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

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], anti-diarrheal [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 (10X), 1.5 µl dNTP mix (25 mmol each), 0.15 µl Taq DNA polymerase (5U/µl), 0.15 µl primer each and 1µl DNA sample. Each amplification was conducted in a PTC-100 thermal cycler (MJ Research).
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 ladder s were used to measure the size of the amplicon.

![Image](https://example.com/image.png)

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

| S. No. | Collection states | Collection sites | Latitude | Longitude | Altitude | Climatic zone |
|--------|-------------------|------------------|----------|-----------|----------|---------------|
| 1      | Haryana           | 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 |
| 3      | HP                | Amritsar         | 31.64° N | 74.86° E | 768 ft   | Humid Sub-tropical |
| 4      | UP                | Una              | 31.49° N | 76.28° E | 1211 ft  | Humid Sub-tropical |
| 5      | MP                | Kulu (Bhuntar)   | 31.88° N | 77.14° E | 3573 ft  | Highland      |
| 6      | Rajasthan         | Agra             | 27.18° N | 78.02° E | 561 ft   | Semi-arid     |
| 7      | Rajasthan         | Varanasi         | 25.28° N | 82.96° E | 2648 ft  | Humid Sub-tropical |
| 8      | Chandigarh        | Tikamgarh        | 24.74° N | 78.83° E | 807 ft   | Humid Sub-tropical |
| 9      | Rajasthan         | Bhopal           | 23.25° N | 77.41° E | 1729 ft  | Humid Sub-tropical |
| 10     | Delhi             | Jodhpur          | 26.90° N | 75.80° E | 1417 ft  | Arid          |
| 11     | Delhi             | Delhi            | 28.61° N | 77.20° E | 712 ft   | Semi-arid     |
| 12     | Chandigarh        | Chandigarh       | 30.74° N | 76.79° E | 1053 ft  | Humid Sub-tropical |

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

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 used 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:

\[
PIC = 1 - \sum p_i^2
\]

Where \( p_i \) 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.
In present study SSR molecular markers were used to evaluate A. nilotica genetic diversity of 14 accessions collected from north India. All 22 primer pairs showed the polymorphism. A total of 137 bands were observed ranging from 70 to 600 base pair showed 2 alleles. Primer AK 18 showed 3 alleles while primer AN 5, AS 13 and AS 14 alleles per primer. Primer AK 22 generated maximum 4 alleles. Alleles were generated by 22 primers with an average of 1.36 in size. The average no. of amplicons per primer was 6.22. PIC was observed for primer no. AN1 and AS10. Table 3 enlists the PIC values, no. of alleles and amplicon size of each primer.

