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Morphological and molecular characterization of Gomphrena genotypes

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ABSTRACT

Gomphrena is one of India's emerging traditional flower crops widely used for garland making. A comparative investigation of morphological and molecular analysis of seventeen Gomphrena genotypes was conducted to characterize and assess their genetic diversity for commercial horticultural traits. Morphological observations have shown drastic variation in growth, flowering, quality and yield parameters in all seventeen genotypes. Clustering based on D_2 statistics assembled the Gomphrena genotypes into seven clusters, demonstrating the existence of wide genetic divergence at the phenotypic level. The major cluster-I included six genotypes, cluster-IV and VI with three genotypes, cluster-II with two genotypes and other clusters with only solo genotype based on similar morphological traits. Further, twenty ISSR primers were used to estimate the genetic diversity and documentation of genotypes through molecular characterization. Thirteen ISSR primers were shown polymorphism with 93 loci across the Gomphrena genotypes. Among these 93 loci, 79 were polymorphic (86.02 %) in nature, portraying the high allelic diversity. All the primers displayed good discriminating power, as indicated by their PIC values, with a mean value of 0.21. Molecular profiling through MegaX and PCoA analyses also separated the *Gomphrena globosa* population into two clusters and placed them into two plots.

Key words: *Gomphrena globosa* L., Characterization, Genetic diversity, Polymorphism.

INTRODUCTION

Gomphrena (*Gomphrena globosa* L.) is an important traditional, annual flower crop grown in India's tropical and sub-tropical zones. It is commonly referred to as a bachelor's button or globe amaranth and is a member of the *Amaranthaceae* family. Central America is regarded as a native of Gomphrena, but now it is known to be grown worldwide (Roriz *et al*., 15). This versatile flower crop is cultivated widely for its attractive coloured round-shaped flower inflorescences, which display shades of purple, fuchsia, white, red, pink, orange mauve, etc. Gomphrena can be used for loose flowers, cut flowers, garland making, borders, beds, wreaths, flower arrangements, pigment extraction, and therapeutic products (Niu *et al*., 11). Notably, it exhibits high heat tolerance and moderate resistance to drought and employs the C4 pathway for carbon fixation (Herold *et al*., 7).

Characterization plays a crucial role in enhancing the heritable improvement of any plant species. Traditionally, morphological characterization has been widely employed to assess genetic variation due to its simplicity. However, molecular characterization is indispensable for a comprehensive understanding of genotypic variation. This enables efficient classification, minimizing errors associated with morphological characterization as environmental changes influence few parameters. Precise identification and characterization of diverse genotypes are of utmost importance for the development of a new variety, certification, and protection of breeders' and farmers' rights (Hale *et al*., 6). So far, a few scientific reports have been documented on correlation and growth behaviour studies (Ashwini *et al*., 1) and minor evaluations of varieties of the Gomphrena crop. However, none of the above studies had classified the varietal diversity and comparison in Gomphrena. To our knowledge, this is the pioneer effort in the field, focusing on the morphological and molecular characterization of G. globosa and aiming to study the diversity among the genotypes using a range of morphological and molecular traits..

MATERIALS AND METHODS

Seventeen different gomphrena genotypes were collected such as AGS-1, AGS-2, AGS-3, AGS-4, AGS-5, AGS-6, AGS-7, AGS-8, AGS-9, AGS-10, AGS-11, AGS-12, AGS-13, AGS-14, AGS-15, AGS-16 and AGS-17 (AGS: Arabhavi Gomphrena Selection) for assessment of genetic diversity. The experimental trial was conducted at the research farm of the Floriculture and Landscape Architecture department, College of Horticulture-Bagalkot, UHS, Bagalkot, Karnataka, in Randomized Block Design (RBD) from November 2020 to March 2021, with three replications and a plot

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size of 1.8 × 1.5 m^2 with plant spacing 30 × 30 cm was followed. The observations were recorded from 5 randomly chosen plants from each genotype, each replication for several vegetative, yield, and quality variables for morphological characterization.

