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Original Article

VALIDATION AND TRANSFERABILITY OF SIMPLE SEQUENCE REPEATS (SSR'S) FROM SOME SPECIES OF ACACIA GENUS TO *ACACIA NILOTICA* L.

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ABSTRACT

Objective: In present study genetic transferability of SSR's from related Acacia species to *Acacia nilotica* was evaluated along with its genetic diversity analysis from north Indian region.

Methods: A total of 30 primers selected from 5 different Acacia species were screened for amplification and polymorphism. Dendrogram and 2 D plot were constructed using NTSys PC version 2.02e. Different diversity parameters like Polymorphism information content (PIC), alleles per primer, number (no.) of amplicons were also calculated for each primer pair.

Results: SSRs from *Acacia tortilis*, *A. senegal* and *A. koa* were highly transferable in *A. nilotica.* Out of 30, only twenty-two primers showed amplification with an average of 1.36 alleles per locus. Polymorphic information content (PIC) values ranged from 0.5 to 0.96 with an average of 0.81. Jaccard similarity coefficient (J) values ranged from 0.04 to 0.67 showing a high level of diversity. Un-weighted pair group method with arithmetic mean (UPGMA), based cluster analysis, divided all accessions into three main clusters.

Conclusion: Geographical and climatic conditions showed a great impact on genetic diversity. The results indicated high transferability of genomic resources from related species and will facilitate more studies to characterize the relatively less studied *Acacia nilotica* genome.

Keywords: *Acacia nilotica*, SSR, Genetic diversity, Transferability, Geographical distance

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INTRODUCTION

Acacia nilotica L. commonly known as Babool or kikar is a popular weed, native to Africa and Indian subcontinent. *A. nilotica* belongs to genus *Acacia* sensu lato (s. l.) which is the largest genus of subfamily Mimosoideae [1]. *A. nilotica* is well-known for fuelwood and forage tree naturalized in many countries [2, 3]. It grows in all types of environments except colder areas [4]. It is a single stemmed, medium sized tree having paired thorns at each node of the stem [5, 6]. It is an important ethnomedicinal plant having antibacterial [7], antioxidant [8], anti-mutagenic [9], antidiarrheal [10], antiviral and many more activities [11]. Various pharmacological compounds like gallates [12], flavonoids [13] and fatty acids [14] have been isolated from the various plant parts. It can be grown for soil reclamation [4], reforestation [15, 16], agro-forestry [17] and wasteland management [18]. It also provides tannins, gum and charcoal [19, 20].

Genetic diversity at molecular level reflects the difference in the DNA of individuals of same species [21]. An understanding of genetic relationship in germplasm is a valuable technique for biodiversity conservation and plant breeding programs [22]. Microsatellites or the SSRs are tandemly repeated sequences of 1-6 base pairs, found in almost all eukaryotic organisms [23]. Microsatellites are usually preferred due to their co-dominant nature, reproducibility, high allelic diversity and cross-species amplification [24, 25]. Microsatellites have been applied to many commercially important plant species and have been proven to be very useful [26, 27].

Genetic diversity of *A. nilotica* has been studied previously by using allozyme markers [28] and RAPD (Random amplified polymorphic DNA) molecular markers [29] and by SSR markers [30] for detecting the origin of *A. nilotica* in Australia. *A. nilotica* is widely distributed and cultivated in north India [18]. Unfortunately only very few molecular resources are available for *A. nilotica*. Genetic diversity studies in India using molecular markers are also limited. Keeping in view of that there is an urgent need to study genetic diversity of *A. nilotica* with new and emerging molecular markers like SSR. Genomic resources or the marker information from other economically important species might be exploited, but less studied in plant species like *A. nilotica*.

The present study focused on assessing the genetic diversity of *A. nilotica* from different accessions of north India collected from different geographical and climatic conditions using SSR molecular markers. The study also focused on providing information about transferability of SSR molecular markers from other well-studied species of genus Acacia to remarkably less exploited *A. nilotica*.

MATERIALS AND METHODS

Sample collection

A total of 14 accessions were collected from wild plants distributed in different states of north India in 2015 (fig. 1). Two collection sites were chosen from each selected state. Climatic and geographical conditions of all collection sites have been depicted in table 1. Healthy, fresh and young leaves of each plant material were harvested and brought to the laboratory in an ice box. Leaves were further stored at -80 °C until DNA extraction.