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-AGGGTCTAGGCTAATACCT                        | (GA)A10       | A. nilotica    | 55                        |
|        | AN11       | AY553917               | R-CAATATCATAGTCTGCTCATGAT                   | (TGC)6        | A. nilotica    | 55                        |
| AN2    | AN12       | AY553918               | F-GGGCTAAGATGGAATGCTAC                      | (CCA)6        | A. nilotica    | 55                        |
| AN3    | AN17       | AY553919               | R-CATATCTCTGATGCTATGCTA                     | (GCT)6 (GTT)2 (GCT)2 | A. nilotica    | 55                        |
| AN4    | AN18       | AY553920               | F-AGTGGGTAGGAGCGCTTGAC                     | (CCG)T5       | A. nilotica    | 55                        |
| AT1    | Acator_01934 | -                     | F-CATATATGCTGCTCTGATGTG                     | (AG)13        | A. tortilis    | 56                        |
| AT2    | Acator_15563 | -                     | R-CAATATCATAGTCTGCTCATGAT                   | (TC)12        | A. tortilis    | 58                        |
| AT3    | Acator_22993 | -                     | F-TGTCTGACCTGCTGATGCTA                     | (AC)21        | A. tortilis    | 60                        |
| AT4    | Acator_24771 | -                     | F-ACTATCTCTGATGCTATGCTA                     | (AC)13        | A. tortilis    | 55                        |
| AS1    | mAsGIR09   | FM883654               | F-CCTTGTAGCAGAAACAAACACG                    | (TA)3 TG (TA)3 TG | A. senegal    | 52                        |
| AS2    | mAsGIR07   | FM883644               | R-CAATATCATAGTCTGCTCATGAT                   | (TA)3         | A. senegal    | 52                        |
| AS3    | mAsGIR10   | FM883645               | F-CACTATCTGCTGAGGAAAGGAGG                   | (GAT)6        | A. senegal    | 54.5                      |
| AS4    | Ame03      | DQ467673               | R-CATATCTCTGATGCTATGCTA                     | (AG)9         | A. senegal    | 52.4                      |
| AS5    | Ame07      | DQ467658               | F-ATGACATTTAAACTACACCTAAGG                 | ([GT]20       | A. senegal    | 56.6                      |
| AS6    | Ab26       | AY843557               | R-ATGACATTTAAACTACACCTAAGG                 | (AG)8(AG)9   | A. senegal    | 54.2                      |
| AM1    | Am389      | -                      | F-GGGCAAAATATATGAGAAGGAAACTG               | (AT)3 (GCT)9 (GCT)2 | A. mangium | 60                        |
| AK1    | Ak08       | -                      | F-AGCGATTAGAAGGAAACTGAGG                   | (TACA)8       | A. koa        | 51.3                      |
| AK2    | Ak36       | -                      | R-CAATATCATAGTCTGCTCATGAT                   | (CA)9         | A. koa        | 47.3                      |
| AK3    | Ak69       | -                      | F-GGGCAAAATATATGAGAAGGAAACTG               | (CT)10        | A. koa        | 45.7                      |
| AK4    | Ak44       | -                      | F-GGTGTGACCTGAGGAAAGGAAACTG               | (CT)13        | A. koa        | 51.3                      |
| AK5    | Ak284      | -                      | F-GACGATTAGAAGGAAACTGAGG                   | (AG)3(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. Kulu. 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 divided into 3 sub-clusters 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.
### 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               |
| AN2   | 220             | 0.75 | 1                 | 7                |
| AN3   | 150             | 0.68 | 1                 | 8                |
| AN4   | 150             | 0.60 | 1                 | 9                |
| AN5   | 600,200         | 0.69 | 2                 | 7                |
| AT6   | 160             | 0.75 | 1                 | 7                |
| AT7   | 90              | 0.96 | 1                 | 3                |
| AT8   | 80              | 0.93 | 1                 | 4                |
| AT9   | 70              | 0.83 | 1                 | 6                |
| AS10  | 250             | 0.50 | 1                 | 10               |
| AS11  | 250             | 0.93 | 1                 | 4                |
| AS12  | 600             | 0.92 | 1                 | 6                |
| AS13  | 400,350         | 0.74 | 2                 | 7                |
| AS14  | 150,400         | 0.74 | 2                 | 9                |
| AS15  | 300             | 0.96 | 1                 | 3                |
| AS16  | 70              | 0.96 | 1                 | 3                |
| AM17  | 120             | 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

| Collection 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               | 0.24 | 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 | 0.38 | 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](image-url)
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], *Logania 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 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.

**REFERENCES**

1. Maslin BR. Classification and phylogeny of *Acacia*. In: Crespi BJ, Morris DC, Mound LA, editors. Evolution of ecological and behavioral diversity: Australian *Acacia* thrives as model organisms. Canberra: Australian Biological Resources Study and Australian National Insect Collection, CSIRO; 2004. p. 97-112.

2. Kriticos DJ, Sutherst RW, Field RJ. Climate change and the potential distribution of an invasive alien plant: *Acacia nilotica* ssp. Indica in N. J Appl Ecol 2003;40:111-24.

3. Orchard AE, Maslin BR. Proposal to conserve the name acacia (Leguminosae: Mimosoideae) with a conserved type. Taxon 2003;52:362-3.