Young fresh leaf samples of all the Gomphrena genotypes were collected for molecular characterization. DNA was extracted using the cetyl-trimethyl ammonium bromide (CTAB) technique (Doyle and Doyle, 4) with some modifications. ISSR (Inter-Simple-Sequence-Repeats) markers were utilized as no prior knowledge of target sequences was known. Then, the molecular data was analyzed by manually scoring the bands, which were recorded as either presence (1) or absence (0) across the sample lanes, and various molecular traits were analyzed using GenALEx software. The dendrogram was generated by MegaX-based software using the neighbour-joining (NJ) technique based on pairwise Euclidean distance values.

RESULTS AND DISCUSSION

Morphological diversity was analyzed for various growth, yield and quality traits representing wide diversity with significant variation (Table 1 & 2). Plant height, a visible variable trait, particularly in flower crops, is used to recommend appropriate genotypes for various purposes such as commercial cultivation, edges, and ground covers. In the present study, the plant height was highest (64.45 cm) in AGS-5 and the lowest, 20.20 cm in AGS-12, which is on par with the AGS-13 and AGS-11. A similar pattern of disparity in the plant's height amongst the considered genotypes was also noted in African marigolds by Narsude *et al*. (11), and this is due to their varied origin and the evolution of specific genotypes as morph types in their definite geographical location. The number of branches is always associated with the flower yield or flower count per plant. This was represented as 8.90 (AGS-13) to 45.90 (AGS-7) branches, which may be accredited due to genetic-makeup of the cultivars as observed in marigold by Naik *et al*. (9). Stem girth ranged from 6.30 mm (AGS-13) to 11.50 mm (AGS-5), plant spread which represents the strength of plant growth calculated in both north-south and east-west directions. The highest plant spread was proved in the genotype AGS-5 (1575.84 cm^2), and lowest in the genotype AGS-13 (211.95 cm^2), and these results are familiar with the interpretations of the Ashwini *et al*. (1) in globe amaranth. A parameter which is considered the ultimate and the utmost valuable trait for commercial cultivation benefits of the crop is

Table 1. Growth and yield traits of gomphrena genotypes.

Genotype	Plant height (cm)	No. of branches	Plant spread (cm^2)	Stem girth (mm)	Number of flowers per plant	Flower yield per plant (g)	Flower yield per m^2 (g)
AGS-1	58.20^{c}	39.1^{bc}	800^{cde}	10.37^{abc}	271.5^{g}	54.30^{f}	603.27^{f}
AGS-2	56.55^{c}	37.2^{c}	718.38^{def}	10.56^{abc}	296.0^{fg}	59.20^{ef}	657.71^{ef}
AGS-3	59.25^{bc}	37.8b^{c}	869.17^{bcde}	10.06^{bc}	367.5^{cd}	73.50^{cd}	816.58b^{cd}
AGS-4	51.40^{d}	37.6b^{c}	1106.28^{bc}	8.37^{de}	418.5^{bc}	83.70^{b}	929.90^{b}
AGS-5	64.45^{a}	41.4^{b}	1575.84^{a}	11.50^{a}	484.0^{a}	96.80^{a}	1075.45^{a}
AGS-6	61.55^{b}	36.4^{c}	1195.2^{b}	10.02^{bc}	467.5^{ab}	81.09^{bc}	900.91^{bc}
AGS-7	59.20^{bc}	50.9^{a}	1183.7^{b}	11.08^{ab}	415.5^{bc}	70.80^{d}	814.36^{cd}
AGS-8	39.70^{e}	29.9^{d}	614.54^{def}	10.14^{abc}	354.0^{d}	70.80^{d}	786.58^{cd}
AGS-9	40.20^{e}	30.6^{d}	717.78^{def}	9.20^{cd}	352.0^{de}	70.40^{d}	782.14^{d}
AGS-10	41.00^{e}	27.9^{d}	546.06^{efg}	9.54^{cd}	261.5^{gh}	52.30^{f}	581.05^{f}
AGS-11	20.45^{g}	11.3^{f}	384.6^{fg}	6.73^{f}	182.5^{i}	36.50^{hi}	405.51^{hi}
AGS-12	20.20^{g}	11.2^{f}	369.28^{fg}	8.50^{de}	157.5^{ij}	31.50^{ij}	349.96^{ij}
AGS-13	20.30^{g}	8.9^{f}	211.6^{g}	6.30^{f}	120.5^{j}	24.40^{j}	271.08^{j}
AGS-14	23.70^{f}	18.1^{e}	620.13^{def}	4.39^{g}	114.5^{j}	22.90^{j}	254.42^{j}
AGS-15	41.90^{e}	30.4^{d}	918.08^{bcde}	7.50^{ef}	209.5^{hi}	41.90^{gh}	465.50^{gh}
AGS-16	52.85^{d}	30.6^{d}	929.3^{bcd}	8.30^{de}	334.5^{def}	66.90^{de}	743.25^{de}
AGS-17	52.95^{d}	31.1^{d}	819.08^{bcde}	7.22^{ef}	296.5^{efg}	49.50^{fg}	549.94^{fg}
S. Em (±)	0.97	1.28	58.25	0.46	18.61	3.28	38.14
C.D. (p=0.05)	2.92	3.83	379.54	1.38	55.80	9.82	114.35

