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Research Paper

Assessment of morphological diversity in *Acorus calamus* L., an economically important endangered medicinal plant species of Kashmir valley

Umar A. Mir[1], Haleema Bano[1], M. Ashraf Bhat[2], Aroosa Khalil[3], Fozia Hamid[1] and Areeba Aijaz[1]

[1]Division of Environmental Sciences; [2]Division of Plant Biotechnology, Division of Fruit Science
Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar-190025, Jammu and Kashmir (India)
*e-mail: *haleemaashraf@gmail.com*

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ABSTRACT

Acorus calamus* L. is an economically important and endangered medicinal plant of wetlands of Kashmir. The present study was carried out for two years. The mapping of genetic diversity of *A. calamus* was assessed in four wetlands *viz*. Hokersar (Srinagar), Shallabugh (Ganderbal), Manasbal lake (Ganderbal) and Anchar lake (Srinagar) of Kashmir Valley using Mahalanobis D^2 analysis. The results revealed the presence of 5 clusters for 40 populations studied with one population in cluster-1; nine in cluster-2; ten in cluster-3; two in cluter-4 and eighteen in cluster-5. Majority of populations were grouped in the cluster-5, followed by cluster-.3. The maximum intra-cluster distance (D^2) of (32.579) was observed in cluster-4 and the maximum inter-cluster distance (D^2) of (32.579) was observed in cluster-1. Present study revealed that plant species possessed sufficient genetic diversity among the populations. Cluster-5 showed superiority with respect of the maximum mean plant height plant^{-1} (93.30 cm), leaf length plant^{-1} (90.92 cm), leaf width plant^{-1}(2.40 cm), fresh weight of rhizome per cm length (2.45 g), rhizome length plant^{-1} (21.60 cm), rhizome diameter plant^{-1} (1.95 cm), dry weight of rhizome per cm length (1.05 g), distance between nodes plant^{-1} (2.08 cm) and number of nodes plant^{-1} (14) as compared to other clusters. Out of five clusters, cluster-5 was promising as the genotypes reflected were better in yield and yield attributing traits., Cluster-5, comprising of eighteen populations were from the undisturbed area, (Shallabugh). The populations from the same cluster can be multiplied for initiating a conservation and breeding program thet can serve as a tool for the scientific community to evolve better contemporary varieties of *A. calamus

Key words: *Acorus calamus*, Cluster distance, Mahalanobis D^2 analysis, Diversity Endangered, Genetic, Wetland plant

Acorus calamus L., commonly known as Sweet flag belongs to family Araceae, is an aromatic perennial herb, indigenous to Central Asia, India and Himalaya. Its distribution in Europe was strongly affected by the Tatars during the 13th to 14th century and now it is native to marshy regions in Northern temperate zones. It grows wild in wetlands, on the edges of swamps, on the banks of rivers and ponds. The rhizomes as well as the leaves have a pleasant, slightly sweet odour due to presence of an essential oil. Dried roots have long been used in medicinal preparations as antispasmodic, antiulcer preparations and for the flavouring of bitter liqueurs and appetizers, headache, loss of appetite and chest infection (pneumonitis) (Bano e*t al.*, 2017).

A. calamus is a common item in the international drug market and the raw material has originated from the natural populations. It is a popular conventional, critically endangered, curative and fragrant plant species (Bano *et al.*, 2022a). In a worldwide survey carried out by McAlpine Thorpe and Warrier Limited (1996), reported that sweet flag is an endangered medicinal plant species and its population in every 7th country has been reported to decrease. In Europe, the populations of *Acorus* have become rare due to its habitat loss caused by drying up of wet areas. This catastrophic decrease of sweet flag populations calls for an immediate conservation of its genetic resources and for starting targeted sweet flag field cultivation, so that raw material demand can be satisfied. Anthropogenic activities have impact on biochemical parameters of

plant (Bano *et al.,* 2021), physico-chemical properties of water (Bano *et al.,* 2022b) and sediment as well (Bano *et al.,*2022c). Little is known about the genetic diversity of the species in Kashmir wetlands, despite the species' strong therapeutic potential and threatened status. As the effectiveness of a species' conservation program depends on a variety of factors, including genetic diversity. Studying the genetic diversity of this plant species is therefore urgently needed. The objective of the present investigation was to find out the morphological diversity in *A. calamus* from the various wetlands in Kashmir. Comparative investigation of the morphological traits vegetative and reproductive components of *A. calamus*, was used to carry out the current study.