DNA extraction

Genomic DNA was extracted from each sample by using a genomic DNA extraction kit (Genetix biotech Asia Pvt. Ltd., India), following the manufacturer's guidelines. Quality and quantity of isolated DNA were checked by 1% (w/v) agarose gel electrophoresis and nanodrop (myspec, Sigma-Svi, Germany). DNA samples were diluted to 20ng/µl and stored at -20 °C for further analysis of genetic diversity.

SSR amplification

Thirty polymorphic primer pairs reported from different species of genus Acacia were selected for amplification [30-35]. Out of these, only 22 primer pairs showed amplification and were selected for further study (table 2). PCR reactions were performed in a final volume of 15 µl; containing 9.35 µl water, 1.5 µl DNA polymerase buffer (10 X), 1.5 µl dNTP mix (2.5 mmol each), 0.15 µl Taq DNA

polymerase at 0.5 U/µl, 1 µl primer (F+R at 1µg/µl) and 1.5 µl genomic DNA. The amplification reactions were performed in a thermocycler (Peqstar; Peqlab Biotechnologie GmbH, Germany) using the cycling profile: an initial denaturation of 10 min. at 95 ° C followed by 35 cycles of denaturation at 94 ° C (30 s), 1 min. at specific annealing temperature (T_a) of each primer (table 1) and extension at 72 ° C for 1 min. and finished by a final extension step at 72 ° C for 10 min. Amplicons were run over a 2.8 % agarose gel for allele size fractionation. Both 50 and 100 bp ladders were used to measure the size of the amplicon.