4. Bargali K, Bargali SS. *Acacia nilotica*: a multipurpose leguminous plant. Nature Sci 2009;7:11-9.

5. Bremen JPM. Manual on the taxonomy of acacia species: a present taxonomy of four species of acacia (A. albida, A. senegal, A. nilotica, A. tortilis). Rome: FAO; 1983.

6. Ali A, Akhtar N, Khan BA, Khan MS, Rasul A, Zaman SU, et al. *Acacia nilotica*: a plant of multipurpose medicinal uses. J Med Plant Res 2012;6:1492-6.

7. Yadav A, Yadav M, Kumar S, Yadav JP. Bactericidal effect of *Acacia nilotica* in vitro antibacterial and time-kill kinetic studies. Int J Curr Res 2015;7:22289-94.

8. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in berries of *Anadricha indica*, *Terminalia arjuna*, *Acacia nilotica* and *Eugenia jambolana* L. Food Chem 2007;104:1-106-14.

9. Meena PD, Kashik P, Shukla S, Soni AK, Kumar M, Kumar A. Anticancer and antimutagenic properties of *Acacia nilotica* (Linn.) on 7, 12-dimethylbenz[a]anthracene induced skin papilloma generation in Swiss albino mice. Asian Pacific J Cancer Prevention 2006;7:627-52.

10. Misar A, Bhagat R, Mujumdar AM. Antidiarrhoeal activity of *Acacia nilotica* Wild. bark methanol extract. Hind Antibiot Bull 2006;49:14-20.

11. Chatterjee P, Das N. Evaluation of the antimicrobial potential of 50 percent aqueous ethanol leaf extract of *Acacia nilotica* Wild. Asian J Pharm Clin Res 2014;7:95-9.

12. Kalavani T, Rajasekaran C, Mathew L. Free radical scavenging, cytotoxic, and hemolytic activities of an active antioxidant compound ethyl gallate from leaves of *Acacia nilotica* (L.) Wild. Ex. Delile Subsp. indica (Benth.) Bremen. J Food Sci 2011;76:144-9.

13. Singh BN, Singh BR, Singh RL, Prakash D, Sarma BK, Singh HB. Antioxidant and anti-quorum sensing activities of a green pond of *Acacia nilotica* L. Food Chem Toxicol 2009;47:778-86.

14. Singh R, Singh B, Singh S, Kumar N, Kumar S, Arora S. Anti-free radical activities of kaempferol isolated from *Acacia nilotica* (L.) Wild. Ex. Del. Toxicol In Vitro re 2008;22:965-70.

15. Sheikh MI. A forestation in waterlogged and saline areas. Pak J For 1974;24:186-92.

16. Marcar NE, Khanna PK. Reforestation of salt-affected and acid soils. Aciar Monogr Ser 1997;43:481-526.

17. Visswanath S, Nair PKR, Kasuahk P, Prasaksam U. *Acacia nilotica* trees in rice fields: a traditional agroforestry system in central India. Agroforestry Systems 2000;50:157-77.

18. Singhal VK, Kaur A, Saggu MIS. Reproductive biology and germplasm evaluation of *Acacia nilotica* (Linn.) Wild. ex. Del. from North India. Muelleria 2008;26:86-94.

19. Dwevedi VP, Bhatia L. Cross-breeding of *Acacia nilotica* for a multipurpose tree of dry areas. Jodhpur: Scientific Publishers; 1993.

20. Patil KN, Ramana PV, Singh RN. Performance evaluation of natural draft based agricultural residues charcoal system. Biomass Bioenergy 2000;18:161-73.

21. Arana MV, Gallo LA, Vendramin GG, Pastorino MJ, Sebastiani F, Marchelli P. High genetic variation in marginal fragmented populations at extreme climatic conditions of the patagonian cypress *Austrocedrus chilensis*. Mol Phylogenet Evol 2010;54:941-9.

