision traits. The machine vision based observations on seed geometric properties are presented in the table- 1, morphology based quantitative traits like plant, leaf, flower and seed traits are presented in table 2 and 3, and qualitative traits are presented in the table-4. Significant difference in genotypes with respect to peduncle length, flower diameter, floret length and width, seed yield and 1000-seed weight was noticed during *rabi* 2015-16 and 2016-17 and the genotypes showed distinct variations in these studied traits during both seasons. The genotypes registered significantly different peduncle length during both seasons of investigation, it was ranged from 14.00 cm (PA) to 7.14 (PBG); A significant difference was also noticed in all the genotypes with regard to the diameter of flower which was varied from 7.26 cm (PNG) to 4.18 cm (GO); Leaf length among the genotypes ranged between 10.95 cm (PBG) to 20.20 cm (PA) whereas, wider and narrow leaves were seen in PA (12.35 cm) and PNG (6.21 cm), respectively; Among the genotypes, floret length ranged from 2.54 cm (PBG) to 1.64 cm (GO) and floret width ranged between 1.74 cm (PA) to 1.26 (PBG). A significant distinction in seed yield plant^{-1} was also registered in all the genotypes and it ranges from 6.18 g (PNG) to 3.77g (GO). All the genotypes found distinctly different in 1000-seed weight, it ranged from 2.85g (PNG) to 2.01g (GO) and it remains constant over the two seasons of study. Among various qualitative characteristics, traits like leaf type, leaf margin, floret shape, floret's margin and flower colour (RHS reference code) shown varied degrees of distinction the genotypes studied. These results were in conformity with the author Singh *et al.* (2008); Pramila (2010) and Gobade *et al.* (2017) who differentiated the marigold verities using the inflorescence descriptors like pedicel length, floret type and shape, incision of margin, and seed weight. Further, the authors opined that marigold genotypes can be distinguished using these traits. Images showing variations in leaf traits like leaflet length and width, leaf venation patterns and distance between two leaflets are presented in (Fig 1a to 1b). Though the leaf appears to be same in terms of length and width but it distinctly differs in leaflet (terminal) length and width, and distance between two leaflets (Fig. 1a, b). Based on the visual recognition, leaflet length and width, and distance between two leaflets is more in PBG than PNG and the leaf venation pattern in PNG was more prominent and distinctly visible with prominent serrations on the leaf margin (dorsal view) and number of oil glands on the leaflets was more (upto 58 in middle leaflets) (Fig. 1a). Whereas in PBG, leaf venation pattern was distinctly less, having moderate serrations on the leaf margin and it possess lesser number of oil glands (up to 45 in the middle leaflets). Genotypes PA and GO were very difficult to differentiate from each other in terms of variations in leaf and leaflet and venation pattern (Fig. 1b). However, PA shows lesser distinctly less venation with near prominent serrations on the leaf margin and the oil glands were up to 27. Whereas, GO shows similar venation pattern alike PA and possessed oil glands up to 34 (middle leaf lets) (Fig. 1b). Similarly, variations for the floret's shape (length and width) and venation patterns were observed among the genotypes and found an indistinguishable venation patterns in florets. However, distinct variations found in flower colour, floret length and width are presented under morphological characterization (Table 2). These results are in similar lines as obtained by Chennem (2016) who reported that variation observed in the leaf patterns (first leaf) can be used to distinguish the hybrids and their parental lines. These results also in sync with the results obtained by Monika *et al.* (2017) who studied the venation pattern in chickpea flowers and suggested that these variations can be used as additional morphological descriptors to distinguish the cultivars. Hence, a simple and careful observation on plant morphology particularly on leaf/leaflet venations becomes as a vital approach and serves as an important tool to identify the closely related cultivars. Based on the seed geometric properties, seed phenotyping (Table 1) made using machine vision system, in which all the seed properties showed a distinct variation the genotype seeds (except eccentricity, and roundness between PBG and PNG). Among various traits, perimeter and axial length are found to be the prominent discriminators followed by area, length, breadth etc. Among all the traits, seed area ranges from 12.34 mm^2 (PNG) to 9.86 mm^2 (GO); length varies from 12.48 mm (PNG) to 9.01 (GO); breadth ranges from 1.56 mm (PA) to 1.35 mm (GO); perimeter recorded from 27.08 mm (PNG) to 20.01 (GO); equivalent diameter ranges from 3.95 mm (PNG) to 3.52 (GO); roundness was more in seeds of GO (0.15) followed by PA (0.12), PNG (0.10) and PBG (0.09); axial length