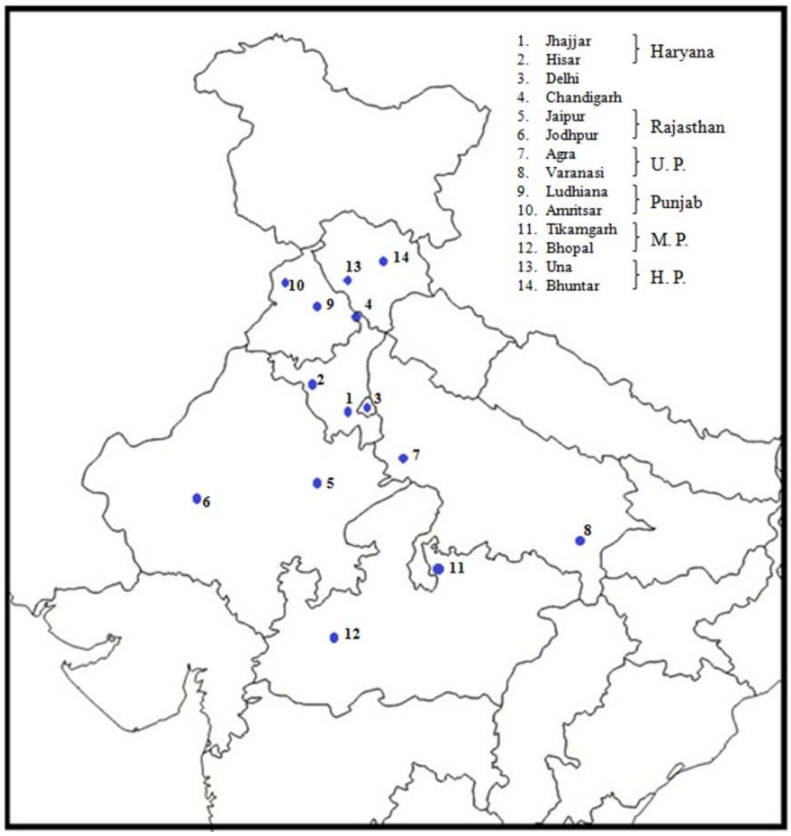

Fig. 1: Map of north India showing different collection sites

Table 1: Collection sites of *A. nilotica* leaves

S. No.	Collection states	Collection sites	Latitude	Longitude	Altitude	Climatic zone
1	Haryana	Jhajjar	28.62 ° N	76.65 ° E	720 ft	Semi-arid
		Hisar	29.15 ° N	75.70 ° E	705 ft	Semi-arid
2	Punjab	Ludhiana	30.91 ° N	75.85 ° E	798 ft	Humid Sub-tropical
		Amritsar	31.64 ° N	74.86 ° E	768 ft.	Humid Sub-tropical
3	HP	Una	31.49 ° N	76.28 ° E	1211 ft	Humid Sub-tropical
		Kullu (Bhuntar)	31.88 ° N	77.14 ° E	3573 ft	Highland
4	UP	Agra	27.18 ° N	78.02 ° E	561 ft	Semi-arid
		Varanasi	25.28 ° N	82.96 ° E	264.8 ft	Humid Sub-tropical
5	MP	Tikamgarh	24.74 ° N	78.83 ° E	807 ft	Humid Sub-tropical
		Bhopal	23.25 ° N	77.41 ° E	1729 ft	Humid Sub-tropical
6	Rajasthan	Jaipur	26.90 ° N	75.80 ° E	1417 ft	Arid
		Jodhpur	26.28 ° N	73.02 ° E	902 ft	Arid
7	Delhi	Delhi	28.61 ° N	77.20 ° E	712 ft	Semi-arid
8	Chandigarh	Chandigarh	30.74 ° N	76.79 °E	1053 ft	Humid Sub-tropical

Data analysis

Amplification data was scored in a binary matrix based on the presence (1) or absence (0) of bands based on their size. Only clear and reproducible bands were included in the study. The similarity between different genotypes was calculated using the SIMQUAL program of numerical taxonomy and multivariate data analysis NTSys-pc version 2.02e [36]. Jaccard similarity coefficient was calculated for different accessions. The dendrogram was constructed using SAHN module based on UPGMA to show a phenetic depiction of the genetic relationship between different accessions. PIC for each primer pair was calculated by using formula:

$$PIC = 1 - \Sigma pi2,$$

Where p is the frequency of i-th allele [37].

Principal coordinate analysis was done using NTSys software. 2D plot of the similarity of different accessions was drawn with Eigen module of the ordinate option of NTSys execute the program. Eigen values were used to draw the 2 D plot along with two ordinates. Correlation between the geographical and genetic distances was calculated using MS Excel program. Relative geographical and genetic distances were calculated relative to Delhi.

