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RESEARCH ARTICLE

Genetic diversity in the natural population of threatened fruit-bearing plant species *Monotheca buxifolia* (Falc.) A.D.

M. Noor[1], U. Nisar[2], K. U. K. Muhammad[1], R. Muhammad[3], A. Niaz[2]

[1]*College of Horticulture, Hebei Agricultural University, Baoding-071001, Hebei, China*
[2]*Department of Botany Hazara University, Mansehra, KP, Pakistan*
[3]*Department of Botany, University of Chitral KP, Pakistan*
Corresponding author E-mail: noorpk_1990@yahoo.com
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Monotheca buxifolia is an ethnomedicinally and economically important threatened fruit bearing plant species in Malakand Division Pakistan. The genetic diversity among the 92 various genotypes of *Monotheca buxifolia* was carried out using sodium dodecyl sulfate poly acrylamide gel electrophoresis (SDS-PAGE) method. A considerable amount of inter districts genetic diversity (66.70%) was observed among the genotypes of *M. buxifolia*. Protein profiling was conducted on 12% gel electrophoresis. A total of 6 protein bands were observed in *M. buxifolia* genotypes. SDS-PAGE practice is a convenient scheme for the examination of both genetic diversity and relationship. Particularly, L-4 and L-5 were monomorphic in the inter districts *Monotheca buxifolia* genotypes and was recognized as species specific. The remaining other loci were polymorphic. In this investigation, the high inter and intra- districts specific diversity was observed demonstrating SDS-PAGE is an authoritative procedure for categorizing the genetically diverse germplasms in *M. buxifolia*. The findings from this study could be useful in the identification and selection of suitable *M. buxifolia* genotypes for future conservation programmes. Today, there is still a need to examine the genetic diversity and protect genetic resources, in particular wild species, for possible benefits in plant conservation programmes. To the best of our knowledge, this is the first ever report that addresses genetic variability in *M. buxifolia.*
Keywords: Genetic variation; Climate change; SDS-PAGE; *M. buxifolia*

Introduction

Monotheca buxifolia is an important ethnomedicinal, fruit-bearing evergreen tree with broad leaves, mostly growing as wild in nature, this species belongs to the family Sapotaceae (Khan et al., 2010; Ullah et al., 2016). This species is mainly found in Pakistan (main center KP province and Northern Pakistan), also found in the hilly areas of Afghanistan, Akhdhar range of Northern Oman and Southwest Saudi Arabia near Zahran al Janub (Shahina and Martin, 1998; Khan et al., 2010). It is common in some places in Pakistan such as Gorakh Hills, Zhob, Loralai, Kohat, Swat, Dir, Chitral Drosh, Chitta Hills Kala, and Attock District (Nasir and Ali, 1972; Khan et al., 2010). Despite other species, *Monotheca buxifolia* is the most preferred species in the hilly areas (Ullah et al., 2016). It is generally used as fuel, forage (for camels and goats), and small timber, due to its thorny nature, particularly used as a fence around cultivated fields and fruit gardens (Khan et al., 2010). *Monotheca buxifolia* is of economic value to local mountain residents, especially in the areas with uneven terrain where conventional horticultural or agronomic cultivation is narrow (Al-Yahyai and Al-Nabhani, 2006; Khan et al., 2010; Ullah et al., 2016). Locally known as Gurguri, black fruit berries are sold to local markets (Ullah et al., 2016). The fresh and dry fruit are quite tasty and has a retail price of between 100 to 150 Pakistani rupees per kg. Ethnomedicinally, fruits are laxative and are used for the treatment of digestive and urinary tract diseases (Ullah et al., 2016).

Limited distribution of *Monotheca buxifolia* was observed in research area (Ullah et al., 2016). Unfair means of collection, cutting for timber and devastation of habitats by man-made activities may have led to extreme deterioration of *Monotheca buxifolia* natural populations (Ullah et al., 2016). Instead of the urgent need for conservation strategy for this species, understanding of population dynamics and diversity that can contribute significantly to reduce its decline in the population. In this study we examined the genetic diversity of *Monotheca buxifolia*, a species threatened in Malakand Division, Pakistan.

