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Comparison of Genotypes, Vegetative Characteristics and Chemical Composition Between two Bamboo Species Grown in the Orman Botanical Garden

Nebal S. Abd Elhamid[1], Amr R. Rabie[1] and Hemat S. Abd EL-Salam[2]

[1]Forestry Dept., Horticulture Res. Institute, Agricultural Research Center(ARC), Giza, Egypt.
[2]Medical and Aromatic Dept., Chemical Analysis Central Lab, Horticulture Res. Institute, Agricultural Research Center (ARC), Giza, Egypt.

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ABSTRACT

This study was conducted on bamboo plants of two species *Denderoclamus giganteus* and *Denderoclamus stricutus*, over 30 years old, grown in the Orman Botanical Garden in Giza during 2021 season. The purpose was to study their vegetative and chemical characteristics. Samples of young leaves were taken for DNA analysis for each of the two species under study. The polymorphic fingerprinting pattern for two *Dendrocalamus* species (*D. giganteus* and *D. stricteus*), generated by five ISSR primers. Primer 844-A exhibited low polymorphism level (12.5%). Otherwise, primer HB-14 exhibited high polymorphism level (77.8%), which is helpful in identifying bamboo species. Primer 844-A showed that eight bands with molecular sizes ranging from 493 to 1587 bp had been amplified. While eight DNA fragments with molecular sizes ranging from 213 to 741 bp were produced using Primer HB-10. Primer HB-14 indicated the amplification of nine DNA fragments with molecular sizes ranging from 232 to 1122 bp. Additionally, seven DNA fragments with molecular sizes ranging from 470 to 1451 bp were observed in the pattern generated by primer UBC-812. High similarity was recorded between *D. giganteus* and *D. stricteus* (0.750), which supports their close phenotypic and genetic relationship. *Denderoclamus giganteus* showed superiority in all vegetative growth parameters in comparison with *Denderoclamus stricutus* with the exception of leaves fresh weight. Stem length, stem diameter, fresh and dry weight for *D. giganteus* were (21.67 m, 11.67 cm, 230.45 and 160.41 gm). *Denderoclamus giganteus* produced the highest values of (N), (P), and Moisture percentage, while *Denderoclamus stricutus* was the highest in potassium (K) percentage.

Keywords: Bamboo, *Denderoclamus giganteus*, *Denderoclamus stricutus*, Genotypes, DNA fragments, phenotypic

Introduction

Bamboo is one of the oldest plants on the earth, the giant grass of subfamily Bambusoideae of Poaceae. Bamboo is a herbaceous plant with woody stems that reaches a height of 40 meters and the diameter of one stem is 20 cm. The number of bamboo species is 1707 species belonging to 128 genera, and its cultivation is widespread in many regions of the world. The global bamboo area is about 36 million hectares, including 16 million in India, 6.5 million in China, and four million in Brazil. The three countries (India, China, and Brazil) contain more than a thousand species of bamboo (Sawarkar *et al.,* 2020 and Sawarkar *et al.,* 2021). According to FAO 40% of the population (about 2.5 billion people) is economically dependent on bamboo; In addition, it provides shelter in the form of traditional bamboo houses to about one billion people (Maxim Lobovikov *et al.,* 2007). Moreover, 80% of the world's populations are depending on NTFP’s for their day-to-day needs such as fuel wood, furniture’s, etc (Trujillo and Lopez 2020). Bamboo plays an important role in numerous ways, from fodder, agricultural implements to providing food, etc. Shoot is the edible parts of bamboo, has high quality vitamins, carbohydrates, proteins and minerals a high nutritive content. The availability of bamboo

Corresponding Author: Nabel S. Abd Elhamid, Forestry Dept., Horticulture Res. Inst., Agricultural Research Center (ARC), Giza, Egypt.

shoot has and its high nutritive content. may help in solving nutritional deficiency of rural (Tripathi, 1998).

Bamboo has excellent chemical, mechanical and physical properties, so it is used in a huge number of applications, reaching ten thousand products. Bamboo is called "the steel of the 21st century", "the poor man's wood", and "green gold" (Azeem *et al.,* 2020). Bamboo is an abundant, renewable and cheap resource that outgrows as compared with most of the other plants (Manandhar *et al.,* 2019 and Suriyapha *et al.,* 2020). Bamboo is one of the most important natural alternatives to hardwoods, which are threatened with extinction. It is one of the most important raw materials used in the manufacture of strong and durable furniture and ornaments. It is also one of the new and important components in the construction industry. Bamboo suits these needs more than ordinary wood, which does not have the same strength and properties as bamboo (Langhelle, 1999). Bamboo is considered the least expensive and most available alternative, as it is highly renewable and environmentally friendly when compared to other types of plants (Bowyer *et al.,* 2014; Agyekum *et al.,* 2017).

