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Diversity Analysis of Marigolds – *Tagetes* (Asteraceae)

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Abstract. Characterization of 20 *Tagetes* cultivars was achieved using morphological and anatomical markers. *Tagetes erecta* 'Maurel orange' (E4) was the most vigorous compared with other genotypes and produced large flowers with high quality and quantity. An anatomical study of leaf secretory cavities differentiated between the cultivars and revealed that foliar secretory cavities were larger and more abundant in *T. erecta* cultivars than in *T. patula* cultivars. The size of oil glands also differed in each cultivar. From this study it is concluded that *T. erecta* cultivars especially *T. erecta* 'Maurel orange' appeared as more promising one to cultivate and more appropriate to be selected as the parental genotype in the hybridization processes for obtaining new varieties and cultivars. It is concluded that different marker systems (morphological, and anatomical) are appropriate to differentiate between the cultivars of Tagetes.

Keywords: marigold, *Tagetes erecta, Tagetes patula,* morphological traits, cultivars, diversity

Introduction

Marigolds belong to the family Asteraceae (Compositae), genus *Tagetes*. Their natural range extends from the southwestern United States into Argentina, with the greatest diversity being in south-central México (Trostle, 1968). The genus *Tagetes* (Asteraceae) contains 56 species, of which only few species are currently cultivated as horticultural crops. Some companies, release new cultivars every year. Examples are, 'Marvel' line, 'Taishan'line of *T. erecta* and 'Bonanza' line, 'Boy' line of *T.patula* which have been widely used in the world (Zhang *et al*., 2011). Most of the cultivars were produced in the traditional hybridization breeding way (Tian *et al*., 2007; Wang, 2003, 2009). Besides, some work has also been done on the breeding of transgenic marigold (Charles *et al*., 2001). Nowadays, the cultivars widely planted throughout the world belong to three species: *T. erecta, T.patula* and *T. tenuifolia*. *T. erecta* flowers are ideal materials for extracting lutein. Therefore, it is very important to study *Tagetes* plant dut to its great economic value. Many cultivars have been available however, little is known about the differences between them, possibly because many have been introduced in different places with different names as well as different documentation of the original identities.

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Plant taxonomy has been mainly based upon morphological, cytological, and molecular biological analysis, etc. Morphological characters, both qualitative and quantitative, have long been used to identify species, varieties and cultivars to evaluate relationships, and discriminate between varieties and cultivars. Morphological traits continue to be the first step in the studies of genetic relationships in most breeding programmes (Van Beuningen and Busch, 1997; Cox and Murphy, 1990). In *Tagetes* breeding programmes, the major emphasis has been on the collection and conservation of genetic pools. There are numerous cultivars that cover a wide spectrum of growth habits, floral traits, environmental responses and varying pest and disease susceptibilities. So a wide range of characters have to be considered to select a superior germplasm that serves as the essential foundation for the breeding of new improved varieties as well as cultivars. Earlier studies on *Tagetes* cultivars using morphological traits such as plant habit, leaf traits, flower size, flower colour and pigmentation are rare. Anatomical characters for the identification of oil glands are more important to characterize the presence of essential oil in leaves. These traits are found to be of great importance to distinguish genetic variability, and can lead to a better classification of *Tagetes* cultivars. Thus, the objectives of the present study were to analyze the diversity of 20 cultivars belonging to two species of Tagetes (*T.erecta and T. patula*) and to classify them into particular groups

based on morphological and anatomical variation. It can be used for cultivar identification and further use in crop improvement through breeding programmes.

Materials and Methods

Plant material. Twelve cultivars of *T. erecta* and eight cultivars of *T. patula* were procured from nurseries for this study. 20 genotypes were grown at the Botany Department Garden, Kariavattom, Thiruvananthapuram (Kerala). All plants were replicated by stem cuttings and these vegetative propagated plants (clones) were used for further study. Crop management was done according to recommended agronomic practices.

Propagation. Clones were produced through stem cuttings. Plants were grown quickly and produce flowers. For seed germination seeds were sown in pots (germination percentage was only 50%).Out of twenty cultivars only twelve were germinated and rest of it was not being germinated at a stipulated period. The seeds were germinated in 3-5 days.

