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Genetic diversity study of *Tagetes* Linn. (Asteraceae)

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ABSTRACT

Present study was conducted to access genetic diversity among 10 genotypes of two species of Tagetes i.e., Tagetes erecta *(6 cultivars) and Tagetes patula (4 cultivars). Though Tagetes possesses great economical value, little attention has been paid for its genetic improvement. Four polymorphic isozyme systems (AAT, MDH, EST and PRX) were selected for cultivar identification. In isozyme analysis, peroxidase was able to differentiate both the species. Esterase showed large number of alleles and polymorphism to differentiate the two species as well as cultivars of the same species. The data would be important in detailing the level of variation and relationship within and between species to plan future domestication trials in Tageties.*

INTRODUCTION

Marigolds belong to the family Asteraceae (Compositae), genus Tagetes. Their natural range extends from the south western United States into Argentina, with the greatest diversity being in south-central México [27]. The genus *Tagetes* (Asteraceae) contains 56 species, of which only few species were currently cultivated as horticultural crops. Some companies, such as, Thompson and Morgan, Pan-American Seed and Sluis Groot etc. cultivate new cultivars every year. Examples are, 'Marvel' line, 'Taishan'line of *T. erecta* L. and 'Bonanza' line, 'Boy' line of *T.patula* L. which have been widely used in the world. Most of the cultivars were produced in the traditional hybridization breeding way [26, 31, 32]. Besides, some works also have been done on the breeding of transgenic marigold [3]. Nowadays, the species widely used throughout the world were *T. erecta* L.*, T.patula* L. and *T. tenuifolia.* The inflorescence of pigment *T. erecta* L. flowers were ideal materials for extracting lutein. Therefore, it was very important to study *Tagetes* plant with their great economic value.

The plant taxonomy was mainly based upon morphological, cytological, and molecular biological analysis, etc. Morphological characters, both qualitative and quantitative, have long been used to identify species, varieties as well as cultivars to evaluate relationships, and to discriminate between varieties and cultivars. The number of morphological traits is limited, most of them are multi-genic, quantitative or continuous characters, and their expression is influenced by environmental conditions. As a complementary approach, biochemical analysis of isozyme markers proves its diagnostic potential [17, 19]. Traditionally, genetic diversity evaluated in crop species is based on differences in morphological characters and qualitative traits [22]. It can also be used as a powerful tool in the classification of cultivars, to study taxonomic status and to study genetic

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relationships in most breeding programmes [4, 28]. 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, quality and yield are rare. These traits are all found to be of great importance to distinguish genetic variability, and can lead to a better classification of Tagetes cultivars. Many cultivars have been available however, little is known about the real difference between them, possibly because many have been introduced to different places with different names with different documentation of the original identities. The discrimination of cultivars is almost always a difficult task, due to phenotypical similarities, which can be overcome by biochemical methods, such as isozyme analysis. The present study was to verify the similarity of 10 cultivars of two species of Tagetes (*T.erecta and T. patula*) and to classify them into particular groups based on morphological variation. And this study also assess the degree of phenotypic variation among the cultivars of *T.erecta* and *T.patula* germplasm. It can use for cultivar identification and further use in improvement through breeding programmes. So the objectives of this study were to analyze diversity for 10 selected cultivars based on morphological and biochemical traits.

MATERIALS AND METHODS

Six cultivars of *T. erecta* and four cultivars of *T. patula* procured from agricultural farm Thiruvananthapuram were used for this study (Table:1). The 10 genotypes were grown in Botany Department Garden, Kariavattom, Thiruvananthapuram (Kerala). All plants were replicated by stem cuttings and these vegetatively propagated plants (clones) were used for further study. Crop