PNG (Ventral View) | PNG (Dorsal View) | PBG (Ventral View) | PBG (Dorsal View)

Fig. 1a: Leaf/leaflet characteristics in African types

PA (Ventral view) | PA (Dorsal view) | GO (Ventral view) | GO (Dorsal View)

Fig. 1b: Leaf and leaflet characteristics in French types

ranges from 9.01 mm (PNG) to 5.83 (GO) and axial width was more in PA (1.48 mm) and less in GO (1.32 mm); Median length ranges from 6.91 mm (PNG) to 4.49 (PA) and median width found higher in GO (1.07 mm) followed by PA (1.04 mm), PNG (0.97 mm) and PBG (0.86 mm) showed distinct difference among the seed genotypes. Reports on these physical traits in marigold were scanty and available reports says seed length can be as a trait to distinguish the marigold seeds as reported by Kennedy (1997), Singh *et al*. (2008) and Pramila (2010). However, these traits (geometric properties) are used to distinguish the varieties by Monika *et al*. (2015) in rice and Munder *et al*. (2017) in sunflower.

Applications of seed image analysis to explore the variations in seed traits, and to overcome from the limitations of conventional approaches. Thus, our results shown distinct differences in most of the seed physical traits studied, this reasonable degree of accuracy in distinction were achieved because of availability of high throughput software based technology like machine vision. Hence, it can be used as potential, most vital and non-destructive tool to identify/distinguish the genotypes at seed level.

Morphological distinction of genotypes showed that genotypes can be distinguished significantly using the morphological descriptors. Whereas, machine vision provided an additional detailed description about leaf and floral morphology (venation pattern and oil glands) and these traits can be considered as additional morphological descriptors for establishing distinctness among closely related genotypes. A data base may be developed in near feature for variations in seed traits (geometric properties), leaf and flower venation patterns. The leaflet traits or venation patterns are not described by the test guidelines provided by PPV and FRA and UPOV. These approaches indicating the significances can be used as promising approaches to identify or to distinguish the cultivars and these can be included in the test guidelines in near feature as promising

Table 1 : Seed phenotyping data obtained from image-based machine vision software

Genotypes	Area (mm^2)	Length (mm)	Breadth (mm)	Eccentricity	Perimeter (mm)	Equivalent diameter (mm)	Roundness	Axial length (mm)	Axial width (mm)	Median length (mm)	Median width (mm)
PBG	10.84	11.98	1.52	0.99	26.17	3.72	0.09	8.73	1.33	6.32	0.86
PNG	12.34	12.48	1.55	0.99	27.08	3.95	0.10	9.01	1.42	6.91	0.97
PA	11.93	10.88	1.56	0.99	23.66	3.87	0.12	6.12	1.48	4.49	1.04
GO	9.86	9.01	1.35	0.99	20.01	3.52	0.15	5.83	1.32	4.56	1.07
LSD (0.05)	**0.79**	**0.50**	**0.07**	**0.001**	**1.07**	**0.14**	**0.01**	**1.42**	**0.07**	**0.39**	**0.09**

Note: values are average of four replicates of ten seeds in each replicate; PBG: Pusa Basanti Gainda; PNG: Pusa Narangi Gainda; PA: Pusa Arpita; GO: Guljafri Orange