22. Govindaraj M, Vettriventhavan M, Srinivasan M. Importance of genetic diversity assessment in crop plants and its recent advances: an overview of its analytical perspectives. Genet Res Int 2015. http://dx.doi.org/10.1155/2015/431487.

23. Jarne P, Lagoda PJ. Microsatellites from molecules to populations and back. Trends Ecol Evol 1996;1:424-9.

24. White G, Powell W. Cross-species amplification of SSR loci in the meliaceae family. Mol Ecol 1997;6:1195-7.

25. This P, Jung A, Bocacci P, Borrejo J, Botta R, Costantini L, et al. Development of a standard set of microsatellite reference alleles for identification of grape cultivars. Theor Appl Genet 2004;109:448-58.

26. Yildiz M, Cuesav HS, Sensoy S, Erdine C, Baloch FS. Transferability of Cytoba SSR markers for genetic diversity assessment of Turkish bottle gourd (*Lagenaria siceraria*) genetic resources. Biochem Syst Ecol 2012;45:49-53.

27. Ngalo S, Shimelis H, Sibuya J, Ameluckow B, Mtunza K. Genetic diversity assessment of Tanzanian sweet potato genotypes using simple sequence repeat markers. S Afr J Bot 2016;102:49-5.

28. Varghese M, Edwards MA, Hamrick JL. Genetic variation within two subspecies of *Acacia nilotica*. For Genet 1999;6:221-8.

29. Ndoye-Ndri K, Samb PI, Chevallier MH. Genetic variability analysis of the polyploid complex of *Acacia nilotica* (L.) Wild. Using RAPD markers. Tropicultura 2006;26:135-40.

30. Wardill TJ, Scott KD, Graham GC, Zalucki MP. Isolation and characterization of microsatellite loci from *Acacia thrips WS* sol. Hind Antibiot Bull 2015;50:221-2.

31. Butcher PA, Decroocq S, Gray Y, Moran GF. Development, inheritance and cross-species amplification of microsatellite markers from *Acacia mangium*. Theor Appl Genet 2000;101:1292-90.

32. Fredua-Agyema R, Adamski D, Liuo RJ, Morden C, Borthakur D. Development and characterization of microsatellite markers for analysis of population differentiation in the tree legume *Acacia koa* (Fabaceae: Mimosoideae) in the Hawaiian Islands. Genome 2008;51:1001-15.
33. Assoumane A, Vaillant A, Mayak AZ, Verhaegen D. Isolation and characterization of microsatellite markers for *Acacia senegal* (L.) Willd., a multipurpose arid and semi-arid tree. Mol Ecol Res. 2009;9:1380-3.

34. Omonti SF, Kireger E, Dangasuk OG, Chikamai B, Odeh DW, Caven S, et al. Genetic diversity and population structure of *Acacia senegal* (L.) Willd. in Kenya. Trop Plant Biol 2010;3:59-70.

35. Winters G, Shklar G, Korol L. Characterizations of microsatellite DNA markers for *Acacia tortilis*. Conserv Plant Genet Resour In Vitro 2013;5:807-9.

36. Rohlf FJ. NTSYS-pc numerical taxonomy and multivariate analysis system. Version 2.02e. EXETER Software: Setauket; 1998.

37. Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, et al. An evaluation of the utility of SSR loci as molecular marker in maize (*Zea mays* L.): comparison with data from RFLPs and pedigree. Theor Appl Genet 1997;95:163-73.

38. Tomar OS, Gupta RK. Performance of some forest tree species in saline soils under shallow and saline water-table conditions. Plant Soil 1985;87:329-35.

39. Kulhari A, Sheorayan A, Bajar S, Sarkar S, Chaudhury A, Kalia RK. Investigation of heavy metals in frequently utilized medicinal plants collected from environmentally diverse locations of north western India. Springer Plus 2013;2:676.

40. Khurana EKTA, Singh JS. Ecology of seed and seedling growth for conservation and restoration of tropical dry forest: a review. Environ Conser 2001;28:39-52.