MATERIALS AND METHODS

The study on estimation of the genetic diversity of *A. calamus* was carried out during 2020-22 by conducting exploration visits to four wetlands namely Hokersar wetland (Srinagar), Shallabugh wetland (Ganderbal), Manasbal Lake (Ganderbal) and Anchar Lake (Srinagar) of Kashmir region. Extensive surveys conducted in the selected wetlands followed by dividing each wetland into three sub locations. The data were recorded on ten competitive plant populations from each sub-location and mean values were calculated. Following morphological characters of the vegetative and reproductive stages were recorded to differentiate the variation in a population.s

(a) Plant height (cm), (b) Leaf width (cm), (c) Leaf length (cm), (d) Fresh weight of Rhizome per cm length (g), (e) Rhizome length (cm), (f) Rhizome Diameter (cm), (g) Dry weight of Rhizome per cm length (g), (h) The dry weight of the rhizome per cm length (h) Distance between nodes (cm), (i) Number of nodes in Rhizome

Statistical Analysis: D^2 statistics were used to determine the competitive representative diversity of plant populations (Mahalanobis, 1936). The differences in transformed values for various characters were computed and D2 -values were calculated according to the following formula: $D^2 = p \sum i{-}1 (Yij - Yik)2$

Where, P is number of characters studied, and Yij and Y_{ik} = are two transformed variables of the i^{th} character for two genotypes.

The data was analysed by Mahalanobis D^2 analysis using Torcher's method as suggested by Rao (1952).

RESULTS AND DISCUSSION

In the present study, variability and diversity (D^2 statistic) at the phenotypic level were estimated at four locations (Hokersar, Shallabugh, Manasbal, Anchar) of Kashmir valley in 40 populations of *A. calamus*. Dendrogram depicting relationship among 40 populations of *A. calamus* grouped genotypes in five clusters (Fig. 1). Genetic diversity was estimated from the data pooled over years using Mahalanobis' D^2-statistics which indicated the presence of sufficient genetic diversity among the populations. D^2-statistics revealed a total of 5 clusters (Fig. 1, Table 1) with one population in cluster-1; nine in cluster-2; ten in cluster-3; two in cluter-4 and eighteen in cluster-5. Majority of populations got grouped in the cluster-5, followed by cluster-.3. Rao (1952) argues that although there are no clear guidelines for how to create clusters, any two genotypes that belong to the same cluster should at least, on average, display a lower D^2 value than two genotypes that fall into several clusters.

Table 1: Classification of *Acorus calamus* populations of wetlands of Kashmir valley into different clusters on the basis of the morphological diversity

Cluster No.	Number of populations	Name of the population
Cluster-1	01	P14
Cluster-2	09	P11,P12,P13,P15,P16,P17,P18,P19,P20
Cluster-3	10	P21,P22,P23,P24,P25,P26,P27,P28,P29,P30
Cluster-4	02	P1,P9
Cluster-5	**18**	P2,P3,P4,P5,P6,P7,P8,P10,P31,P32,P33,P34, P35,P36,P37,P38,P39,P40

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Ahmad and Borah (1996), reported that the pattern of clustering has a significant impact on genetic diversity. Geographic location, the contributions of diverse genotypes in numerous different clusters over time, and genetic diversity are not comparable. In his study (*Phaseolus volgaris* L.), the genetic diversity of 36 six genotypes were also evaluated for 13 yield and yield attributive properties and grouped into six clusters via Mahalanobis D^2 analysis. The largest cluster-1 was followed by cluster-6 and cluster-2 and -3 with 19 different genotyping types. The genetic divergence of sesame (*Sesamum indicum* L) in a set of 96 advancing breeding lines of genotype was classified in 15 clusters, by Hukumchand and Parameshwarappa (2019). Cluster-I was having the highest number of genotypes (68), cluster-2 (11), cluster-5 (4), and so on.