Table 2. Floral and quality traits of gomphrena genotypes.

Genotype	Days to first flowering	Flower diameter (cm)	Individual flower weight (g)	Duration of flowering	Display life (days)	Colour code	Colour group
AGS-1	33.20^{cd}	1.52^{h}	0.40^{abcd}	48.00^{a}	37.50^{ab}	N78A	Strong reddish purple
AGS-2	34.80^{a}	1.71^{defg}	0.31^{bcdef}	45.00^{bc}	37.00^{bc}	N78C	Deep purplish pink
AGS-3	32.50^{de}	1.9^{bc}	0.33^{bcde}	45.00^{bc}	34.50^{cd}	155A	Pale yellow green
AGS-4	33.90^{abc}	1.8^{cdef}	0.30^{cdefg}	43.00^{cde}	32.00^{de}	63C	Strong purplish pink
AGS-5	32.10^{ef}	1.89^{bc}	0.47^{a}	49.00^{a}	40.00^{a}	N78A	Strong reddish purple
AGS-6	33.80^{bc}	1.87^{bcd}	0.44^{ab}	47.00^{ab}	38.00^{ab}	N78C	Deep purplish pink
AGS-7	34.70^{ab}	1.83^{cde}	0.42^{abc}	47.50^{a}	34.50^{cd}	155A	Pale yellow green
AGS-8	31.00^{gh}	1.97^{abc}	0.22^{efg}	40.50^{f}	37.00^{bc}	N78C	Deep purplish pink
AGS-9	31.30^{fg}	2.09^{a}	0.20^{fg}	42.00^{def}	33.50^{d}	N78A	Strong reddish purple
AGS-10	27.80^{j}	1.93^{abc}	0.20^{efg}	40.50^{f}	30.50^{e}	155A	Pale yellow green
AGS-11	27.60^{j}	2.03^{ab}	0.23^{efg}	40.50^{f}	27.50^{f}	N78C	Deep purplish pink
AGS-12	28.90^{i}	2.09^{a}	0.22^{efg}	32.00^{h}	25.00^{f}	155A	Pale yellow green
AGS-13	30.10^{h}	1.66^{efgh}	0.17^{g}	35.00^{g}	26.50^{f}	N78A	Strong reddish purple
AGS-14	27.30^{j}	1.56^{gh}	0.29^{defg}	41.50^{ef}	30.50^{e}	N45	Moderate red
AGS-15	32.20^{ef}	1.67^{efgh}	0.21^{efg}	37.00^{g}	25.50^{f}	76C	Very pale purple
AGS-16	33.70^{c}	1.63^{fgh}	0.27^{efg}	45.00^{bc}	27.50f	155A	Pale yellow green
AGS-17	30.30^{h}	2.02^{ab}	0.18^{g}	44.00^{cd}	25.50f	N78A	Strong reddish purple
S. Em (±)	0.32	0.06	0.06	0.67	0.98		
C.D. (p=0.05)	0.95	0.17	0.13	2.02	2.94		

flower yield per plant, signified by a value of 22.90g (AGS-14) to 96.80g (AGS-5), which is governed by genetic nature and interrelated with many other characters like plant spread, plant height, branch count per plant and are familiar with the conclusions of Narsude *et al*. (10) in Marigold.