Morphological study. Twenty-cultivars belonging to two species of *Tagetes* were selected for this study. To avoid phenological differences between individuals, plants that had started to bloom were chosen for the study of vegetative traits and flowers were collected at the anthesis stage. For the study of leaf traits, the third leaf from the apex was chosen. Thirty morphological characters; 20 qualitative and 10 quantitative characters (Table 1), were considerd. Five plants were measured for each trait.

The terminology of Hickey and King (2000) was adopted to describe the qualitative characters. Morphological and floral traits were studied with the help of hand lens. Photographs were taken with a digital camera (DP11, Olympus, Tokyo, Japan). A datasheet was designed, and information was recorded for 10 quantitative and 20 qualitative characters (Table 2-3). To evaluate significant differences in quantitative traits, one-way ANOVA was performed. A correlation based PCA was performed to select taxonomically significant qualitative and quantitative characters and to detect outliers. The relative taxonomic distance between the cultivars was calculated by cluster analysis. Pair-wise relationships were estimated by the Euclidean distance coefficient. The distance matrix was represented as a phenogram by the UPGMA clustering method (Sneath and Sokal, 1973). All statistical tests except ANOVA were performed with the MultiVariate Statistical Package (MVSP) version 3.1 (Kovach Computing Services, Wales, UK). ANOVA was carried out with SPSS 7.5 (SPSS, 1999).

Table 1. Morphological characters of *Tagetes* taken for the study

Parameters Qualitative characters	Observation
Stem surface	1. Ribbed; 2. Ribbed and Hairy; 3. Stem Reddish and Ribbed
Petiole	1.Short; 2. Long
Leaf position	1. Opposite; 2. Alternate
Leaf Shape	1.Lanceolate; 2.Ovate; 3.Acute
Leaf apex	1.Acute; 2. Ovate
Leaf base	1.Acute; 2. Ovate
Leaf margin	1.Serrate ; 2. Non serrate
Leaf colour	1. Green; 2. Not green
Lamina symmetry	1. Symmetry; 2. Non symmetry
Head type	1. Heterozygous; 2. Homozygous
Inflorescence type	1. Corymbose; 2. Not corymbose
Inflorescenceposition	1. Solitary; 2. Axillary
Involucre type	1. Uni serrate; 2. Multi serrate
Pappus type	1. Awns; 2. Capillary; 3. Bristles
Style colour	1.Yellow; 2. Orange; 3. Pale yellow 4. Pale orange
Stigma colour	1.Yellow; 2. Orange; 3. Pale yellow 4. Pale orange
Stigma type	1. Bifid; 2.Not Bifid
Quantitative traits	
Leaf length	cm
Leaf breadth	cm
Leaf area	cm^2
Leaf perimeter	cm
Peduncle length	cm
Internode length	cm
Involucre length	mm
Pappus length	mm
Ovary length	mm
Style length	mm

Anatomical study. For the study of some anatomical traits, morphologically distinct accessions were selected based on the UPGMA cluster analysis, PCA and one-way ANOVA. They were *T. erecta* 'Indian orange'(E1), *T. erecta* 'Maurel orange' (E4), *T. erecta* 'Antigua white (E6), *T. erecta* 'Discovery yellow' (E7), *T. erecta* 'Antigua Orange' (E9), *T. erecta* ' Safari Tangerine' (E11), *T. patula* 'Double Eagle' (P1), *T. patula* 'Double Orange' (P5), *T. patula* 'Inca Cream'(P6) and *T. patula* 'Safari Yellow' (P8). For the leaf anatomy analysis, fully developed leaves were collected and fixed in ethanol. Transverse sections of the midrib, as well as