Nowadays, there are numerous tools available to determine genetic diversity among plant species' germplasms (Khan et al., 2020). Morphological characterization was initially used, but in the majority of cases morphological description is unstable due to environmental fluctuations (Noor et al., 2018; Muhammad et al., 2018; Khan et al., 2020). Evaluation of germplasms and genetic variation by DNA-based molecular marker is very powerful and influential way, but is more expensive (Muhammad et al., 2018; 2019). Especially in comparison to DNA marker evaluation of germplasms, the characterization of seed storage protein is free from environmental fluxes and easy to handle in developing countries like Pakistan (Muhammad et al., 2018).

Monotheca buxifolia is one of the major medicinal plant species and has been believed to be involved in the treatment of several diseases such as urinary and digestive tract disorders (Ullah et al., 2016). This study was planned to study the genetic diversity in threatened species *Monotheca buxifolia*, using SDS-PAGE characterization among 92 genotypes of *Monotheca buxifolia*. *Monotheca*

buxifolia has an important local adaptation and widespread use by people for fruit, medicinal and fuel purposes (Khan et al., 2010). The main objective of this study is to evaluate the intraspecific diversity of *Monotheca buxifolia* in order to emphasize the diversity amongst the compatible genotypes. This study is the first ever report from Pakistan.

Materials and Methods

Collection sites

In the present study, various sites of four districts (Swat, Buner, Dirlower and Chitral) of Malakand Division were visited for the collection 92 different genotypes of *Monotheca buxifolia* for characterization of genetic diversity in seed storage protein. These sites are shown in Table 1. Sodium dodecyl sulfate poly acrylamide gel electrophoresis SDS-PAGE (seed storage protein) was used for genetic diversity assessment (Noor et al., 2008). For seed storage protein characterization, 2 to 3 seeds of each genotype were crushed into fine flour and 400µl of Protein Extraction Buffer were added to each samples following the method of Laemmli, (1970) modified by Noor et al. (2018) and Khan et al. (2020).

Data analysis

The protein data was analyzed using MS Excel 2010 and PC-ord software (McCune and Mefford, 1997).

Table 1. Collection sites of *Monotheca buxifolia* genotypes.

District	Genotypes	Collection Sites	District	G	CS	District	G	CS	District	G	CS
Swat	Mb1	Dool	Buner	Mb24	Swarai	Dir lower	Mb47	Khawas	Chitral	Mb70	Arundu
	Mb2	Chargu Tangy		Mb25	Pir Baba		Mb48	Shalam Baba		Mb71	Drosh
	Mb3	Khazana		Mb26	Elum		Mb49	Och		Mb72	Ayun
	Mb4	Gamkot		Mb27	Babosar		Mb50	Purighar		Mb73	Bamborat
	Mb5	Kohay		Mb28	Gokand		Mb51	Gedar		Mb74	Lutkho
	Mb6	Rangila		Mb29	Chagharzo		Mb52	Shagukas		Mb75	Barum
	Mb7	Shenkay		Mb30	Malka		Mb53	Khadagzai		Mb76	Shahgram
	Mb8	Banda		Mb31	Nanser		Mb54	Pengal		Mb77	Ustur
	Mb9	Nimogram		Mb32	Elai		Mb55	Sorkamar		Mb78	Beshgram
	Mb10	Sabunkhfa		Mb33	LoyPitaw		Mb56	Katan Payen		Mb79	Kessu
	Mb11	Merata		Mb34	Kohay		Mb57	Jawaro		Mb80	Warinji
	Mb12	Jabagai		Mb35	Koga		Mb58	Nawagai		Mb81	Booni
	Mb13	Ziarat		Mb36	Rega		Mb59	Bezobanr		Mb82	Ujnu
	Mb14	Swegalai		Mb37	Nawagai		Mb60	Darra		Mb83	Darkot
	Mb15	Daam		Mb38	Turwarsak		Mb61	Asbanr		Mb84	Eizh
	Mb16	Parrai		Mb39	Kalpanai		Mb62	Kuhay		Mb85	Ashrait
	Mb17	Dara		Mb40	Chena		Mb63	Derai		Mb86	Beohi
	Mb18	Nagoha		Mb41	Karapa		Mb64	DeranKashmir		Mb87	Baroghil
	Mb19	Zawra		Mb42	Sultanwas		Mb65	Girotangay		Mb88	Mastuj
	Mb20	Kasai		Mb43	Ambela		Mb66	Palusin		Mb89	Reshun
	Mb21	Chinar		Mb44	ShamshadTangi		Mb67	Chal Kamar		Mb90	Kosht
	Mb22	Yakhtangay		Mb45	YakhTangi		Mb68	Manrogay		Mb91	Khot
	Mb23	Naray Tanagay		Mb46	GataGaray		Mb69	Zar		Mb92	Noshaq