Wide variations were also depicted among the genotypes for the floral traits in Gomphrena, such as days to first flowering ranging from 27.30 (AGS-14) to 34.80 (AGS-2). In flower crops, the genotype takes a short duration for flower initiation, which is one of the essential criteria for considering it as an early variety, which is highly preferred in the national and international markets. Flower quality traits like flower diameter, which determines the size of the bloom, can divide the flowers into various grades, and a variation was reported from 1.52 (AGS-1) to 2.10 cm (AGS-11). Individual flower weight, which ultimately reveals the power of the total yield of the crop, shows a significant variation of 0.23g (AGS-13) to 0.53g (AGS-5). Display life, measured as the total number of days until the flowers remained unwithered, is a highly preferred trait for flower shows and exhibitions. It also shows a dignified disparity for various genotypes. It ranged from 25.00 days for AGS-12 to 40.00 days for AGS-5. The duration of flowering, an important parameter to be considered for having more flower pickings, represented a very low 32.00 days in AGS-12 and the highest in AGS-5 (49.00). Parallel outcomes were conveyed in marigold by Naik *et al*. (8).

Flower colour plays a crucial role in defining the market value of flower crops. In case of Gomphrena, there are six different shades observed by the use of RHS colour chart. Genotypes, AGS-1, AGS-5, AGS-9, AGS-13, AGS-17 represented strong reddish-purple group with colour code N78A. Additionally, AGS-2, AGS-6, AGS-8, AGS-11 fall into deep purplish pink group (N78C). AGS-4 is characterized by colour code 63C, denoting a strong purplish pink shade. On the other hand, AGS-3, AGS-7, AGS-10, AGS-12, AGS-16 exhibit a pale yellow-green hue (155A). Whereas, AGS-15 displayed very pale purple group (76C) and AGS-14 recorded colour code N45, indicating strong reddish-purple group. Similar studies have reported the use of flower colour as a distinguishing factor for genotypes by Panwar *et al*. (13) in which 32 rose genotypes divided into 13 different colour categories.

D2 clustering divided the 17 gomphrena genotypes into seven separate clusters representing (Table 3)

Table 3. Cluster composition of 17 gomphrena genotypes in D^2 analysis.

S. No.	Cluster No.	No. of genotypes	Genotype name
1	Cluster-I	6	AGS-5, AGS-3, AGS-7, AGS-6, AGS-15, AGS-2
2	Cluster-II	2	AGS-12, AGS-13, AGS-11
3	Cluster-III	1	AGS-17
4	Cluster-IV	3	AGS-1, AGS-16,
5	Cluster-V	1	AGS-4
6	Cluster-VI	3	AGS-8, AGS-10, AGS-9
7	Cluster-VII	1	AGS-14

the wide diversity at morphological level. The major cluster-I included six genotypes, cluster-IV and VI with three genotypes, cluster-II with 2 genotypes and other clusters with single genotypes based on the distribution of various mutual morphological features. Genotypes which are grouped are sharing a similar type of plant height and yield traits, whereas the clusters with solo genotypes are unique and are little divergent from others. Patil *et al*. (14) also reported that 26 marigold genotypes also divided into seven clusters with maximum genetic divergence contribution was given by flower yield per plant, followed by other traits.

In molecular characterization, among twenty ISSR primers used for the evaluation, thirteen primers shown polymorphism with the total of 93 loci across gomphrena genotypes, while few displayed monomorphisms and five primers did not amplify at all. Out of these 93 loci, 79 loci (86.02 %) demonstrated polymorphism, portraying the high allelic diversity and were congruence with the findings of Satya (16) in Hibiscus in which they have reported 67.76% polymorphism in the considered species. The magnitude of the amplified bands ranged from 100 to 1100 bp, which is spotted in agreement with the reporting of Ogras *et al*. (12), in Rose genotypes, where the amplification range was almost same with a magnitude of 150 to 1100 bp. The primer UBC899, indicated the significant polymorphism with 6 bands followed by UBC880, UBC829, UBC809, UBC8405 with 5 bands, whereas, minimum bands (2) were detected by UBC 864 primer (Table 4).