Table 2: SSR primers used to detect diversity among *A. nilotica* genotypes

S. No.	Primer	Genbank accession no.	Sequence	Repeat motif	Origin species	Annealing Temp. (Ta in °C)
AN1	AN06	AY553916	F-AGCGTCATGGCATAACCT R-CGAATTCATAATCTGTCTCGAT	(GAA)10	*A. nilotica*	55
AN 2	AN11	AY553917	F-AGAAACCAAGGGGAGTAA R-TCCTCTCTAATCATCTCAAC	(TGC)6	*A. nilotica*	55
AN 3	AN12	AY553918	F-GGCCATAATTGGTAATGCATTC R-CAATCCAGTGTTGCCAGTGT	(CCA)6	*A. nilotica*	55
AN 4	AN17	AY553919	F-CATAATCCCTTTGATGTCCA R-AAATTCTCCTGATTCTGCG	(GCT)6. (GTT)2(GCT)2	*A. nilotica*	55
AN 5	AN18	AY553920	F-CAGGTAGCGGAGTAGGACGG R-AAAGAGACAAAGCTAGAAGCGATT	(CCCT)5	*A. nilotica*	55
AT 6	Acator_01934	-	F-CACATTTAACTCGCTTTGGCG R-CACATATGGCTGCTGTGGAG	(AG)13	*A. tortilis*	56
AT 7	Acator_15563	-	F-TCGTACTCATCGCCAAGACC R-CGAAGAAGCTACAATGGCTG	(TC)12	*A. tortilis*	58
AT 8	Acator_22993	-	F-CATCGGCCCAGTTATGAGTTG R-TCCAAAGTTCTGATACTGGTAAGC	(AC)21	*A. tortilis*	60
AT 9	Acator_24771	-	F-ACCGTTGAGCATATTTCAGTTG R-AAGTACTGCTTAATCTGAGTGTG	(AC)13	*A. tortolis*	55
AS 10	mAsCIRB09	FM883654	F-CCTTATGAACCAAAACCAGC R-CAACGAGCATTAACACCAAC	(TA)3 TG (TA)3 TG (TA)3	*A. senegal*	52
AS 11	mAsCIRE07	FM883644	F-GAAGCAGAAGCAGAAGCAGC R-CCCCCCTCACACTCATCTC	(GGA)2 (GAA) (GGA)4	*A. senegal*	52.3
AS 12	mAsCIRE10	FM883645	F-GAGAAACTGGAGAGGGGAAG R-GCGACAAAAGTAGTAAAGGGC	(GAT)6	*A. senegal*	54.5
AS13	mAsCIRF03	FM883651	F-CACTTTTACTTTGTGATCTCCC R-CAGTCTTGTGTGCGTCTTAC	(TA)4 (N)55 (AC)10	*A. senegal*	52.4
AS14	Ame03	DQ467673	F-GAACAATATCAGCAATCACT R-CCTCATGCACACACAAGAT	(AG)9	*A. senegal*	52.4
AS15	Ame07	DQ467658	F-ATAAAAACAAAAACCCAACTAAATG R-GTCCAAAACTCTTCAATGTCAA	((GT)20	*A. senegal*	56.6
AS16	Ab26	AY843557	F-ATATTCTGCTTTAGTCTA R-GGGGCATAAATATGAG	(AG)8(AG)9	*A. senegal*	52.4
AM17	Am389	-	F-AATCCTTCCGAAAGTTATACATGG R-GCACTTGTAAGTCGGAACTGC	(AT)3(GT)9GC(GT)2	*A. mangium*	60
AK18	Ak08	-	F-ACAGTTCCACCTCACCGTTC R-CGACCCTATCACCTTCTTGC	(TACA)8	*A. koa*	51.3
AK19	Ak36	-	F-GCAGGACTTGACGAACCTTT R-TTGGCTCCATCTTTTCCTTG	(CA)9	*A. koa*	47.3
AK20	Ak99	-	F-GCTGGTCCAACGTAGAAGGA R-CCAGCATGAGAACGAACAAT	(CT)10	*A. koa*	45.7
AK21	Ak44	-	F-TTCTGTGTTCACCGTCGTTC R-CTGCATCCAACCTTTGACCT	(CT)13	*A. koa*	51.3
AK22	Ak284	-	F-ACAACCATCGGCAACTTAGC R-CCCAACGAAGAAGCTAGACG	(AG)3A(AG)11	*A. koa*	51.3

RESULTS

Genetic diversity by SSR

In present study SSR molecular markers were used to evaluate genetic diversity of 14 accessions of *A. nilotica* collected from north India. All 22 primer pairs showed the polymorphism. 30 alleles were generated by 22 primers with an average of 1.36 alleles per primer. Primer AK 22 generated maximum 4 alleles. Primer AK 18 showed 3 alleles while primer AN 5, AS 13 and AS 14 showed 2 alleles.

A total of 137 bands were observed ranging from 70 to 600 base pair in size. The average no. of amplicons per primer was 6.22. PIC was calculated for each SSR primer. PIC was higher than 0.5 in all cases. PIC values ranged from 0.5 to 0.96 with an average of 0.81. Lowest PIC was observed for primer no. AN1 and AS10. Table 3 enlists the PIC values, no. of alleles and amplicon size of each primer.

Genetic relationship

Genetic relationship between 14 *A. nilotica* accessions was measured by Jaccard similarity coefficient. Values of J ranged from 0.04 to 0.67. Maximum similarity (67 %) was observed between Jhajjar and Delhi accession while minimum similarity was observed between Jodhpur and Amritsar accession. Table 4 enlists the J values of all accessions with respect to each other.

Phylogenetic analysis

The dendrogram was constructed using UPGMA method based on values of Jaccard similarity coefficient (fig. 2). Cluster analysis divided all 14 accessions into three main clusters i.e. A, B and C. cluster A consisted of 8 accessions and was further divided into 3 sub-clusters A1, A2 and A3 based on their similarity. Sub-cluster A1 included Jhajjar, Hisar, Delhi and Chandigarh accessions. Sub-cluster A2 included accessions from Agra and Tikamgarh; sub-cluster A3 included Jaipur and Jodhpur accessions. Cluster 2 was further divided into 3 sub-clusters B1, B2 and B3; B1 comprising only one accession i.e. Kullu. Ludhiana, Varanasi and Bhopal, Una accessions were included in B2 and B3 sub-clusters respectively.