41. Nanda RM, Nayak S, Rout GR. Studies on genetic relatedness of *Acacia* tree species using RAPD markers. Bologna 2004;59:115-20.

42. Singh K, Kadyan S, Panghal M, Yadav JP. Assessment of genetic diversity in *Tinospora cordifolia* by inters simple sequence repeats (ISSR) and expressed sequence tagged-simple sequence repeats (EST-SSR). Int J Pharm Pharm Sci 2014;6:520-4.

43. Kumar S, Yadav M, Yadav A, Yadav JP. Molecular assessment of genetic diversity in Indian accessions of *Aloe vera* using SSR marker. Int J Pharm Pharm Sci 2015;7:149-54.

44. Zhang Z, Gao J, Kong D, Wang A, Tang S, Li Y, et al. Assessing genetic diversity in *Ziziphus jujuba* 'Jinsixiaozao' using morphological and microsatellite (SSR) markers. Biochem Syst Ecol 2015;61:196-202.

45. Khadivi-Khob A, Yarahmadi M, Jannatizadeh A, Ebrahimzadeh A. Genetic relationships and diversity of common apricot (*Prunus armeniaca* L.) based on simple sequence repeat (SSR) markers. Biochem Syst Ecol 2015;61:366-71.

46. Abrah MT, Shemelis H, Laing M, Asefa K, Amelewok B. Assessment of the genetic relationship of tef (*Eragrostis tef*) genotypes using SSR markers. S Afr J Bot 2016;105:106-10.

47. Shinta NS, Abberton MT, Adesoye AL, Adewale DB, Oyatomi O. Analysis of genetic diversity of African yam bean using SSR markers derived from cowpea. Plant Genet Res 2016;14:50-6.

48. Satya P, Passwan PK, Ghosh S, Majmundar S, Ali N. Consitolerability of simple sequence repeat (SSR) markers from cotton (*Gossypium hirsutum* L.) and jute (*Corchorus olitorius* L.) to twenty two Malvaceae species. 3 Biotechnology 2016;6:1-7.

49. Maslin BR, Miller JT, Siegler DS. Overview of the genetic status of *Acacia* (Leguminosae: Mimosoideae). Aust Syst Bot 2003;16:1-18.

50. El Ayadi F, Abd NA, El Finti A, Msanda F, Baniameur F, El Mousadik A. Genetic variability of wild provenances of *Acacia tortilis* ssp. *raddiana* (Sav) brenan in South of morocco. Asian J Plant Sci 2011;10:119-34.

51. Whitesell CD. *Acacia koa* Gray. In: Burns RM, Honkala BH. editors. Silvics of North America; 2, Hardwoods. Agricultural Handbook No. 594. Washington DC. USDA Forest Service; 1990.

52. Midgley SJ, Turnbull JW, Pinyopasart K. Industrial *Acacias* in Asia: Small brother or big competitor? In: Wei P, Xu D. editors. Eucalyptus Plantations: Research, Management and Development. Proceedings of the International Symposium, Guangzhou, China: World Scientific; 2003. p. 19-20.

53. Josiah CC, George DO, Eleazar OM, Nyanu WF. Genetic diversity in Kenyan populations of *Acacia senegal* (L.) Willd revealed by combined RAPD and ISSR markers. Afr J Biotech 2008;7:2333-40.

54. Cloutier S, Miranda E, Ward K, Radovanovic N, Reimer E, Walichnowski A, et al. Simple sequence repeat marker development from bacterial artificial chromosome end sequences and expressed sequence tags of flax (*Linum usitatissimum* L.). Theor Appl Genet 2012;125:685-94.

55. Ge XJ, Yu Y, Yuan YM, Huang HW, Yan C. Genetic diversity and geographic differentiation in endangered *Ammopiptanthus* (Leguminosae) populations in desert regions of northwest China as revealed by ISSR analysis. Ann Bot 2005;95:843-51.

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