All the primers displayed good discriminating power, as indicated by their polymorphic Information Content (PIC) values, with mean value of 0.21. The highest PIC value observed with UBC903 (0.34), while the lowest (0.08) obtained with UBC862. Parallel consequences were also conveyed earlier with the use of ISSRs, with a PIC value range of 0.29 to 0.66, by Baliyan *et al*. (2), in chrysanthemum, suggesting that the ISSR primers used were capable to distinguish the genotypes. The genetic polymorphic analysis results indicated, the mean observed number of alleles (Na) was 1.86, the maximum number of effective alleles (Ne) are 1.39, Shannon's information index (I) of 0.37, Expected Heterozygosity (He) of 0.24, Unbiased Expected Heterozygosity (uHe) of 0.24 and Polymorphic Loci PPL as 86.02, signifying the incidence of rare microsatellite alleles. This result was in agreement with the observation of

Table 4. Sequences of ISSR primer and their features for characterization of Gomphrena genotypes.

Primer Code	Primer Sequence	AT °C	FR	TB	PB	PPB	PIC
AG_n based Primer-809	(AG) 8G	52	200-600	7	5	71.43	0.19
UBC817	CACACACACACACAA	50	350-850	8	8	100	0.18
UBC827	ACACACACACACACACT	48	300-750	6	5	83.33	0.22
UBC829	ACACACACACACACACG	50	250-700	10	10	100	0.31
UBC836	TGTGTGTGTGTGTGTGC	50	250-700	9	9	100	0.28
UBC840	TGTGTGTGTGTGTGTGG	48	450-1050	6	6	100	0.21
UBC841	GAGAGAGAGAGAGAGAY	52	300-700	5	3	60	0.10
UBC862	(AGC) 6	55	400-1050	4	3	75	0.08
UBC864	TCTCTCTCTCTCTCTCRT	55	400-1100	6	6	100	0.21
UBC880	ATGATGATGATGATGATG	55	250-1050	9	9	100	0.19
UBC899	GAGCAACAACAACAACAA	55	400-1100	8	6	75	0.13
UBC901	GAGCAACAACAACAACAC	54	400-1100	4	4	100	0.24
UBC903	ACACACACACACACACG	54	200-1100	7	7	100	0.34

AT: Annealing temperature; **FR**: Fragment range; **TB**: Total no bands; **PB**: No. of polymorphic bands; **PPB**: Percentage of polymorphic bands; **PIC**: polymorphic information content

Cole (3) as reported that the He and uHe values to be 0.113 and 0.150 for rare and common species, correspondingly. The banding patterns across the population of gomphrena genotypes shown that maximum number of bands (93) were found and all the bands showing frequency > =5 and 93 private bands also noticed.

Gomphrena genotypes were also clustered on the basis of principal co-ordinates, contributing to first and second axes, determined from the genetic distances of ISSR band similarities and disclosed a total variation of 49.94% with axis 1, 2 and 3 exhibiting 24.65%, 13.14% and 12.15% variation respectively. The PCoA divided 17 genotypes into two components as shown in Fig. 1. In PCoA analysis, coordinate 1 shown ten different genotypes clustered into one group, comprising of all the flower colours *viz*., purple, pink, light pink, white, bicolored genotypes used for the present investigation. Similarly, the coordinate 2 comprising six genotypes which were not shown any similarity pattern among them. However, association with the all-other genotypes of co-ordinate 2, as it shown various diverse traits like red coloured inflorescence and prostate growth habit. Band similarities might be either due to the naturally occurred hybridization or spontaneous mutation. Such parallel reports have also been reported in *Jasminum* spp. by Ghehsareh *et al*. (5).

The result attained from PCoA is in agreement with that of the dendrogram procured by neighbour joining phylogenetic tree by the MegaX based software, an extra validation of relationships portrayed by cluster-analysis in gomphrena. 17 genotypes, were clustered into two major clads with 16 genotypes in one clad and one solitary clad (Fig 2.). This clustering pattern confirms the phylogeny and flower colour traits. Namita *et al*. (9) also