Bamboo has a great ability to absorb carbon from the atmosphere and store it. It is also more capable of fixing atmospheric nitrogen, which makes it more beneficial from an environmental standpoint. (Dwivedi *et al.,* 2019). The properties of papers made from bamboo and wood have the same properties, and the properties of paper made from bamboo do not deteriorate. Therefore, many countries, such as India and China, rely on bamboo only in making paper (Win *et al.,* 2012).

Due to environmental threats, the high prices of raw materials used in engineering industries, and the unsustainability of natural resources, we have become in need of using recyclable materials. Therefore, it has become necessary at the present time to rely on bamboo-based industries and develop them and their many value-added applications, while shifting the focus from engineering industries based on chemicals (Sawarkar *et al.,* 2020).

Dendrocalamus stricteus is a densely tufted, sympodial bamboo. Culm much curved, 16-20 m tall Its diameter ranges from 8 to 12.5 cm. Its walls are thick and hard, shiny when young, and turn pale green or yellowish as it matures. The internodes are 30 to 45 cm long and are located at the nodes, which are often swollen. Basal nodes usually develop aerial roots. Dried bamboo poles are used in various industries such as building materials, furniture, and woven utensils. They are also used to make baskets, mats, rafts, sticks, and some agricultural tools. The young shoots and seeds can be used for human nutrition, while the leaves are used as animal feed (Dransfield and Widjaja, 2001).

Dendrocalamus giganteus (giant bamboo) can reach a diameter of 30 cm and length of 40 m, also this bamboo species can be used for erosion control and also has an aesthetic value (Ramanayake and Yakandawala, 1997). The native habitat of (*D. giganteus*) is Southeast Asia. It is a huge species of bamboo called giant bamboo. It is a tropical or subtropical plant. It is also a woody perennial plant that belongs to the Bambusoideae of the Poaceae family. Giant bamboo is a fast-growing species that can generate large biomass in a short time, making it an important economic option due to its many uses.

The International Bamboo and Rattan Organization (INBAR) has recommended further research into its potential as a raw material for the pulp and paper industries, as well as the construction and handicraft industries. (Razvi *et al.,* 2011).

Traditionally, the use of morphology and vegetative characteristics alone to identify bamboo varieties and species has limited the ability to study genetic variation and distinguish genetic differences between them. (Tian *et al.,* 2011). Intersecting simple sequence repeats (ISSR) is the most commonly used form of AFLP and RFLP as it requires a small amount of DNA and has other advantages such as speed, reliability and no need for sequence information. (Lin *et al.,* 2008). Performing ISSR analysis enables genetic differentiation that reveals differences at the internal and intergenic levels, as it works to amplify them and more specifically to repeat genomic regions. (Golkar *et al.,* 2011). The distribution of ISSR throughout the genome allows genomic DNA to be amplified with much larger numbers of fragments per primer compared to RAPD markers. (Li *et al.,* 2010). Genetic studies of some bamboo species have been conducted using ISSR markers, but the use of these applications in molecular studies and genetic characterization is still limited. (Sharma *et al.,* 2008 and Liu *et al.,* 2010).

Bamboo is considered a promising lignocellulosic material relatively as its low-cost material, strong, renewable, light, so it can be used in construction and building materials especially in scaffolding, walling, roof, flooring and foundation (Supriadi and Trisatya, 2021).

As a sustainable building material, bamboo complements wood in the furniture and construction industries as a sustainable material. Some bamboo species excel in mechanical properties such as

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strength, flexural strength, stiffness, compression, flexibility, and tensile strength over most hard and soft woods. Bamboo's durability allows it to withstand varying forces and loads on structural elements. Hollow and solid bamboo are equally applicable to both furniture and construction applications. (Onyeka *et al.,* 2019).

The objective of this study was to investigate the phenotypic and genetic relationship for *D. giganteus* and *D. stricteus* in addition to some morphological features.