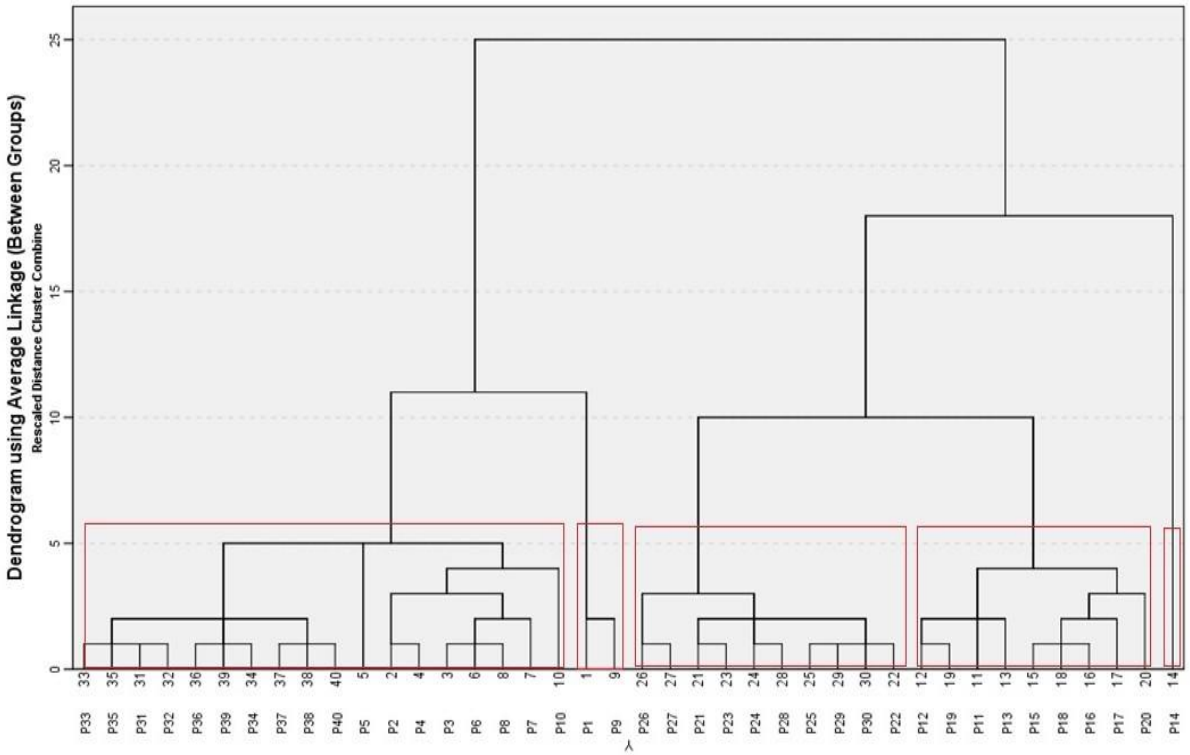


Fig. 1. Dendrogram depicting genetic relationships amongst 40 populations of *Acorus calamus* in wetlands of Kashmir valley

Cluster mean performance of morphological, yield and yield attributing traits in populations of *A. calamus* of wetlands of Kashmir valley: The Mean performance of various traits of a phenotype in a cluster and different phenotypes falling within a cluster are presented in the Table 2. The maximum mean plant height plant^{-1} (93.30 cm) was recorded in Cluster-5 followed by Cluster-4 (90.60 cm),and the lowest mean plant height plant^{-1} was found in Cluster-2 (67.90 cm).The maximum mean leaf length plant^{-1} (90.92 cm) was in the Cluster-5 followed by Cluster-4 (82.09 cm), and the lowest mean leaf length plant^{-1} was found in Cluster-3 (69.37cm).The maximum mean leaf width plant^{-1} (2.40cm) was recorded in Cluster-5 followed by Cluster-4 (2.10cm), and the lowest mean leaf width plant^{-1} was found in Cluster-2(0.60cm).The maximum mean fresh weight of rhizome per cm length (2.45g) was recorded in Cluster-5 followed by Cluster-4(2.18g), and the lowest mean fresh weight of rhizome per cm length was found in Cluster-2(1.52g).The maximum mean rhizome length plant^{-1} (21.60cm) was recorded in Cluster-5 followed by Cluster-4 (20.80cm), and the lowest mean rhizome length plant^{-1} was found in Cluster-3 (13.30cm).

The maximum mean rhizome diameter plant^{-1} (1.95cm) was recorded in Cluster-5 followed Cluster-4 (1.86 cm), and the lowest mean rhizome diameter plant^{-1} was found in Cluster-3(1.24cm).The maximum dry weight of rhizome per cm length (1.05g) was recorded in Cluster-5 followed Cluster-4(1.00g), and the lowest dry weight of rhizome per cm length was found in Cluster-3(0.62g).