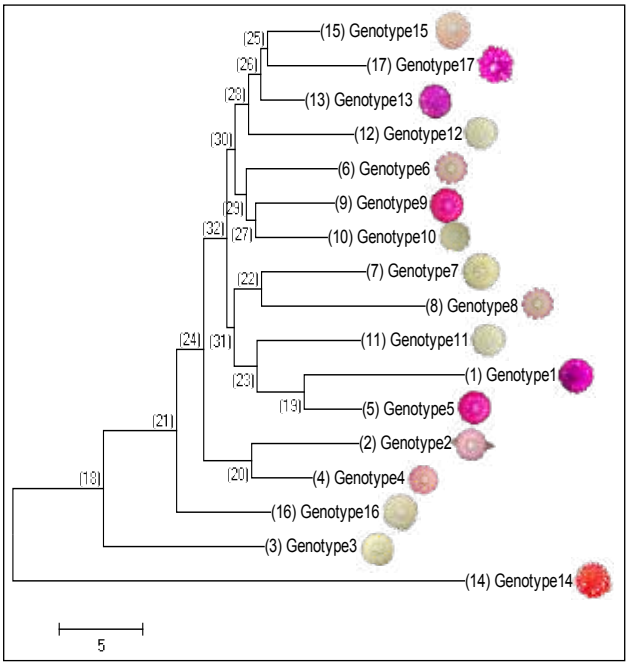

Fig. 2. Neighbour joining phylogenetic tree formed for various gomphrena genotypes.

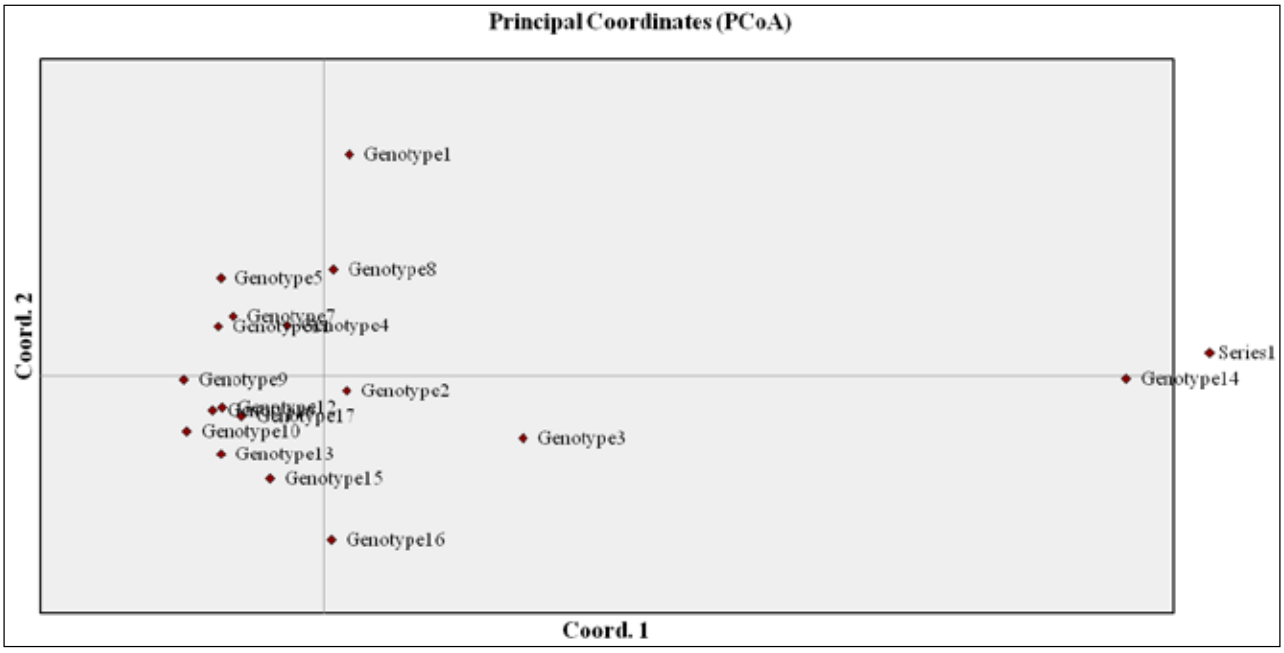

Fig. 1. Principal co-ordinate analysis obtained for various gomphrena genotypes.

reported that Marigold genotypes are divided into two clusters based on association of species level by use of ISSR and RAPD markers. Genotype 14 is divergent morphologically and genetically with novel flower colour (*i.e.,* red) as it belongs to *Gomphrena haageana* whereas, the all-other genotypes belong to same species *i.e*., *G. globosa*. Further, these distant genotypes could be highly recommended to use in crop improvement programs for gaining a broad spectrum of variation.