Table 1. Cultivars used for the morphological study

No:	Code	Name
1.	TE1	*Tagetes erecta* 'Indian Orange'
2.	TE2	*Tagetes erecta* 'Maurel Orange'
3.	TE3	*Tagetes erecta* 'Antigua White'
4.	TE4	*Tagetes erecta* 'Discovery Yellow'
5.	TE5	*Tagetes erecta* 'Antigua Orange'
6.	TE6	*Tagetes erecta* ' Safari Tangerine'
7.	TP1	*Tagetes patula* 'Double Eagle'
8.	TP2	*Tagetes patula* 'Double Orange'
9.	TP3	*Tagetes patula* 'Inca Cream'
10.	TP4	*Tagetes patula* 'Safari Yellow'

Table 2. Morphological characters taken for the study.

Qualitative characters	
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.OvateAcute
Leaf apex	1. Acute; 2.Ovate
Leaf base	1. Acute; 2.Ovate
Leaf margin	1.Serrate; 2.Non serrate
Leaf color	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	
Internode length cm	
Involucre length mm	
Pappus length mm	
Ovary length mm Style length mm	

Table 3: Qualitative characters of *Tagetes*

Characters	TE1	TE2	TE3	TE4	TE5	TE6	TP1	TP2	TP3	TP4
Habit	H	H	H	H	H	H	H	H	H	**H**
Stem surface	RH	RH	RH	RH	R	RR	R	R	RR	**RR**
Branching nature	SB	PB	NB	SB	SB	PB	B	PB	PB	**PB**
Petiole	L	L	L	L	L	L	S	S	S	**S**
Leaf position	OP	OP	AL	OP	AL	AL	OP	AL	AL	**OP**
Leaf shape	OV	LA	AC	AC	AC	AC	LA	OV	OV	**OV**
Leaf apex	AC	AC	OV	OV	OV	OV	AC	AC	AC	**AC**
Leaf base	AC	OV	OV	AC	OV	AC	OV	AC	AC	**AC**
Leaf margin	SE	SE	SE	SE	SE	SE	SE	SE	SE	**SE**
Leaf colour	G	G	G	G	G	G	G	G	G	**G**
Lamina symmetry	SY	SY	SY	SY	SY	SY	SY	SY	SY	**SY**
Flower colour	O	DO	WH	DY	PO	YO	YR	DR	YC	**DY**
Head type	HO	HO	HO	HO	HO	HO	HE	HE	HE	**HE**
Inflorescence type	CO	CO	CO	CO	CO	CO	CO	CO	CO	**CO**
Inflorescence position	AX	AX	SO	SO	AX	SO	SO	AX	CO	**CO**
Involucre type	UN	UN	UN	UN	UN	UN	MU	MU	MU	**MU**
Pappus type	CB	CB	CB	CB	CB	CB	AW	AW	AW	**AW**
Style colour	y	PO	O	WH	WH	Y	Y	PO	PO	**O**
Stigma colour	Y	PO	O	WH	WH	Y	Y	PO	PO	**O**
Stigma type	**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) Serrate, (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.

management was done according to the 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. The seeds were germinated in 3-5 days. Germinated seeds were grown quickly, and produced flowers (approximately within 45 to 50 days after seedling). The biggest flowerings form at *Tagetes erecta* and the smallest ones at *T. patula.* There were also the intermediary varieties as size of the flowering.

Morphological study

The study was confined to the clones at flowering period. To avoid phenological differences between individuals, plants that had started to bloom were chosen, and the flowers for study were collected at the anthesis stage. Thirty morphological characters [20 qualitative and 10 quantitative characters (Table: 2)], were taken under consideration. The terminology of Hickey and King [10] was adopted to describe the qualitative characters. Morphological and floral traits were studied with the help of hand lens. A datasheet was designed, and information was recorded for 20 qualitative and 10 quantitative characters (Table 3 and 4). To evaluate significant differences in quantitative traits, one-way ANOVA was performed. ANOVA was carried out with SPSS 7.5 (SPSS, Chicago, IL, USA).