2. Materials and Methods

This study was conducted on bamboo plants of two species *Denderoclamus giganteus* and *Denderoclamus stricutus*, over 30 years old, growing in the Orman Botanical Garden in Giza during 2021 season. The purpose was to study their vegetative and chemical characteristics. Samples of young leaves (the fifth leaf from the top) were taken for DNA analysis for each of the two species under study in the Chemical Analysis Central Lab, Horticulture Research Institute.

2.1. Vegetative Characteristics

Each hole was divided into four sections, each duplicated, and the required measurements were taken.

Length in meters (m) and diameter in centimeters (cm) were measured using a Vernier Caliper, as well as fresh and dry leave weight in grams.

2.2. Chemical Analysis

Moisture content (%): Leaf samples were dried at 70ºC till constant weight and determined with: Moisture percentage = Fresh weight - Dry weight / fresh weight x 100

Mineral content: Samples were finely ground about 0.5g dry matter was digested in 5 ml sulfuric and perchloric acid 2:1 (v/v) until the digestive solution became colourless, then transferred quantitatively to 100 ml volumetric flask. The mineral content were determined in samples as follows:

- Nitrogen was determined by Micro-Kjeldahle method as described by Jackson, (1967).
- Phosphorus was determined according to A.O.A.C. (1970).
- Potassium was determined by flame photometry (Brown and Lilleland, 1946).

2.3. Inter Simple Sequence Repeat (ISSR) analysis

Liquid nitrogen was used to finely powder plant tissues, and the DNeasy Plant Mini Kit (QIAGEN) was then used to extract bulked DNA. Five primers were used in the PCR amplification process: HB-10 (5` GAGAGAGAGAGACC 3`), HB-14 (5` CTCCTCCTCGC 3`), UBC-811 (5` GAGA GAGAG AGAGA GAC3`), UBC-812(5`GAGAGAGAGAGAGAGAGAA3`), and 844A (5` CTCT CTCTC TCTCTCT CTCTCTCTCTCTCTAC3`). 2.5 µl of dNTPs (2.5mM), 2.5 µl of MgCl2 (25 mM), 2.5 µl of a 10 x buffer, 2.5 µl of primer (10pmol), 2.5 µl of DNA template (25 ng/µl), 1µl of Taq polymerase (1U/µl), and 11.5 µl of sterilized dd H_2O were all included in the 25 µl volume of the amplification reaction. Initial denaturization took place at 94°C for 1.5 minutes, followed by 35 cycles of 45 seconds at 94°C (denaturation), 45 seconds at 45°C (annealing), and 1.5 minutes of extension at 72°C. Thereafter, there were 45 seconds at 94°C, 45 seconds at 44°C, 5 minutes at 72°C for final extension, and finally, storage at 4°C (Hu *et al*., 2006). The 1500 bp ladder marker was used to assess the fragment sizes after the PCR results were separated on 1.5% agarose gels. The SPSS Windows (Version 10) application was used to analyze the connections between genotypes. The pairwise difference matrix between lines was calculated using the DICE computer program (Yang and Quiros, 1993).

2.4. Statistical analysis:

Experimental design was complete randomized blocks and data were statistically tested according to Snedecor and Cochran, (1980). The individual comparisons between the values obtained were carried out at the 5% level of L.S.D. according to (Waller and Duncan, 1969).

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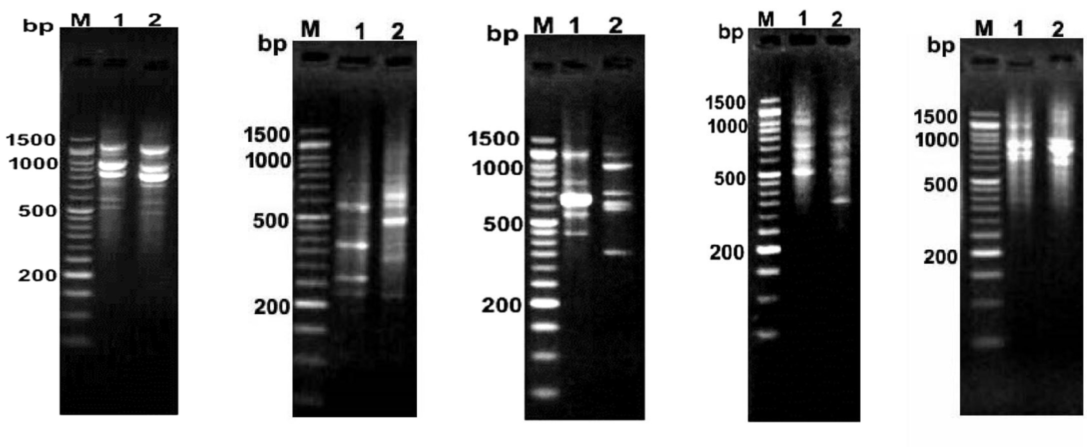
3. Results and Discussion