Table 2. Variations in quantitative characters of *Tagetes*

Name	Morphological Characters										
	Cultivar code	Leaf length (cm)	Leaf breadth (cm)	Leaf area (cm^2)	Leaf perimeter (cm)	Peduncle length (cm)	Intermodal length (cm)	Involucres length (cm)	Pappus length (mm)	Ovary length (mm)	Style length (cm)
T. erecta'Indian Orange'	E1	6.2 ±0.08	1.6±-0.04	7.3±-0.04	9.2±-0.1	6.3±-0.06	1.3±-0.04	3.2±-0.07	2.2±-0.1	1.4±-0.04	2.1±-0.06
T. erecta'Atlantis Orange'	E2	6.3±0.06	1.6±-0.04	7.2±-0.04	9.3±-0	6.1±-0.07	1.2±-0.04	3.1±-0.04	2.2±-0.05	1.2±-0.08	2.1±-0.04
T. erecta'Antigua Yellow'	E3	6.5±0.07	1.5±-0.04	7.3±-0.04	9.4±-0.9	6.2±-0.08	1.4±-0.04	3.1±-0.04	2.6±-0.09	1.2±-0.04	2.2±-0.04
T. erecta'Maurel Orange'	E4	6.4±0.08	1.6±-0.04	7.2±-0.85	8.2±-0.1	5.5±-0.15	1.2±-0.04	3.1±-0.06	2.3±-0.06	1.1±-0.07	2.1±-0.06
T. erecta'Maurel Yellow'	E5	6.4±0.05	1.5±-0.06	7.1±-0.08	9.2±-0.08	6.3±-0.08	1.4±-0.08	3.1±-0.08	2.6±-0.04	1.2±-0.04	2.3±-0.10
T. erecta'Antigua White'	E6	6.3±0.04	1.4±-0.04	7.2±-0.6	9.1±-0.6	6.4±-0.1	1.3±-0.04	3.1±-0.08	2.1±-0.06	1.4±-0.10	2.1±-0.07
T. erecta'Discovery Yellow'	E7	6.5±0.04	1.6±-0.06	7.3±-0.6	9.1±-0.7	6.3±-0.06	1.2±-0.06	3.1±-0.06	2.6±-0.04	1.3±-0.04	2.2±-0.09
T. erecta'Sweet White'	E8	6.0±0.06	1.5±-0.04	7.3±-0.	9.3±-0.06	6.3±-0.08	1.2±-0.09	3.2±-0.06	2.6±-0.06	1.1±-0.07	2.2±-0.09
T. erecta'Antigua Orange'	E9	6.6±0.04	1.6±-0.06	7.2±-0.6	9.3±-0.04	6.3±-0.06	1.4±-0.10	3.2±-0.06	2.4±-0.04	1.2±-0.02	2.1±-0.04
T. erecta'Inca Yellow'	E10	6.4±0.06	1.3±-0.04	7.4±-0.08	9.4±-0.08	6.65±-0.1	1.5±-0.08	3.1±-0.06	2.4±-0.04	1.4±-0.06	2.1±-0.06
T. erecta' Safari Tangerine'	E11	6.4±0.07	1.2±-0.07	7.2±-0.7	9.2±-0.06	6.5±-0.1	1.4±-0.06	3.4±-0.1	2.7±-0.01	1.3±-0.04	2.1±-0.04
T. erecta' Sweet Cream'	E12	6.6±0.06	1.4±-0.04	7.4±-0.04	9.4±-0.1	6.3±-0.07	1.2±-0.10	3.3±-0.08	2.5±-0.04	1.3±-0.06	2.2±-0.0
T. patula 'Double Eagle'	P1	5.3±0.07	1.2±-0.08	6.9±-0.07	8.3±-0.08	5.0±-0.14	1.2±-0.00	2.1±-0.04	3.0±-0.08	2.1±-0.06	2.1±-0.06
T. patula'Inca Orange'	P2	5.4±0.08	1.2±-0.08	7.1±-0.06	8.3±-0.07	5.3±-0.08	1.2±-0.07	2.3±-0.04	3.1±-0.06	2.2±-0.09	2.2±-0.09
T. patula 'Orange Eagle'	P3	5.3±0.08	1.3±-0.06	7.1±-0.06	8.2±-0.10	5.4±-0.09	1.2±-0.08	2.1±-0.06	3.2±-0.06	1.1±-0.04	2.1±-0.04
T. patula 'Safari'	P4	5.4±0.08	1.4±-0.04	7.2±-0.02	8.2±-0.09	5.6±-0.06	1.2±-0.04	3.2±-0.00	2.1±-0.06	1.3±-0.06	1.3±-0.06
T. patula 'Double Orange'	P5	5.2±0.04	1.1±-0.06	6.9±-0.04	8.4±-0.11	5.6±-0.06	1.3±-0.04	3.2±-0.01	2.1±-0.06	1.2±-0.07	1.2±-7.07
T. patula 'Inca Cream'	P6	5.7±0.05	1.3±-0.06	6.9±-0.04	8.3±-0.06	5.3±-0.00	1.2±-0.08	3.0±-0.06	2.4±-0.02	1.1±-0.0	1.1±-0.08
T. patula 'Mesa Gold'	P7	5.3±0.09	1.0±-0.06	7.0±-0.08	8.5±-0.07	5.3±-0.09	1.2±-0.08	3.0±-7.5	2.2±-0.08	1.1±-0.06	1.1±-6.29
T. patula'Safari Yellow'	P8	5.2±-0.08	1.3±-0.02	6.7±-0.06	8.4±-0.08	5.6±-0.04	1.2±-0.08	3.1±-0.08	2.1±-0.06	1.1±-0.06	1.1±-6.29