Biochemical study

Ten cultivars were used for this study. The isozyme analysis was carried out with native PAGE without inactivating the native form of the enzyme. Native PAGE was done in a Genei Mini Model Slab gel system (Biorad, U.S.A.) following the procedure of Laemmli [7, 14]. Leaf samples were used for isozyme analysis.

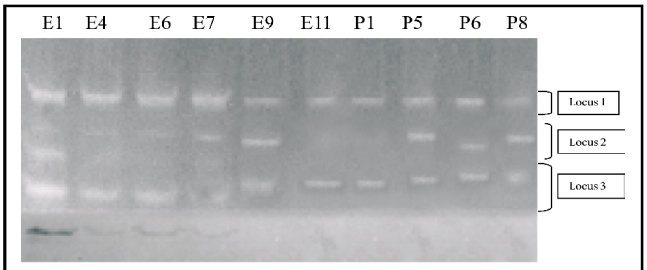

Fig. 1a: Isozyme Profiling by Catalase Enzyme

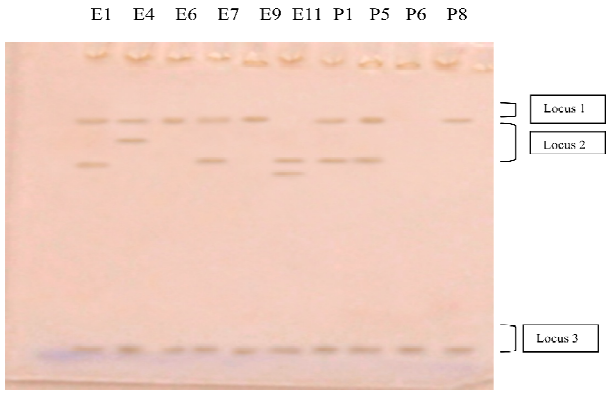

Fig. 1b: Isozyme Profiling by Aspartate Amino Transferase

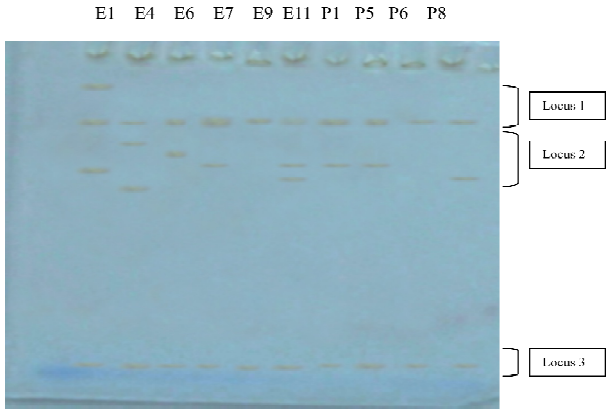

Fig. 1c: Isozyme Profiling by Esterase

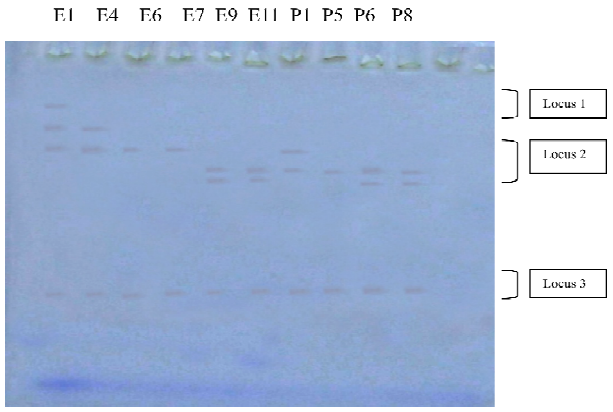

Fig. 1d: Isozyme Profiling by Malate Dehydrogenase

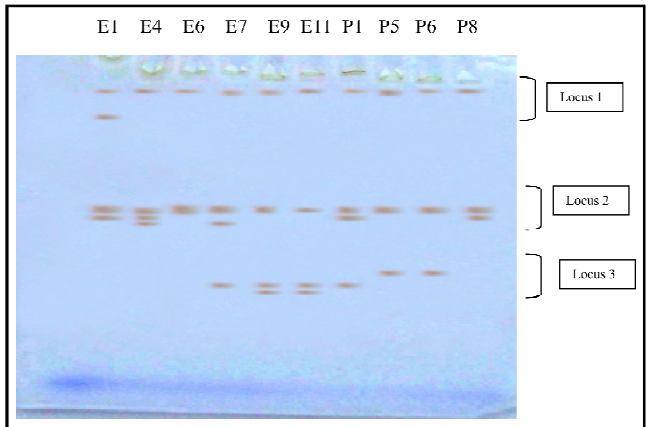

Fig. 1e: Isozyme Profiling by Peroxidase

Isozyme analysis

Aspartate amino transferase, catalase, esterase, malate dehydrogenase and peroxidase were isolated to assess the genetic variability. For the isolation of the isozymes, fresh leaves of plants were collected and washed in tap water followed by distilled water. Water droplets were removed with the help of tissue paper. 500 mg leaves were weighed and crushed in minimum amount of extraction buffer (0.1 M tris + 2% â mercaptoethanol) with the help of mortar and pestle under chilled conditions. The extracts were quickly transferred to pre-cooled eppendorf tubes and kept in a refrigerator. Tubes were centrifuged at 12000 rpm for 30 min at 40 ^{0}C in a refrigerated centrifuge. The supernatant was transferred to fresh tubes. One part extract: one part glycerol: one part bromophenol blue (0.05 mg/ml) were mixed and then the samples were stored at -200 ^{0}C for further use. The experiments were carried out in order to