Amritsar was found separated from all other accessions and was included in Cluster C.

Table 3: Allele size, PIC, no. of alleles, no. of amplicons of each SSR locus used for *A. nilotica* diversity analysis

Locus	Allele size (bp)	PIC	Alleles per locus	No. of amplicons
AN1	150	0.50	1	10
AN 2	220	0.75	1	7
AN 3	150	0.68	1	8
AN 4	150	0.60	1	9
AN 5	600,200	0.69	2	7
AT 6	160	0.75	1	7
AT 7	90	0.96	1	3
AT 8	80	0.93	1	4
AT 9	70	0.83	1	6
AS 10	250	0.50	1	10
AS 11	250	0.93	1	4
AS 12	600	0.92	1	6
AS13	400,350	0.69	2	7
AS14	150,400	0.74	2	9
AS15	300	0.96	1	3
AS16	120	0.96	1	3
AM17	70	0.96	1	3
AK18	450,400,300	0.90	3	8
AK19	200	0.96	1	3
AK20	250	0.93	1	4
AK21	360	0.83	1	6
AK22	420, 400,210,120	0.88	4	10

Table 4: Jaccard similarity coefficient values for different *A. nilotica* accessions

Collec-tion sites	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14
C1	1													
C2	0.56	1												
C3	0.67	0.64	1											
C4	0.41	0.42	0.58	1										
C5	0.33	0.26	0.26	0.28	1									
C6	0.23	0.36	0.19	0.20	0.43	1								
C7	0.33	0.18	0.18	0.28	0.25	0.11	1							
C8	0.11	0.20	0.20	0.28	0.15	0.07	0.15	1						
C9	0.21	0.18	0.24	0.32	0.21	0.21	0.13	0.38	1					
C10	0.08	0.06	0.05	0.06	0.05	0.04	0.17	0.05	0.08	1				
C11	0.40	0.20	0.33	0.43	0.25	0.07	0.50	0.25	0.29	0.09	1			
C12	0.15	0.18	0.24	0.39	0.31	0.13	0.13	0.30	0.20	0.08	0.22	1		
C13	0.17	0.24	0.24	0.36	0.22	0.29	0.10	0.23	0.38	0.06	0.17	0.53	1	
C14	0.12	0.16	0.23	0.25	0.20	0.09	0.09	0.31	0.19	0.08	0.21	0.27	0.09	1

C1-Jhajjar, C2-Hisar, C3-Delhi, C4-Chandigarh, C5-Jaipur, C6-Jodhpur, C7-Agra, C8-Banaras, C9-Ludhiana, C10-Amritsar, C11-Tikamgarh, C12-Bhopal, C13-Una, C14-Kullu

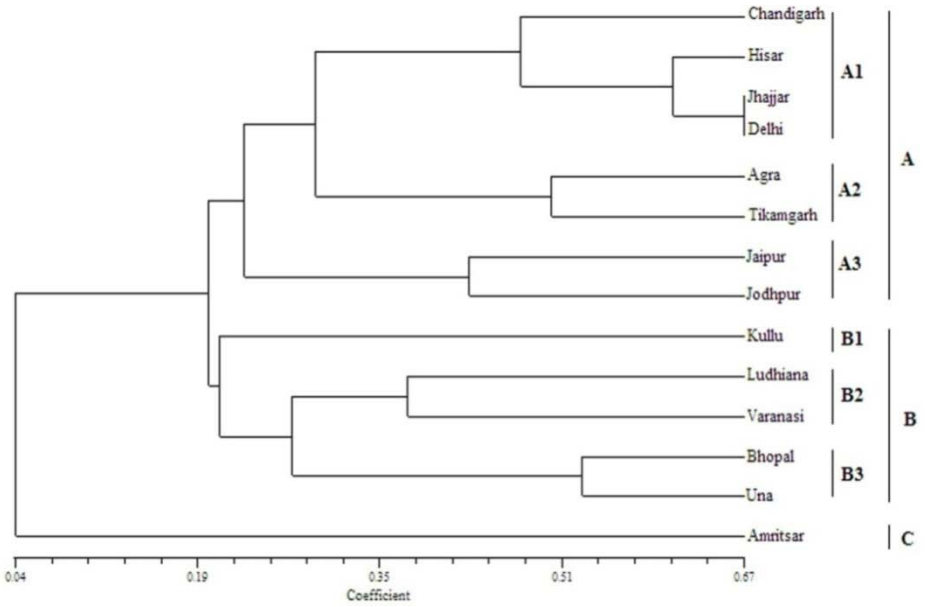

Fig. 2: Dendrogram generated using UPGMA cluster analysis for 14 *A. nilotica* accessions