The polymorphic fingerprinting pattern produced by five ISSR primers for two Dendrocalamus species (*D. giganteus* and *D. stricteus*) is shown in Figure (1). The degree of polymorphism varied depending on the primer. The polymorphism level of primer 844-A was modest (12.5%). The high polymorphism level (77.8%) of primer HB-14 is helpful in identifying bamboo species. Primer 844-A showed that eight bands with molecular sizes ranging from 493 to 1587 bp had been amplified. Eight DNA fragments with molecular sizes ranging from 213 to 741 bp were also produced using primer HB-10. The amplification of nine DNA fragments with molecular sizes ranging from 232 to 1122 bp was revealed by the primer HB-14 findings. Ten DNA fragments with molecular sizes ranging from 358 to 1232 bp were produced by primer UBC-811. Ten DNA fragments with molecular sizes ranging from 470 to 1451 bp have been identified in the pattern formed by primer UBC-812 (Table 1). It's interesting to note that *D. giganteus* and *D. stricteus* showed a high degree of similarity (0.750), indicating an extensive genetic and phenotypic link. *D. giganteus* and *D. sikkimensis*, which shared the same morphological characteristics and were assigned to the same group, can be explained similarly. In this concern, **Basumatary *et al.* (2017)** reported that, *D. giganteus* and *D. hamiltoni*i were assigned to the same subcluster.



844A HB-10 HB-14 UBC-811 UBC-812

Fig. 1: The polymorphic fingerprinting pattern of *D. giganteus* and *D. Stricteus*.

Table 1: Features of ISSR primers used for ISSR−PCR of *D. giganteus* and *D. Stricteus*.

Primer	Scorable bands	Polymorphic bands	Polymorphism (%)
844-A	8	1	12.5
HB-10	8	4	50
HB14	9	7	77.8
UBC-811	10	4	40
UBC-812	10	2	20

3.1. Vegetative Characteristics

Data presented in table (2) showed that, there were superiors in all vegetative growth parameters of *Denderoclamus giganteus* in comparison with *Denderoclamus stricutus* exception of leaves fresh weight. Stem length, stem diameter, fresh and dry weight for *D. giganteus* were (21.67 m, 11.67 cm, 230.45 and 160.41 gm), whereas the same parameters for *D. stricutus* were (13.73 m, 5.37cm, 210.53 and 120.91gm), respectively.

Table 2: Some vegetative parameters on *Denderoclamus giganteus* and *Denderoclamus stricutus*.

	Stem length (m)	Stem diameter (cm)	Leaves fresh weight (gm)	Leaves dry weight (gm)
Denderoclamus giganteus	21.67	11.67	230.45	160.41
Denderoclamus stricutus	13.73	5.37	210.53	120.91
New L.S.D. at 5%	**3.531**	**5.179**	**N.S**	**2.233**

3.2. Chemical properties

As regards data presented in table (3), it can be concluded that *Denderoclamus giganteus* produced the highest values of Nitrogen (N), Phosphorus (P), and Moisture percentage, while *Denderoclamus stricutus* was the highest in potassium (K) percentage.

Table 3: Nitrogen (N), Phosphorus (P), Potassium (K) and Moisture percentage content on *Denderoclamus giganteus* and *Denderoclamus stricutus* under this study.

	N (%)	P (%)	K (%)	Moisture (%)
Denderoclamus giganteus	1.97	0.27	0.73	30.23
Denderoclamus stricutus	1.13	0.11	0.97	42.17
New L.S.D. at 5%	**0.237**	**0.0371**	**0.139**	**4.67**

4. Conclusion

Genetic finger printing techniques were used to differentiate between the two bamboo species, the results revealed significant genetic variation between them, confirming clear genetic divergence and highlighting the effectiveness of molecular analysis as a precise tool for species identification.

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