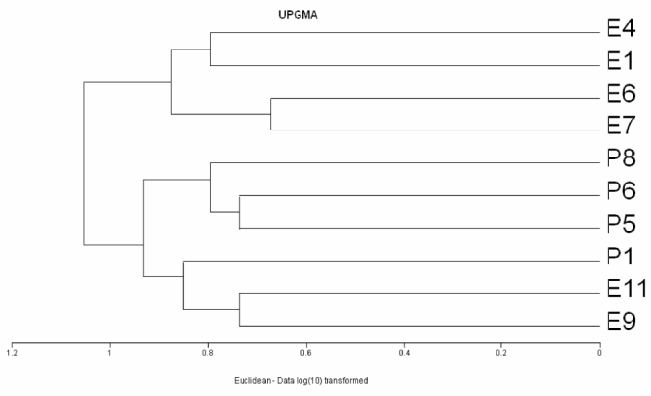

Fig. 2 UPGMA phenogram for 10 cultivars of *Tagetes* based on isozymes

characterize the genetic variability by Native Polyacrylamide Gel Electrophoresis (PAGE). Electrophoresis was conducted at 20mA at 10ºC until the tracking dye reached 0.5 cm from the gel bottom. The staining procedure for catalase, peroxidase, esterase, Aspartate amino transferase and malate dehydrogenase isozymes were done [33].

RESULTS

Morphological study

Morphological characters

The plants were herbs and the shoot length of *T. erecta* ranged between 60 to 70 cm, while the shoot length of *T. patula* ranged between 55 to 60 cm. Stem colour was reddish or green and stem surface was ribbed or ribbed and hairy. Both were observed in *T. erecta* and *T. patula*. Some genotypes were unbranched as TE3 while the other genotypes were sparingly and profusely branched. Internodal length varies from 1.2 to 1.4 cm in *T. erecta* and 1.2 to1.3 cm in *T. patula*. The leaves were green, compound and symmetrical as well as opposite or alternate. Leaves were lanceolate, acute or ovate. The leaves were serrated and the 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 1.1 to 1.3 cm in *T. patula*. Leaf area and perimeter in *T. erecta* ranged between 7.2 to 7.3 cm^2 and 8.2 to 9.3 cm respectively. In *T. patula* it was ranged between 6.7 to 6.9 cm^2 and 8.3 to 8.4 cm respectively.