Table 3. Qualitative characters of *Tagetes*

Characters	Cultivars																			
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	P1	P2	P3	P4	P5	P6	P7	P8
Habit	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Stem surface	RH	R	R	RH	RH	RH	RH	RH	R	R	RR	RH	R	RR	RH	RR	R	RR	RR	RR
Branching nature	SB	PB	NB	PB	NB	NB	SB	SB	SB	PB	PB	PB	B	B	PB	B	PB	PB	PB	PB
Petiole	L	L	L	L	L	L	L	L	L	L	L	L	S	M	S	S	S	S	S	S
Leaf position	OP	OP	AL	OP	AL	AL	OP	OP	AL	OP	AL	OP	OP	OP	AL	AL	AL	AL	OP	OP
Leaf shape	OV	LA	LA	LA	OV	AC	AC	AC	AC	AC	AC	AC	LA	OV	OV	OV	OV	OV	AC	OV
Leaf apex	AC	AC	AC	AC	AC	OV	OV	OV	OV	OV	OV	OV	AC	AC	AC	AC	AC	AC	OV	AC
Leaf base	AC	AC	AC	OV	AC	OV	AC	OV	OV	OV	AC	AC	OV	AC	OV	AC	AC	AC	OV	AC
Leaf margin	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE	SE
Leaf colour	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
Lamina symmetry	SY	SY	SY	SY	SY	SY	SY	SY	SY	SY	SY	SY	SY	SY	SY	SY	SY	SY	SY	SY
Flower colour	O	LO	PY	DO	FY	WH	DY	CW	PO	LY	YO	C	YR	YO	DO	DY	DR	YC	GY	DY
Head type	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HO	HE	HE	HE	HE	HE	HE	HE	HE
Inflorescence type	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO	CO
Inflorescence position	AX	AX	SO	AX	AX	SO	SO	SO	AX	AX	SO	SO	SO	SO	SO	SO	AX	CO	CO	CO
Involucre type	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	UN	MU	MU	MU	MU	MU	MU	MU	MU
~~Pappus type~~	~~CB~~	~~CB~~	~~CB~~	~~CB~~	~~CB~~	~~CB~~	~~CB~~	~~CB~~	~~CB~~	~~CB~~	~~CB~~	~~CB~~	~~AW~~	~~AW~~	~~AW~~	~~AW~~	~~AW~~	~~AW~~	~~AW~~	~~AW~~
Style colour	y	PY	Y	PO	Y	O	WH	O	WH	O	Y	O	Y	O	PY	O	PO	PO	Y	O
Stigma colour	Y	Y	Y	PO	Y	O	WH	O	WH	O	Y	YO	Y	O	PY	O	PO	PO	Y	O
Stigma type	BF	BF	BF	BF	BF	BF	BF	BF	BF	BF	BF	BF	BF	BF	BF	BF	BF	BF	BF	BF

R = Ribbed; RH = Ribbed and Hairy; RR = Stem Reddish and Ribbed; SB = Sparingly branched; PB = Profusely branched; NB = Not branched; S = Short; L = Long; OP = Opposite; AL = Alternate; LA = Lanceolate; OV = Ovate; AC = Acute; S = Serate; G = Green; S = Symmetry; HE = Heterozygous; HO = Homozygous; C = Corymbose; SO = Solitary; AX = Axillary; UN = Uni serrate; MU = Multi serrate; AW = Awns; CB = Capillary bristles; Y = Yellow; O = Orange; PY = Pale yellow; PO = Pale orange; BF = Bifid.

transverse sections of the leaf-blade in the intercostal region and in the margin were obtained. Hand-sections were performed using a razor blade, cleared with sodium hypochlorite, washed with water, stained with astrablau-safranin (Bukatsch, 1972) and mounted in 50% glycerol. Anatomical features were studied by using a stereo zoom microscope (SZ61, Olympus, Tokyo, Japan). Photographs were taken with a digital camera (DP11, Olympus, Tokyo, Japan) attached to the microscope.