Principle coordinate analysis (PCoA)

Two-dimensional plot of accessions was generated using principle coordinate analysis to know about the similarity among accessions. 2 D plot also segregated all accessions in 3 major clusters as clustered by cluster tree analysis. All three clusters on their respective coordinates have been shown in fig. 3.

Correlation analysis

Correlation between geographical and genetic distances was calculated to show the effect of geographical distances on diversity. Correlation analysis showed a positive correlation but the effect was not so pronounced. Correlation analysis resulted in correlation coefficient value of 0.464 (fig. 4).

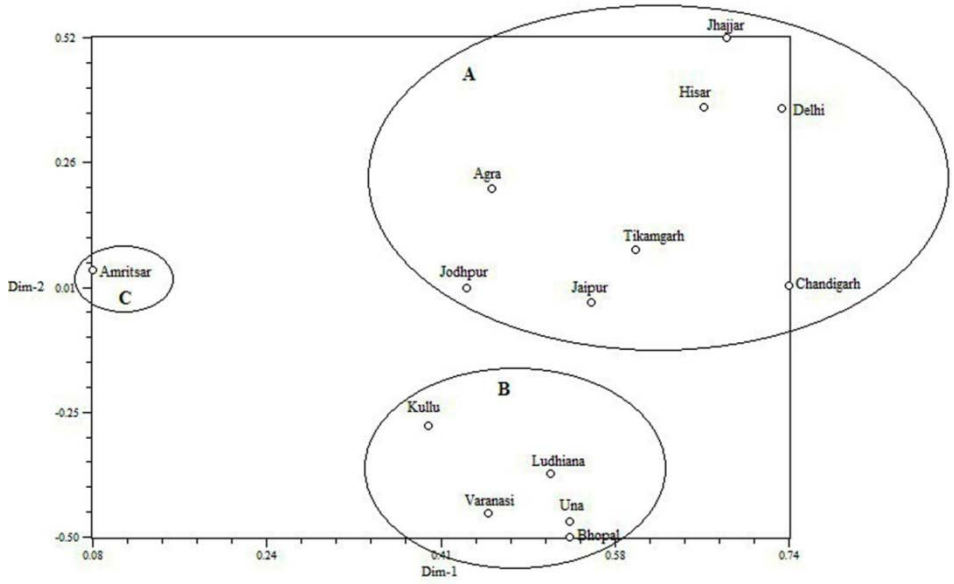

Fig. 3: 2 D plot of 14 *A. nitotica* accessions generated by PCoA

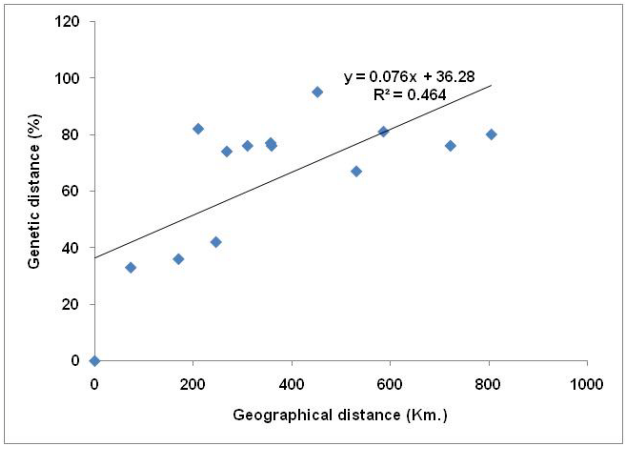

Fig. 4: Correlation analysis between geographical and genetic distances between *A. nilotica* accessions collected from north India

DISCUSSION

A. nilotica is a relatively less admired weed occurring throughout India. *A. nilotica* is widely grown in saline soils [38], heavy metal contaminated soils [39] and drought conditions [40]. Various plant parts are known to have pharmacological effects [11]. Therefore it is very important to characterize the germplasm of plant from various locations in India. Arid and semi-arid regions of north India host populations of *A. nilotica* in varied climatic conditions. So different geographical locations were selected from north India; covering the four climatic zones i.e. arid, semi-arid, humid sub-tropical and highland.