The inflorescence consists of numerous ray and disc florets in head or capitulam. Head type was homogamous in *T. erecta* but herterogamous in *T. patula.* Variations could be noted in the flower colour. It was orange in TE1, TE2 and TE5. Various yellowish shades were seen in TE4 and TE6. creamy white colour was found in TE3. Some cultivars of *T. patula* showed distinct colours in outside and inside of the head inflorescence. Reddish yellow outside and yellowish inside in TP1 and whitish cream outside and yellow inside in TP3. Dark red coloured ray florets were found in TP2. 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.* The morphological characters were represented in the tables 3. 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 3.0 cm in *T. patula*. Ovary and style length of *T. patula* were found to be much variation compared to *T. erecta*. In *T. patula* ovary length ranged between 1.1 to 2.1 cm where as in *T. erecta* it was 1.1 to 1.4 cm. Style length ranged between 1.2 to 2.1 cm in *T. patula* where as in *T. erecta* it was 2.1 to 2.2 cm. Stigma bifid in both species. All the quantitative characters were found to be significant (Table 4).

Table 4: Variations in and quantitative floral characters of *Tagetes erecta* cultivars

Culti-varcode	Leaf Length	Leaf Breadth	Leaf Area	Leaf Perimeter	Peduncle Length	Internodal Length	Involucre Length	Pappus Length	Ovary Length	Style Length
TE1	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
TE2	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
TE3	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
TE4	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
TE5	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
TE6	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
TP1	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
TP2	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
TP3	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
TP4	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

Biochemical study

Genetic diversity based on isozyme markers

A total of 33 alleles were observed at the 15 isozyme loci in all 10 cultivars of both *Tagetes erecta* and *T patula*. All allelic distribution is shown in table 5. The ten cultivars were clearly separated on the basis of the five different isozyme system.

Catalase

Three loci were observed and named as CAT-1, CAT-2 & CAT-3. Only one allele was observed in CAT-1 locus and it was found in all samples. The other two loci were polymorphic. Three alleles (CAT-2^a CAT-2^b & CAT-2^c) were observed at CAT-2 locus. Allele CAT-2^a was polymorphic and was present in samples 1, 2, 3, 4, 8 &10. Allele CAT-2^b was observed in samples 5&9. Allele CAT-2^c was observed in samples 1&4. Three alleles (CAT-3^a CAT-3^b & CAT-3^c) were present in CAT-3 locus. Allele CAT-3^a was present in samples 8, 9 &10. Allele CAT-3^b was observed in samples 5,6&7. Allele CAT-3^c was polymorphic and observed in samples 1, 2, 3&4.

Aspartate aminotransferase

Three loci were observed and named as AAT-1, AAT-2 & AAT-3. AAT-1 was monomorphic among the studied loci and the single allele was found in all samples except 6&9. The next locus, AAT-2 was polymorphic. Three alleles (AAT-2^a AAT-2^b & AAT-2^c) were observed. Allele AAT-2^a was present only in sample 2. Allele AAT-2^b was observed in samples 1, 4, 6, 7 & 8. Allele Aat-2^c was observed only in sample 6. Only one allele was observed at AAT-3 locus and that was present in all samples.

Esterase

Three loci were observed and named as EST-1, EST-2 & EST-3. Two alleles at EST-1 locus (EST-1^a and EST-1^b) first allele was observed in sample 1 and second allele was found in all other samples. Six alleles (EST-2^a EST-2^b EST-2^c & EST-2^d EST-2^e and EST-2^f) were present in locus EST-2. Allele EST-2^a was detected in sample 2 only. Allele EST-2^b was present in sample3. Allele EST-2^c was observed in 4, 6,7 and 8 samples. Sample 1 alone showed EST-2^d allele. Two samples (6 and 10) showed EST-2^e allele. EST-2^f allele was observed in sample 2. Only one allele observed in EST-3 and that allele was present in all samples.