Results and Discussion

Morphological study. ***Morphological characters.*** The plants were herbs and the shoot length of *T.erecta* ranged between 12 to 16 cm, while the shoot length of *T. patula* ranged between 8 to 10 cm. Stem colour was reddish or green and stem surface was ribbed or ribbed and hairy. Some genotypes were unbranched as E3, E5 and E6 while the other genotypes were sparingly or profusely branched. Internodal length varied from 1.2 to 1.5 cm in *T. erecta* and 1.2 to 1.3 cm in *T. patula*. The leaves were green, compound and symmetrical, lanceolate, acute or ovate with serrate margin. Leaves were opposite or alternate. Leaf length ranged from 6.2 to 6.6 cm in *T. erecta* and 5.2 to 5.7 cm in *T. patula*. Leaf width ranged between 1.2 to 1.6 cm in *T. erecta* and 1.0 to 1.4 cm in *T. patula*. Leaf area and perimeter in *T. erecta* ranged between 7.1 to 7.4 cm^2 and 8.2 to 9.4 cm respectively. In *T. patula* it ranged between 6.7 to 7.2 cm^2 and 8.2 to 8.5 cm respectively.

The inflorescence consists of numerous ray and disc florets in head or capitulum. Head type was homogamous in *T.erecta* but herterogamous in *T. patula.* Variations could be noted in the flower colour. It was orange in E1, E2, E4 and E9. Various yellowish shades were seen in E3, E5, E7, E10 and E11. White, creamy white and cream colours were found in E6, E8 and E12 respectively. Some cultivars of *T.patula* showed distinct colours in outside and inside of the head inflorescence. Reddish yellow outside and yellowish inside in P1, orange outside and yellow inside in P3 and whitish cream outside and yellow inside in P6. Dark red coloured ray florets were found in P5. Involucre type in *T.erecta* was uniserrate but in *T. patula* it was multi serrate. Capillary bristles were found in *T. erecta* but awns in *T. patula.* Involucre length of *T. erecta* ranged between 3.1 to 3.4 cm where as in *T. patula* it ranged between 2.1 to 3.2 cm. Pappus length of flowers ranged between 2.1 to 2.7 cm in *T. erecta* and 2.1 to 3.2 cm in *T. patula*. Ovary and style length of *T. patula* were found to be more variable compared to *T. erecta*. In *T. patula* ovary length ranged between 1.1 to 2.2 cm where as in *T. erecta* it was 1.1 to 1.4 cm. Style length ranged between 1.1 to 2.2 cm in *T. patula* where as in *T. erecta* it was 2.1 to 2.3 cm. Stigma was bifid in both species.

Morphometric analysis. ANOVA. All the quantitative characters were found to be significant.