Results

SDS-PAGE profiling

Six bands were detected in the electrophoregram (Figure 1). The protein (0, 1) data of 92 genotypes of *M. buxifolia* based on SDS-PAGE was analyzed for the construction of phylogram (Figure 2). This tree demonstrates the diversity and similarity of various genotypes and the 92 genotypes of the *M. buxifolia* were studied and the tree was built (Appendix, Fig. 2); the phylogenetic tree divided 92 genotypes of *M. buxifolia* into four regions (R-I – R-IV). Each region was comprised of 23 genotypes. The region I is comprised of the all genotypes collected from Distrct Swat (Mb-1 to Mb- 23). Similarly the region II (R-II) is comprised of all 23 genotypes collected from District Buner whereas the region III (R-III) and region IV (R-IV) are comprised of the genotypes collected District Dir lower and District Chitral respectively. This was further confirmed by principal component analysis (PCA) as shown in Fig. 3.

Intra and inter Districts genetic diversity within the genotypes *M. buxifolia*

The overall inter district (Swat, Buner, Dir lower and Chitral) genetic diversity among 92 genotypes of *M. buxifolia* is shown in Table 2 and remarkably, Locus 4 and 5 (L-4 and L-5) were present in the total genotypes of *M. buxifolia* and were considered as monomorphic loci. These two loci (L-4 and L-5) were treated as species specific. L-1, L-2, L-3 and L-6 were polymorphic. The total genetic diversity in *M. buxifolia* genotypes was 66.70% (Table 2).

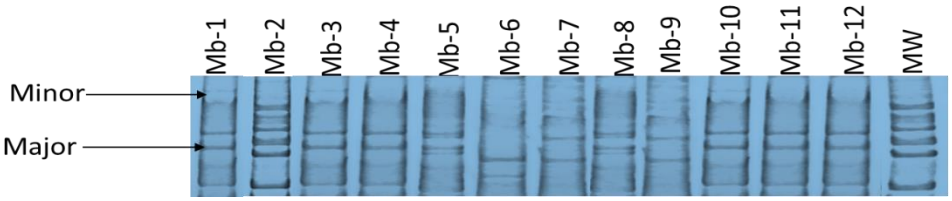

Fig. 1. The electrophoregram displaying the genetic diversity in 12 genotypes of *M. buxifolia.*

Table 2. Inter-districts genetic diversity within the genotypes of *Monotheca buxifolia*.