Malate Dehydrogenase

Three loci were observed and named as MDH-1, MDH-2 & MDH-3. Two alleles (MDH-1^a & MDH-1^b) were observed at MDH-1 locus. MDH-1^a observed only in 1^{st} sample. MDH-1^b was detected in sample 1&2.Three alleles (MDH-2^a MDH-2^b & MDH-2^c) were present in locus MDH-2. Allele MDH-2^a was detected in samples1, 2, 3, 4 and 7. Allele MDH-2^b was polymorphic and present in samples 5, 6, 7, 8, 9&10. Allele MDH-2^c was observed in sample5,6,9&10. Only one allele observed in MDH-3 and that allele was present in all samples.

Peroxidase

Three loci were observed and named as PRX-1, PRX-2 & PRX-3. Two alleles (PRX-1^a & PRX-1^b) were observed at PRX-1 locus. Allele PRX-1^a was not polymorphic and was present in all samples. Allele PRX-1^b was observed only in 1^{st} sample. Three alleles (PRX-2^a, PRX-2^b & PRX-2^c) were present in PRX-2 locus. Allele PRX-2^a was present in all samples. Allele PRX-2^b was observed in samples 1, 2, 7&10. Allele PRX-2^c was detected in samples 2&4. Three alleles (PRX-3^a, PRX-3^b & PRX-3^c) were present in PRX-3 locus. Allele PRX-3^a was present in samples 8 & 9. Allele PRX-3^b was observed in samples 4, 5, 6 & 7. Allele PRX-3^c was detected in samples 5&6.

Dendrogram was prepared on the basis of banding pattern obtained by five different isozyme patterns. Cultivars of both the species (*T.erecta* and *T.patula*) were grouped into two major clusters. First cluster include TE2, TE1, TE3 and TE4. Second cluster comprise TP4, TP3, TP2, TP1, TE6 and TE5. Two cultivars of *T. erecta* TE6 and TE5 constitute a sub cluster and they were closely associated to TP1.

Table 5: Isozyme banding patterns of ten Tagetes genotypes.

(a): *Catalase enzyme*

Allele CAT	Code numbers of Cultivars used for this study									
	TE1	TE2	TE3	TE4	TE5	TE6	TP1	TP2	TP3	TP4
1	+	+	+	+	+	+	+	+	+	+
2^a	+	+	+	+	-	-	-	+	-	+
2^b	-	-	-	-	+	-	-	-	+	-
2^c	+	-	-	+	-	-	-	-	-	-
3^a	-	-	-	-	-	-	-	+	+	+
3^b	-	-	-	-	+	+	+	-	-	-
3^c	+	+	+	+	-	-	-	-	-	-
Total	4	3	3	4	3	2	2	3	3	3

(b): *Aspartate amino transferase*

Allele EST	TE1	TE2	TE3	TE4	TE5	TE6	TP1	TP2	TP3	TP4
1	+	+	+	+	+	-	+	+	-	+
2^a	-	+	-	-	-	-	-	-	-	-
2^b	+	-	-	+	-	+	+	+	-	-
2^c	-	-	-	-	-	+	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+
Total	3	3	2	3	2	3	3	3	1	2

(c): *Malate dehydrogenase*

Allele	TE1	TE2	TE3	TE4	TE5	TE6	TP1	TP2	TP3	TP4
1^a	+	-	-	-	-	-	-	-	-	-
1^b	+	+	-	-	-	-	-	-	-	-
2^a	+	+	+	+	-	-	+	-	-	-
2^b	-	-	-	-	+	+	+	+	+	+
2^c	-	-	-	-	+	+	-	-	+	+
3	+	+	+	+	+	+	+	+	+	+
Total	4	3	2	2	3	3	3	2	3	3