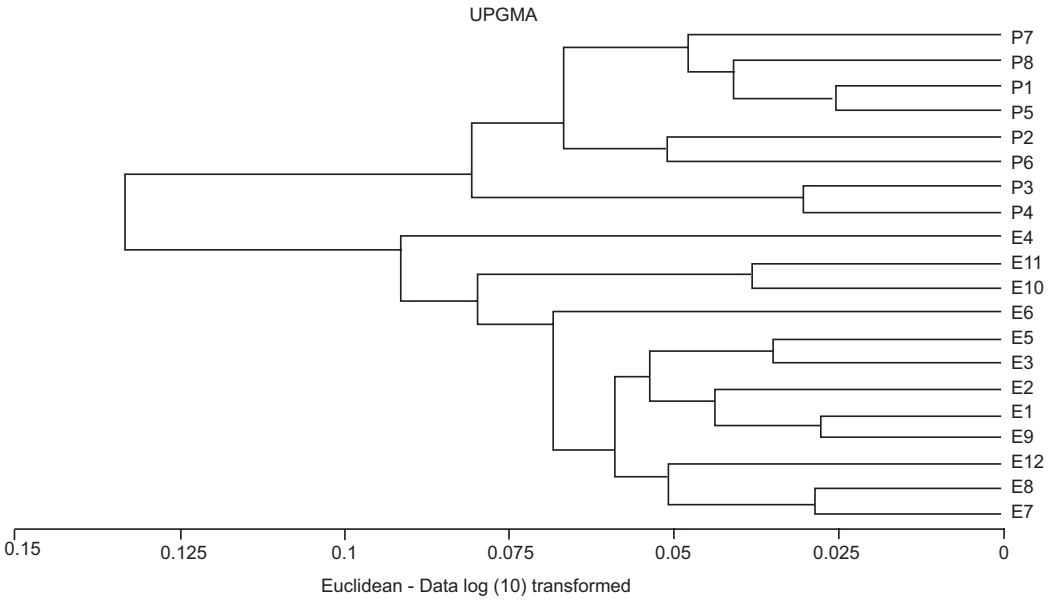
PCA. ***Qualitative traits.*** In the PCA of qualitative data of *T.erecta*, 56.8% of the phenetic variance was accounted for by the first principal axis, followed by 17.3% for the second, 8.2% for the third, 5.5% for the fourth and 5.3% for the fifth principal component (Table 4). Most of the selected qualitative traits were found principally influential in the PCA. Branching nature, leaf morphology and flower colour were found influential in the most variable first principal component.

In the PCA of qualitative data of *T.patula*, 38.6% of the phenetic variance was accounted for by the first principal axis, followed by 25.5% for the second, 16.2% for the third, 8.6% for the fourth and 6.9% for the fifth principal component (Table 4). Most of the selected qualitative traits were found principally influential in the PCA. Leaf position, leaf morphology and flower colour were found influential in the most variable first principal component.

Quantitative traits. In the PCA of quantitative data of *T.erecta*, 43.3% of the phenetic variance was accounted for by the first principal axis, followed by 23.2% for the second, 15.8% for the third and 10.3% for the fourth principal component (Table 5). The first principal component accounted for 62.7% of phenotypic variance and the second component for 21.1% (Table 5) in *T.patula*. All the quantitative traits except leaf characters were found to have significant loadings in PCA.

Cluster analysis. The UPGMA phenogram based on the morphological characters shows two main principal clusters (Fig. 1) separating the two species. In the *T.patula* cluster, the cultivars P3 and P4 are separated out with others, and again P2 and P6 are separated out. Cultivar P7 is more distinct from P2, P6, P3 and P4 but more close to P8, P1 and P5. In the *T. erecta* cluster cultivar E4 is more distant from others. E8 and E7 as well as E10 and E11 are more close to each other.

Anatomical study. Anatomical studies revealed that foliar secretory cavities were more abundant in *T. erecta* cultivars than in *T. patula* cultivars. The secretory structures were found in the lamina between the palisade



E1 *T. erecta* 'Indian Orange'
E2 *T. erecta* 'Atlantis Orange'
E3 *T. erecta* 'Antigua Yellow'
E4 *T. erecta* 'Maurel Orange'
E5 *T. erecta* 'Maurel Yellow'
E6 *T. erecta* 'Antigua White'
E7 *T. erecta* 'Discovery Yellow'
E8 *T. erecta* 'Sweet White'
E9 *T. erecta* 'Antigua Orange'
E10 *T. erecta* 'Inca Yellow'
E11 *T. erecta* ' Safari Tangerine'
E12 *T.erecta* ' Sweet Cream'

P1 *T. patula* 'Double Eagle'
P2 *T. patula* 'Inca Orange'
P3 *T. patula* 'Orange Eagle'
P4 *T. patula* 'Safari'
P5 *T. patula* 'Double Orange'
P6 *T. patula* 'Inca Cream'
P7 *T. patula* 'Mesa Gold'
P8 *T. patula* 'Safari Yellow'

Fig. 1. UPGMA phenogram *Tagetes* based on morphological characters.