Morphological evaluation separated the Gomphrena genotypes easily but ISSR markers proved to be highly valuable in identifying polymorphism and investigating the diversity among different gomphrena genotypes. Good amount of variability was noticed among gomphrena genotypes because of available gene pool. The dendrograms created from molecular data effectively distinguish between genotypes by reflecting their genetic affinities and disparities. The ISSRs used in the current study is beneficial in identifying a cultivar, and for documentation of genetic diversity of gomphrena germplasm. Characterization also helpful to divide the usage of genotypes for distinctive uses like loose flower (AGS-5, AGS-6, AGS-7) and pot plant (dwarf genotypes like AGS-11, AGS-12, AGS-13).

AUTHORS' CONTRIBUTION

Conception of research (BSK, PP); plotting of experiments (PP, KDL); Input of experimental materials (BSK, PP); Implementation of field or lab trails (BSK, PP); Analysis of data and interpretation (KDL, PP, CRB); Preparation of manuscript (KDL, PP). All the authors read the manuscript and approved the final draft of it.

DECLARATION

The authors confirm no conflict of interest with them.

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REFERENCES

1. Ashwini, A., Kurubar, A. R., Patil, S. and Kumar, C. S. 2019. Evaluation of bachelor's button (*Gomphrena globosa* L.) genotypes for growth and yield parameters. *J. Pharmogn. Phytochem.* **8**: 541-45.
2. Baliyan, D., Sirohi, A., Kumar, M., Kumar, V., Malik, S. and Sharma, S. 2014. Comparative genetic diversity analysis in chrysanthemum: A pilot study based on morpho-agronomic traits and ISSR markers. *Sci. Hortic*. **167**: 164-68.
3. Cole, C. T. 2003. Genetic variation in rare and common plants. *Annu. Rev. Ecol. Evol. Syst.* **34**: 213-37.
4. Doyle, J. J. and Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus*., **12**:13-15.
5. Ghehsareh, M. G., Salehi, H., Khosh-Khui, M. and Niazi, A. 2015. Application of ISSR markers to analyze molecular relationships in Iranian jasmine (*Jasminum* spp.) accessions. *Mol Biotechnol*. **1**:65-74.
6. Hale, A. L., Farnham, M. W. and Menz, M. A. 2006. Effectiveness of pcr-based markers for differentiating elite broccoli inbreds. *J. Am. Soc. Hortic. Sci*. **131**: 418-23.
7. Herold, A., Lewis, D. H. and Walker, D. A. 1976. Sequestration of cytoplasmic orthophosphate by mannose and its differential effect on photosynthetic starch synthesis in C3 and C4 species. *New Phytologist*. **76**: 397-07.
8. Naik, H., B., Patil, A. A., Basavaraj, N. and Patil, V. S. 2005. Stability analysis for growth, yield and flower colour (xanthophyll) in African marigold (*Tagetes erecta* L.). *Kar. J. Hortic*. **1**: 28-36.
9. Namita, Panwar, S., Sonah, H., Singh, K. P. and Sharma, T. M. 2013, Genetic diversity analysis of marigold (*Tagetes* sp.) genotypes using RAPD and ISSR markers. *Indian J. Agric. Sci.,* **83**: 484-90.
10. Narsude, P. B., Kadam, A. S. and Patil, V.K. 2010. Studies on the growth and yield attributes of different African marigold genotypes under Marathwada conditions. The *Asian J. Hortic*. **5**: 284-86.
11. Niu, Y. F., Han, C. M., Shao, Y. and Tao, Y.D. 2009. Comparative study on eight trace elements in twelve flower medicines. *Spectroscopy Spectral Anal*. **29**:1997-2000.
12. Ogras, T., Başţanlar, E. K., Metin, O. K., Kandemir, I. and Ozçelik, H., 2017. Assessment of genetic diversity of rose genotypes using ISSR markers. *Turk. J. Bot.* **41**: 347-55.