(d): *Esterase*

Allele	TE1	TE2	TE3	TE4	TE5	TE6	TP1	TP2	TP3	TP4
1^a	+	-	-	-	-	-	-	-	-	+
1^b	+	+	+	+	+	+	+	+	+	+
2^a	-	+		-	-	-	-	-	-	-
2^b	-	-	+	-	-	-	+	-	-	-
2^c	-	-	-	+	-	+	+	+	-	-
2^d	+	-	-	-	-	-	-	-	-	-
2^e	-	-	-	-	-	+	-	-	-	+
2^f	-	+	-	-	-	-	-	-	-	-
3	+	+	+	+	+	+	+	+	+	+
Total	4	4	3	3	2	4	4	3	2	4

(e): *Peroxidase*

Allele	TE1	TE2	TE3	TE4	TE5	TE6	TP1	TP2	TP3	TP4
1^a	+	+	+	+	+	+	+	+	+	+
1^b	+	-	-	-	-	-	-	-	-	-
2^a	+	+	+	+	+	+	+	+	+	+
2^b	+	+	-	-	-	-	+	-	-	+
2^c	-	-	+	+	-	-	-	-	-	-
3^a	-	-	-	-	-	-	-	+	+	-
3^b	-	-	-	+	+	+	+	-	-	-
3^c	-	-	-	-	+	+	-	-	-	-
Total	4	3	3	4	4	4	4	3	3	3

"+" indicate the presence of bands.

T.erecta 'Indian orange'(TE1) *T.erecta* 'Maurel orange' (TE2)
T.erecta 'Antigua white (TE3) *T.erecta* ' Discovery yellow' (TE4)
T.erecta 'Antigua Orange' (TE5) *T.erecta* ' Safari Tangerine' (TE6)
T.patula 'Double Eagle' (TP1) *T.patula* 'Double Orange' (TP2)
T.patula 'Inca Cream'(TP3) *T.patula* 'Safari Yellow' (TP4)

DISCUSSION

Morphological characters

In the present study, analysis of morphological characters of Tagetes species revealed that each cultivar is different. The two species of Tagetes (*T. erecta* and *T. patula*) used for this study were morphologically dissimilar. *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'(TE1), *T. erecta* 'Maurel orange' (TE2), *T. erecta* 'Antigua Orange' (TE5) and *T.erecta* ' Safari Tangerine' (TE6). yellowish shades were seen in *T. erecta* ' Discovery yellow' (TE4). White flowers were found in *T. erecta* 'Antigua white (TE3). The most attractive flowers were observed in *T. patula* and head was heterogamous. The biggest flowerings form at *T. erecta* (*Tagetes erecta* 'Maurel Orange'[TE2]) and smallest ones at *T. patula*.

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 [18, 20, 30]. Ethnobotanical studies on *T. erecta* were also reported by many workers [1, 16]. Vasudevan et al. [29] has ranked *T. erecta* as a multipurpose herb.

A marigold flower contains abundant amounts of a valuable antioxidant compound called lutein [15]. The extract with only the purified form with a lutein content of known concentration 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 [8]. Xanthophyll content varies in the range of 9 to 11 g/Kg. Total lutein esters have been reported to be in the range of 3.8 to 791 mg/Kg of flower [24]. The dark orange colour

flowers are observed in *T.erecta.* From the present study it was evident that *T.erecta* 'Maurel orange' (TE2) have large and dark orange coloured flowers.