Table 4. PCA variable loading of morphology, (qualitative) in *Tagetes* cultivars

Anatomical features	*Tagetes erecta*					*Tagetes patula*				
	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5	Axis 1	Axis 2	Axis 3	Axis 4	Axis 5
Stem surface	-0.058	-0.470	-0.458	-0.112	0.560	0.077	0.397	-0.466	0.209	-0.115
Branching nature	-0.148	-0.370	0.703	0.341	0.138	0.077	0.397	-0.466	0.209	-0.115
Petiole	0.000	0.000	0.000	0.000	0.000	-0.019	-0.066	-0.175	0.263	0.497
Leaf position	-0.070	-0.382	0.274	-0.265	0.059	0.378	-0.048	-0.041	-0.088	-0.465
Leaf Shape	0.307	-0.259	-0.084	-0.235	0.206	0.121	0.346	-0.064	-0.275	0.288
Leaf apex	0.267	-0.200	-0.128	-0.170	-0.200	-0.130	0.195	0.021	-0.393	0.068
Leaf base	0.196	0.046	0.184	0.166	0.521	0.247	0.029	0.246	0.444	0.507
Leaf margin	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Leaf colour	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Lamina symmetry	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Flower colour	0.383	-0.549	-0.058	0.225	-0.489	0.223	0.618	0.392	-0.275	0.130
Head Type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Inflorescence type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
~~Inflorescene position~~	~~0.080~~	~~0.058~~	~~0.374~~	~~-0.782~~	~~-0.033~~	~~0.259~~	~~-0.063~~	~~0.373~~	~~-0.002~~	~~-0.142~~
Involucre type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pappus type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Style colour	0.584	0.246	0.101	0.164	0.261	0.484	-0.365	-0.411	-0.500	0.325
Stigma colour	0.521	0.148	0.104	-0.016	-0.034	0.634	-0.021	0.076	0.289	-0.154
Stigma type	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Eigenvalues	2.639	0.804	0.383	0.256	0.248	1.403	0.927	0.587	0.313	0.251
Percentage	56.784	17.305	8.244	5.504	5.342	38.640	25.540	16.168	8.627	6.913
Cum. Percentage	56.784	74.089	82.333	87.837	93.179	38.640	64.180	80.348	88.975	95.888

Table 5. PCA variable loading of morphology, (quantitative) in *Tagetes* cultivars

Anatomical features	*Tagetes erecta*				*Tagetes patula*	
	Axis 1	Axis 2	Axis 3	Axis 4	Axis 1	Axis 2
Leaf length	-0.006	0.050	-0.292	-0.154	-0.084	0.169
Leaf breadth	0.649	0.590	0.403	-0.052	0.004	0.648
Leaf area	-0.002	0.035	0.014	0.101	0.040	0.183
Leaf perimeter	-0.229	0.194	0.083	0.330	0.001	-0.017
Petiole length	-0.240	0.219	0.185	0.561	0.085	-0.050
Internode length	-0.515	0.017	0.679	-0.401	-0.344	-0.139
Involucre length	-0.030	-0.101	-0.239	0.070	0.465	0.577
Pappus length	-0.440	0.692	-0.373	-0.154	-0.396	0.138
Ovary length	-0.078	-0.153	0.193	0.543	0.646	-0.284
Style length	0.057	0.222	-0.128	0.235	-0.275	0.253
Eigenvalues	0.012	0.007	0.005	0.003	0.017	0.006
Percentage	43.266	23.210	15.845	10.372	62.740	21.126
Cum. Percentage	43.266	66.476	82.321	92.693	62.740	83.867

and spongy parenchymatic tissue and in the cortical region of the petiole. The size of oil glands differed among the cultivars. In the cultivars of *T. erecta* the oil glands were larger in size compared with the oil glands in *T. patula* cultivars.

In the present study, an analysis of morphological characters of *Tagetes* species revealed that each cultivar is distinct. The two species of *Tagetes* (*T. erecta* and *T. patula*) used for this study were morphologically dissimilar. The shoot length of *T. erecta* ranged between 12 to 16 cm, while the shoot length of *T. patula* ranged between 8 to 10 cm suggests that *T. patula* plants were smaller than *T. erecta*. Branching nature was also different. Most of the cultivars from *T. patula* were shown profusely branched nature. Variations could be noted in the flower colour. It was orange in *T. erecta* 'Indian orange'(E1), *T. erecta* 'Atlantis orange' (E2), *T.erecta* 'Maurel orange' (E4), *T. erecta* 'Antigua Orange' (E9) and *T. erecta* 'Safari Tangerine' (E11). Various yellowish shades were seen in *T.erecta* 'Antigua yellow' (E3), *T. erecta* 'Maurel yellow (E5), *T. erecta*' Discovery yellow' (E7) and *T. erecta* ' Inca Yellow' (E10).White, creamy white and cream colours were found in *T. erecta* 'Antigua white (E6), *T. erecta* 'Sweet white' (E8) and *T. erecta*' Sweet Cream' (E12) respectively. The most attractive flowers were observed in *T. patula* and head was heterogamous. The biggest flowerings form at *T. erecta* (*Tagetes erecta* 'Maurel Orange'[E4]) and smallest ones at *T.patula* (*T.patula* 'Inca Orange' [P2]).