Locus	Present	Absent	Status	GD%= (Poly loci/total loci*100)
L-1	68 (73.91%)	24 (26.08%)	Poly	
L-2	68 (73.91%)	24 (26.08%)	Poly	
L-3	67 (72.82%)	25 (27.17%)	Poly	
L-4	92 (100%)	0	Mono	
L-5	92 (100%)	0	Mono	
L-6	47 (51.08)	45 (48.91%)	Poly	GD=66.70%

Intra-Districts genetic diversity within the genotypes *M. buxifolia*

The intra genetic diversity in the 23 genotypes of *M. buxifolia* collected from Swat was 66.70%. The L-4 and L-5 were found as monomorphic while L-1, 2, 3 and 6 were polymorphic. Whereas the genotypes collected from district Buner represented 16.70% genetic diversity. Among the six loci, L-6 was polymorphic while rest of the other loci were monomorphic. The L-1 was absent in the genotypes collected from the district Dir lower, L-4 and L-5 were monomorphic. L-2, 3 and 6 were polymorphic. These genotypes represented 50.00% genetic diversity. Similarly, the genotypes collected from district Chitral showed 16.70% genetic diversity. The L-2 was absent in the genotypes collected from Chitral while the remaining other loci were polymorphic (Table 3).

Table 3. Genetic diversity within the genotypes of *Monotheca buxifolia*.

	Locus	Present	Absent	Status	GD%= (Poly loci/total loci*100)
District Swat	L-1	22 (95.65%)	1 (4.34%)	Poly	
	L-2	22 (95.65%)	1 (4.34%)	Poly	
	L-3	14 (63.63%)	9 (39.13%)	Poly	
	L-4	23 (100%)	0	Mono	
	L-5	23 (100%)	0	Mono	
	L-6	20 (86.95%)	3 (13.04)	Poly	**66.70%**

	Ls	P	A	S	GD%
Distrct Buner	L-1	23 (100%)	0	Mono	
	L-2	23 (100%)	0	Mono	
	L-3	23 (100%)	0	Mono	
	L-4	23 (100%)	0	Mono	
	L-5	23 (100%)	0	Mono	
	L-6	22 (95.65%)	1 (8.69%)	Poly	**16.70%**

	Locus	Present	Absent	Status	GD%= (Poly loci/total loci*100)
District Dir lower	L-1	0 (0.00%0	23 (100%)	Mono	
	L-2	22 (95.65%)	1 (4.34%)	Poly	
	L-3	21 (91.30%)	2 (8.69%)	Poly	
	L-4	23 (100%)	0	Mono	
	L-5	23 (100%)	0	Mono	
	L-6	21 (91.30%)	2 (8.69%)	Poly	**50.00%**

	Ls	P	A	Status	GD%
District Chitral	L-1	23 (100%)	0	Mono	
	L-2	0	23 (100%)	Mono	
	L-3	11 (47.82%)	12 (52.17%)	Poly	
	L-4	23 (100%)	0	Mono	
	L-5	23 (100%)	0	Mono	
	L-6	23 (100%)	0	Mono	**16.70%**

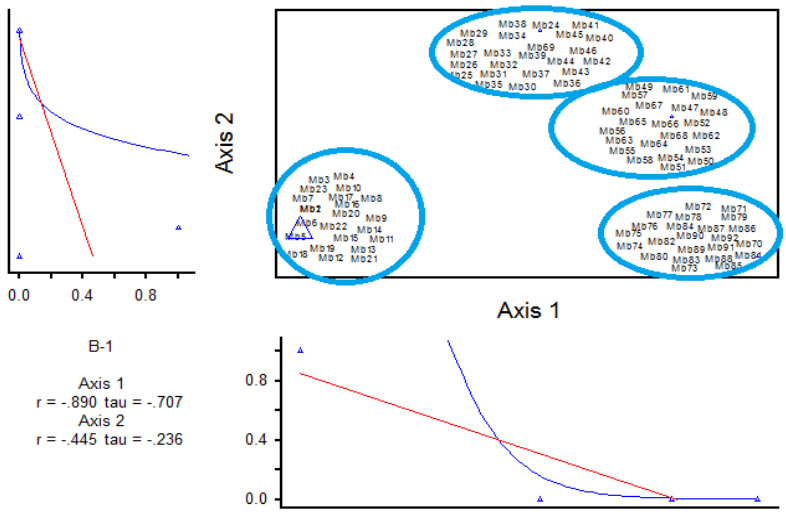

Fig. 3. Confirmation of cluster analysis by scattered plot detected through PCA (analysis).