Polymorphism detected by biochemical markers (Isozymes)

In the present study not much variation was observed in catalase zymogram. However high polymorphism was observed in AAT at second locus. Cultivar TE3, TE5 and TP3 showed no bands in this locus. Isozyme esterase exhibited one of the most complicated zymograms. In this study, it appeared to be controlled by 3 loci. On the other hand, esterase showed large number of alleles in second locus and polymorphism to differentiate the two species as well as cltivars of the same species. Samples TE5 and TP3 were not shown bands in this locus. Maximum diversity was observed in locus EST-1, which differentiated cultivars TE1 and TE2, while the other loci differentiate other cultivars. Esterase (EST), can be used as a diagnostic tool for cultivar identification in view of its extensive polymorphism for this enzyme. Previous studies showed that esterase produced low polymorphic bands [12], while Fachinello et al. [6] found that esterase is most reliable enzyme for identification of pear cultivars. In this study esterase was active to differentiate between the ten Tagetes genotypes.

The more differential MDH isozyme expression observed in cultivars of *T. erecta.* In the present study variation was observed in first locus. The MDH-1 locus expressed only in TE1 and TE2. Two alleles (MDH-1^a & MDH-1^b) were observed at MDH-1 locus. MDH-1^a observed only in 1^{st} sample (E1). MDH-1^b was detected in TE1 and TE2. In the present study revealed that MDH can be used for cultivar identification of Tagetes.

Three zones of peroxidase (PRX), activity were present on acrylamide gels. It appeared to be coded by three loci (expressed as 8 different alleles at three loci). Locus–2 and locus–3 exhibiting three alleles per locus. All these alleles showed polymorphism in both species. Among the three loci genetic diversity per locus for peroxidase followed the trend, PRX-2> PRX-3> PRX-1. In the present analysis, peroxidase was able to differentiate cultivars of the same species.

Variation at isozyme loci revolutionized the research and evolution [22, 23, 33]. Only the polymorphic bands are actually of use in genetical, physiological or taxonomical studies [2]. The isozyme data showed inter- and intra specific variability in Tagetes higher than in other plant species analysed earlier, like pea, field bean, lupin [17, 34]. Intra specific variation was observed for 5 isozyme systems (CAT, AAT,MDH, EST and PRX) in analysed species. The presence of intra specific isozyme variation was expected in Tagetes. According to Hamrick et al. [9], asexually reproducing plants exhibit less isozyme variation than sexually reproducing ones. Moreover, the most variable species are those capable of both sexual and asexual propagation [9]. In addition, somatic mutations can create genotypic variation. Clonal off springs are expected in Tagetes because of the lack of a mechanism of recombination in asexual organisms, but DNA can be changed at various stages of plant development. Mutations in undifferentiated meristematic tissue can be further incorporated into the germ line, so mutation in the parent is passed to the offspring [13]. Hong [11] reported that allozymes usually display insufficient polymorphisms among cultivars in *Allium*. However, in this study of the variability in Tagetes cultivars , assayed with isozymes, was high enough for dendrogram construction. The purpose of this study, to analyse isozyme polymorphism, as compared with morphological data, was fulfill for making more reliable inferences on genetic dissimilarity in cultivars.

Systematic inferences were made usually from observations and comparisons of phenotypes [5]. However, several genes and the environment can influence the development of morphological characters. The assumption that 2 plants lacking a feature are more similar to each other than to a third plant produces an additional error. In the case of isozymes, alternative alleles are always visible. The isozymes were historically the first application of markers, but they still show some advantages over other marker systems, e.g. simplicity and speed of analysis. The reliability of this method

allows the verification of results by other researchers. The isozyme investigations of Tagetes collections showed the strength of this technique. Isozyme analysis of *Tagetes* cultivars are rare, hence the results of this present investigation become more helpful for future researchers and breeding programmes.

It is concluded from these results that the different marker systems (morphological, and biochemical markers) 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 colours can be used in further hybridization programme because of high carotenoide content. In isozyme analysis, peroxidase was able to differentiate both the species. Esterase showed large number of alleles and polymorphism to differentiate the two species as well as cultivars of the same species. Selection of better cultivar can be made for species or varietal improvement on the basis of percent similarity with other species.

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