Cluster analysis substantiated the existence of diversity among the 20 cultivars for the morphological traits studied. The clustering pattern showed that two principal clusters for two species, *T. erecta* 'Maurel orange' (E4) was distant from other cultivars of *T. erecta*. The dendrogram constructed on the basis of the data generated from the morphological traits of *T. erecta* also showed E4 was distinct from others. The colour of the flower was more attractive in this cultivar and other notable characters were the ribbed and hairy stem surface, long petiole, opposite leaves with serrate margin, leaf shape lanceolate and lamina symmetrical. Peduncle was found to be short (5.5 cm) compared to other cultivars. Biggest flowers with dark orange colour confirm its high pigment content rather than attractiveness. Anatomical characterization of leaf showed the presence of large oil glands in this cultivar. Both these characters make up this cultivar more economical.

Anatomical studies revealed that foliar secretory cavities were more in *T. erecta* cultivars than in *T. patula* cultivars. The size of oil glands differ in each cultivar. In the cultivars of *T. erecta,* the oil glands were larger in size compared with the oil glands in *T. patula* cultivars. From this present study it is clear that *T. erecta* cultivars are more appropriate to select as the parental genotype in the hybridization processes for obtaining different varieties and cultivars. Since many reports show that *T. erecta* are good source of yellow pigments and essential oil content useful for various activities (Verghese,1998). Ethnobotanical studies on *T. erecta* were also reported by many workers (Balick

and Cox, 1996; Neher, 1968;). Vasudevan *et al*. (1997) has ranked *T. erecta* as a multipurpose herb.

A marigold flower contains abundant amount of a valuable antioxidant compound called lutein (Lavececchia *et al*.,2004). The extract with only the purified form with a lutein content of known concen-tration and a pure crystalline lutein isolated from marigold flower especially from *T. erecta* is allowed for food use. Dark coloured flowers contain about 200 times more lutein than the light coloured flowers. The concentration of lutein varies in different shades of marigold flowers, viz.; greenish yellow to bright yellow and orange brown (Gregory *et al*., 1986). The dark orange colour flowers are observed in *T. erecta* cultivars. From the present study it was evident that *T. erecta* 'Maurel orange' (E4) have large and dark orange coloured flowers.

Because of the wide range of activity marigolds are cultivated by farmers, but in India mostly for its yellow flowers. After harvesting they destroy the plants without utilizing its full benefits. Each and every part of the plant is very much useful. Simple distillation of the plant parts achieve good amount of essential oil which can be used for various purposes. Still the identification of superior cultivar is a difficult task. Various characters are spread in different cultivars and breeders can not find these characters in a single plant. Different genes which are distributed in different cultivars if pooled together in single plant will definitely create a superior plant which can be achieved through breeding. This study of preliminary screening of these cultivars by morphological and anatomical traits revealed that *T. erecta* cultivars are more appropriate to be selected as the parental genotype in the hybridization processes. Among this *T.erecta* 'Maurel orange' (E4) is superior in floral traits as well as large oil glands.

It is concluded from these results that different marker systems are appropriate to differentiate between the cultivars of *Tagetes*. Also, these marker systems could be complementary to each other and should be followed by molecular characterization using PCR-based markers to establish an integrated data about these cultivars. The maximum variability was present in flower colour. The cultivars with dark orange colour flowers can be used in further hybridization programme because of high carotenoid content. Selection of better cultivar can be made for species or varietal improvement on the basis of percent similarity with other species. Two more prominent characters, i.e., large oil glands and dark orange flowers can be chosen for the purpose.

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