The maximum distance between two successive nodes plant^{-1} (2.08cm) was in Cluster-5 followed by Cluster-4(1.90cm), and the lowest distance was found in Cluster-3 (0.67cm).The maximum number of nodes in a rhizome (14) was recorded in Cluster-5 followed by Cluster-4 (13), and the lowest number of nodes in a rhizome was found Cluster-2(06) (Table 2).

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Table 2. Classification of *Acorus calamus* populations of wetlands of Kashmir valley into different clusters on the basis of the morphological

Cluster No.	Cluster-1	Cluster-2	Cluster-3	Cluster-4	Cluster-5
Cluster 1	0.00	30.741	19.189	**32.579**	9.324
Cluster 2	-	3.891	11.760	9.367	22.907
Cluster 3	-	-	3.780	16.252	11.290
Cluster 4	-	-	-	2.261	26.391
Cluster 5	-	-	-	-	4.810

The maximum Cluster mean of different phenotypic traits, comprising of plant height, leaf width, leaf length, fresh weight of rhizome, rhizome length, rhizome diameter, dry weight of rhizome, distance between nodes and number of nodes, were predominately found at undisturbed areas recorded in cluster-5 and the lowest values were found at more disturbed areas recorded in cluster-2 and cluster-3 (Table 2). Hukumchand and Parameshwarappa, in 2019 studied genetic diversity in *Sesamum indicum* L., and reported that the traits like seed yield per plant (42.43%), 1000 seed weight (18.44%), number of capsules per plant (9.25), and height to the first capsule (6.82) and contributed most to genetic diversity. In addition, their work on *Phaseolus vulgaris* L., revealed that traits *viz* green pod yield contributed diversity (40.17%) followed by plant height (18.89 per cent). The highest green pod yield was 165 g per plant, with an average number of pods of 23.85. The cluster pattern might be utilized to choose parents for the hybridization procedure to give the greatest potential diversity for various economic characteristics.

The mean intra-cluster distances (D^2) values for the data was maximum (32.579) in cluster-4. Maximum inter –cluster distance (D^2) (32.579) was observed in cluster-1 (Table 3). Among the clusters with more than one population, the maximum intra cluster distance included high yielding populations.

Table 3: Cluster mean performance of morphological, yield and yield component traits of populations of *Acorus calamus* at four wetlands of Kashmir valley

Cluster No.	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Fresh weight of rhizome cm^{-1} length (g)	Rhizome length (cm)	Rhizome diameter (cm)	Dry weight of rhizome cm^{-1} length (g)	Distance between nodes (cm)	No. of nodes in rhizome
1	83.20	76.42	1.50	1.93	16.40	1.58	0.78	1.48	10
2	67.90	74.60	0.60	1.52	14.90	1.26	0.68	0.70	06
3	75.40	69.37	0.90	1.78	13.30	1.24	0.62	0.67	08
4	90.60	82.09	2.10	2.18	20.80	1.86	1.00	1.90	13
5	93.30	90.92	2.40	2.45	21.60	1.95	1.05	2.08	14

Selection of parents from different clusters having wider inter-cluster distance and also revealing good intra-cluster diversity for a set of economic traits have been useful in creating broad genetic base after hybridization (Singh *et al.*, 1996). Cluster means of different characters help in choosing the diverse parents for hybridization and the divergent parents are likely to broaden the genetic base (variability) and make available transgressive segregants with high heterotic effects (Qian and He, 1991; Rao and Gomathinayagam, 1997). Cluster means and genotypic variation, showed an intriguing picture of the nature of variety and have the greatest chance of allowing genotypes from different clusters to cross, which is anticipated to result in the greatest amount of heterosis (Sardana *et al.*, 1997).

CONCLUSION

This study demonstrated that populations of *A. calamus* obtained from four different wetlands in Kashmir had significant morphological variability. Cluster 5 contained the majority of the population groups. The selection of diverse parents for hybridization is aided by cluster methods of various characters which help in selection of parents for desired economic traits from different clusters for initiating an effective hybridization programmes.

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Cluster-5 depicted a promise for improved yield and traits that influence yield. Eighteen populations from cluster-5 of the Shallabugh, the undisturbed area, can be multiplied for an initial conservation and breeding program, which can help the scientific community to develop better modern *A. calamus* varieties with beneficial traits like a higher yield of rhizomes and economically attributed traits.

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