Discussion

Genetic diversity is of paramount importance in the continuity of the species, as it provides the required adaptation to the prevailing biotic and abiotic stresses and ensures changes in the genetic makeup to cope with environmental changes (Noor et al., 2018). The incorporation of new varieties of cash crop in agriculture ultimately leads to an overall decline in genetic variability, although the data are incompatible within the released varieties themselves and no overall narrowing of the genetic base can be distinguished (Muhammad et al., 209). The genetic erosion situation, in landraces and crop wild relatives is equally complex. Although many latest studies have proven that diversity in farmland and protected areas has deteriorated (Silva et al., 2017; Noor et al., 2018). The conservation of the species both *ex situ* and *in situ* necessitates understanding of the target and genetic diversity of the species; therefore, a knowledge of this nature is required in the study of the remaining *M. buxifolia*s' populations and also it is crucial to fight the increasing reduction of the areas of natural occurrence, also the genetics of a species is an essential factor in the survival of populations in variable environments (Silva et al., 2017), it is also considered as a key component of biodiversity (Silva et al., 2017; Khan et al., 2020). Awareness of how genetic variations are divided among populations may therefore have important consequences, not only in evolutionary biology and ecology, but also in conservation biology of *M. buxifolia* growing Malakand Division, Pakistan.

A broad genetic base is key to adaptation and will predict the potential extent of climate change impact (Noor et al., 2018). Genetic bottlenecks threaten the efficiency of crop species for sustainable farming and make them vulnerable to stress (Mujeeb-Kazi et al., 2017; Noor et al., 2018). Continuous developments in DNA-based technologies and computational tools could enable more objective measurements of genetic diversity at the genomic level and a precise mapping of important loci (Khan et al., 2020), However, not all of these techniques are currently available to plant breeders in developing countries who are home to major food-insecure citizens (Noor et al., 2018).

In the case of a clearly defined character identifiable in the phenotype, plant breeders still rely on conventional approaches to check entries. When a line with the desired trait is identified, an elite cultivar is crossed to incorporate exotic donor genes into the cultivated type (Noor et al., 208). Proteins are end products of gene expression and the measurement of the diversity of total seed proteins using the SDS-PAGE method is reliable, cheap and essential in practice for plant species (Khan et al., 2020). We still have little knowledge of the potential and objective diversity of wild plant species that need to be enhanced (Muhammad et al., 208). To date, little attention has been paid to wild plants and therefore the main aim of the current work was carried out to determine intraspecific genetic variation within *M. buxifolia* naturalized in the various geographical regions.

In our current study, 92 genotypes *M. buxifolia* revealed a significant genetic diversity (in inter districts and intra districts genotypes) assessed through SDS-PAGE profiling. The utmost genetic diversity in inter districts genotypes of *M. buxifolia* have been observed. The phylogenetic tree based on SDS-PAGE differentiated all the genotypes into four regions. R-I comprised of the genotypes of *M. buxifolia* collected from Swat district, R-II, III and IV comprised of genotypes collected from Buner, Dir lower and Chitral districts respectively. Due to high inter district genetic diversity within the genotypes of *M. buxifolia*, SDS-PAGE is a reliable method for documentation of the genotypes of *M. buxifolia* and the overall genetic diversity was 66.70%, principally L-4 and L-5 were monomorphic in *M. buxifolia* and was considered as species specific. While the intra district genetic diversity was 66.70, 16.70, 50.00 and 16.70% for genotypes collected from Swat, Buner, Dir lower and Chitral district respectively.