Table 2 : Quantitative traits of genotypes: flower associated traits

Genotypes	Peduncle length (cm)			Flower diameter (cm)			Floret length (cm)			Floret width (cm)		
	2015-16	2016-17	Mean	2015-16	2016-17	Mean	2015-16	2016-17	Mean	2015-16	2016-17	Mean
PBG	7.22	7.06	**7.14**	6.12	7.31	**6.72**	2.62	2.46	**2.54**	1.28	1.23	**1.26**
PNG	8.08	8.28	**8.18**	6.78	7.74	**7.26**	2.30	2.18	**2.24**	1.10	1.24	**1.17**
PA	13.80	14.20	**14.00**	4.52	4.41	**4.47**	1.66	1.82	**1.74**	1.76	1.71	**1.74**
GO	12.56	13.78	**13.17**	4.28	4.08	**4.18**	1.62	1.66	**1.64**	1.50	1.56	**1.53**
LSD (0.05)	**1.38**	**1.35**		**0.23**	**0.65**		**0.11**	**0.24**		**0.25**	**0.27**	

Note: PBG: Pusa Basanti Gainda; PNG: Pusa Narangi Gainda; PA: Pusa Arpita; GO: Guljafri Orange

Table 3 : Quantitative traits of genotypes: leaf and seed traits

Genotypes	Leaf length (cm)			Leaf width (cm)			Seed yield plant^{-1} (g)			1000-seed weight (g)		
	2015-16	2016-17	Mean	2015-16	2016-17	Mean	2015-16	2016-17	Mean	2015-16	2016-17	Mean
PBG	10.68	11.22	**10.95**	7.16	6.94	**7.05**	5.23	5.69	**5.46**	2.14	2.11	**2.13**
PNG	12.52	12.04	**12.28**	6.34	6.08	**6.21**	6.12	6.25	**6.18**	2.83	2.86	**2.85**
PA	19.82	20.58	**20.20**	12.52	12.18	**12.35**	4.76	4.51	**4.64**	2.71	2.69	**2.70**
GO	16.24	14.56	**15.40**	11.20	10.28	**10.74**	3.58	3.96	**3.77**	1.99	2.02	**2.01**
LSD (0.05)	**1.97**	**1.52**		**0.85**	**1.31**		**0.50**	**0.45**		**0.04**	**0.08**	

Note: PBG: Pusa Basanti Gainda; PNG: Pusa Narangi Gainda; PA: Pusa Arpita; GO: Guljafri Orange

Table 4 : Qualitative characteristics of genotypes

Genotypes	Leaf type	Leaf margin : Depth of indentation	Ligulate floret: Shape	Ligulate floret: Incision of margin	Flower colour:RHS reference code	Flower head: Floret type
PBG	Pinnate	Shallow	Intermediate	Absent	Yellow Group 9A	All ligulate
PNG	Pinnate	Medium	Intermediate	Present	Orange Group 28A	All ligulate
PA	Pinnate	Medium	Flat	Absent	Orange Group N25A	All ligulate
GO	Pinnate	Medium	Flat	Absent	Orange Group N25B	All ligulate

Note: PBG: Pusa Basanti Gainda; PNG: Pusa Narangi Gainda; PA: Pusa Arpita; GO: Guljafri Orange descriptors for identifying or distinguishing cultivars. A simple and careful observation on plant morphology, particularly on leaf or leaflet venations becomes a vital approach and serves as an important tool to identify the closely related cultivars (PBG and PNG)

The results suggest that software aided image analysis is a promising technique and it can be employed as a first approach to investigate seed morphological traits. Standardizing such methodologies integrated with conventional testing methods highlight the future prospects in the area of seed biology. A standard database may be developed to integrate image analysis data with taxonomic and bio-morphological features of seed plant species. However, accepting the little unavoidable errors machine vision can be used for seed phenotyping with reasonable degree of accuracy even in minute variable seed traits. Thus, our results provide basic information on variations in leaf/leaflet and they can be used as a reference for development of data base for varietal identification and further to validate with large number of genotypes.

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