Molecular techniques are very useful in characterization, domestication, and estimation of genetic relationship among different accessions. *A. nilotica* genotypes are not very well characterized by molecular markers, especially in India. *A. nilotica* genotypes have been characterized by RAPD and SSR markers previously [29, 30]. Some studies have focused on the interspecific genetic relatedness of *A. nilotica* but intra-specific genetic similarity has not been studied so much [41]. SSR molecular markers have been proven very useful in diversity analysis due to high polymorphism levels and their codominant nature [25]. SSRs have been used for diversity analyses in many medicinally and commercially important crops like *Tinospora cordifolia* [42], *Aloe vera* [43], *Lagenaria siceraria* [26], *Ziziphus jujube* [44], *Prunus armeniaca* [45], *Eragrostis tef* [46], *Sphenostylis stenocarpa* [47] etc.

The present study focused on genetic diversity analysis of 14 *A. nilotica* accessions collected from north India with the help of 22 SSR molecular primers. Out of these 22 SSR primers, 5 were developed for *A. nilotica* in Australia [30]. Rest 17 primers were selected from other species of genus *Acacia*. Transferability of genomic resources from one species to another depends on upon the phylogenetic relationship between different species [48]. Genus Acacia is presently divided into three main subgenera: Acacia, Aculiferum and Phyllodinae (syn. Heterophyllum) [49]. *A. nilotica* and *A. tortilis* belong to subgenus Acacia [49, 50]; *A. mangium* and *A. koa* belong to sub-genus Phyllodinae [51, 52]; *A. senegal* belongs to sub-genus Aculiferum [53]. SSR loci from *Acacia tortilis*, *A. senegal* and *A. koa* were highly transferable in *A. nilotica*. SSR primers from *A. mangium* were not so much transferable. Only one SSR loci showed transferability. The result of our study is in concordance with the finding of Butcher *et al.* (2000) that SSR loci developed for *A. mangium* are not transferable to *A. nilotica* [31].

In the present investigation, PIC values of 22 SSR loci ranged from 0.5 to 0.96 with an average of 0.81. Codominant markers usually have a PIC value from 0.5 to 1 [54]. It implies that selected SSR loci were highly informative. High polymorphism levels were detected among accessions. Jaccard similarity coefficient values ranged from 0.04 to 0.67. A positive correlation was observed between genetic and geographical distances of different accessions. The cluster tree analysis revealed that most of the accessions were clustered according to the geographical distance between them. Ge *et al.* (2005) also reported previously that a positive correlation was

observed between geographical and genetic distances in populations of *Ammopiptanthus mongolicus* [55]. In the present study, *A. nilotica* populations of Jhajjar and Delhi were genetically most similar with a J value of 0.67. Climatic conditions also affect the genetic diversity. Kumar *et al.* (2015) showed that climatic conditions are one of the major factors affecting genetic diversity in *Aloe vera* [43]. Cluster analysis clustered accessions from arid and semi-arid regions in cluster A except for Tikamgarh which lies in the humid sub-tropical zone. Cluster B included accessions from the humid sub-tropical zone and one from the highland.

Genetic diversity is responsible for the existence of a species and its evaluation is helpful in conservation strategies. *A. nilotica* is a very important plant used for agroforestry and reclamation of soils in India [16, 18]. This study proves the potential of SSR as a tool to detect genetic diversity. The high value of detected diversity indicated the adaptability of species to various geographical and climatic conditions. It also provides information that genetic resources from other species of genus Acacia are highly transferable to *Acacia nilotica*.

CONCLUSION

SSR is a powerful tool for assessing genetic diversity in wild population of *A. nilotica*. Results of our finding indicate the rich genomic resources of *A. nilotica* in north India. Present study findings can be further imposed in germplasm management; marker assisted selection in breeding programs and agroforestry systems. Study also implies the high transferability of genetic resources from other related species of *A. nilotica*. Further research is required for developing more SSR molecular markers as well as exploiting genomic resources from related species to study the diversity of Indian germplasms of *A. nilotica*.

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CONFLICT OF INTERESTS

All authors declare that they have no conflict of interest

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