Conclusion

Genetic diversity is of prime significance in the continuity of the species, as it guarantee the necessary adaptation to the prevailing en vironmental stresses and ensures changes in the genetic makeup to deal with climatic changes (Khan et al., 2020). SDS-PAGE (of Seed storage protein) is an influential procedure for evaluation of genetic diversity and this technology is particularly believed to be a consistent technique, as the seed storage proteins are mainly independent of environmental variabilities (Muhammad et al., 2018). Genetic variation assessment using seed storage protein is extremely significant for the conservation strategies of threatened and endangered plant species. A significant amount of inter districts genetic diversity (66.70%) was detected among the genotypes of *M. buxifolia*. Protein profiling was conducted on 12% gel electrophoresis. A total of 6 protein bands were observed in *M. buxifolia* genotypes. Particularly, L-4 and L-5 were monomorphic in the inter districts *Monotheca buxifolia* genotypes and was recognized as species specific. The remaining other loci were polymorphic. Considerable genetic variation may help to identify *M. buxifolia*'s elite germplasms that could ensure its survival under unfavorable climatic conditions. This study could help to conserve this important genetic resource.

Conflict of Interest

Authors have no competing interests to declare.

Author's Contributions

NM did the experimental work and wrote the paper, NU, MKUK and MR help in analysis, NA conceived the project.

References

Al-Yahyai and Al-Nabhani. (2006). Botanical description and propagation of *Monotheca buxifolia* (13-19 August) 27 international Horticulture exhibitions (IHC 200). Korea (ABS.)

Khan, M.K.U., Muhammad, N., Uddin, N., Ali, N., Umer, M., Ullah, S. (2020). Genetic diversity in threatened plant species Alnus nitida (Spach.) Endel. Plant Science Today, 7(3), 314-318.

Khan, N., Ahmed, M., Wahab, M., Ajaib, M., & Hussain, S. S. (2010). Studies along an altitudinal gradient in *Monotheca buxifolia* (falc.) ad, forest, District Lower Dir, Pakistan. Pak. J. Bot, 42(5), 3029-3038.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680-685.

McCune, B. and M. J. Mefford. (1997). PC-ORD. Multivariate Analysis of Ecological Data. Version 5. MjM Software Design, Gleneden Beach, Oregon, U.S.A, 1997.

Muhammad, N., Uddin, N., Liu, M., Ali, N. (2019). Ethno medicine, antioxidant potential and inter-specific variations of Punica L. species growing in Swat District, KP, Pakistan. International Journal of Botany Studies. 4 (4):36-46.

Muhammad, N., Wadood, S.F., Khan, W, Ali, N., Nisar, M. (2018) Intra-species profiling of Cleome viscosa growing in Swat District

(Pakistan). Biosystems Diversity, 26(1),52-55.
Mujeeb-Kazi, A., Ali, N., Ibrahim, A., Napar, A. A., Jamil, M., Hussain, S., Mahmood, Z., Delgado, R., Rosas, V., Cortes, A., Rajaram, S. (2017). Tissue culture mediated allelic diversification and genomic enrichment of wheat to combat production constraints and address food security. – Plant Tissue Culture and Biotechnology, 27, 89-140.
Nasir, E., Ali, S.I. (1972). Flora of West Pakistan. Karachi. Fakhri.
Noor, M., Ali, N., Nisar, M., Abd Allah, E.F., Hashem, A. (2018). Genetic diversity within natural populations of the medicinal plant Rhynchosia minima (L.) DC. Applied Ecology and Environmental Research, 16(5), 5633-5651.
Shahina, A.G. and F. Martin. (1998). Vegetation of Arabian Peninsula. Kluwer Academic publisher. Netherlands.
Silva, A. V. C. D., Amorim, J. A. E., Vitória, M. F. D., Ledo, A. D. S., & Rabbani, A. R. C. (2017). Characterization of trees, fruits and genetic diversity in natural populations of mangaba. Ciência e agrotecnologia, 41 (3), 255-262.
Ullah, I., Khan, J. A., Shahid, M., Khan, A., Adhikari, A., Hannan, P. A., ... & Farooq, U. (2016). Pharmacological screening of *Monotheca buxifolia* (Falc.) A. DC. for antinociceptive, anti-inflammatory and antipyretic activities. BMC complementary and alternative medicine, 16 (1), 273.

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Appendix

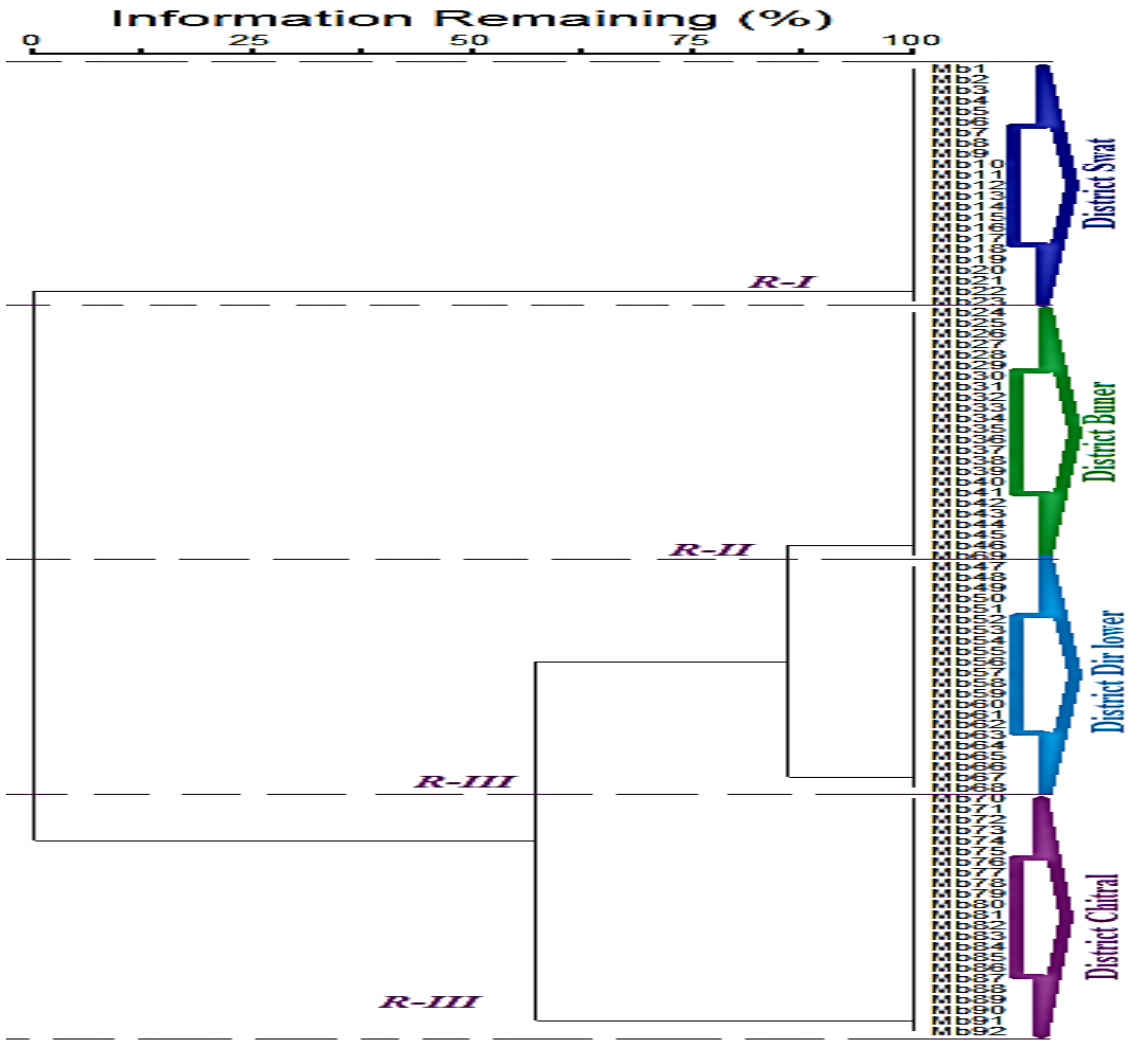

Fig. 2. Phylogram based on Seed storage protein characterization in 92 genotypes of *Monotheca buxifolia*.