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Arrowroot (*Maranta arundinacea*): Variation in Morphological and Yield Traits Across Sri Lanka's Agro-Climatic Zones and Genetic Diversity Assessment

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Abstract: Arrowroot (*Maranta arundinacea*) is an underutilized tuber crop in Sri Lanka. Morphological characterization of arrowroot is necessary for its selection, improvement, and utilization. This study evaluated thirteen quantitative and four qualitative plant morphological traits across the arrowroot populations in Sri Lanka's seven agro-climatic zones. Principal component analysis was done to identify the lead plant morphological traits for arrowroot plant and cluster analysis was performed to evaluate the similarity level among collected plant populations. Twenty *M. arundinacea* genotypes from different agro ecological regions of Sri Lanka were screened for genetic diversity using ISSR markers. Nine of the thirteen quantitative morphological traits were found to be significantly distinct from one another. Most plant populations had high similarities, indicating that planting materials can be collected from all of the country's agro-climatic zones and used for breeding programmes. Seven out of thirteen quantitative plant morphological traits were identified as lead plant morphological traits for production of quality rhizomes for crop selection, improvement, and application in Sri Lanka. Outcome of this first study on morphological characterization of arrowroot in Sri Lanka suggests that, for effective utilization of arrowroot, plant populations can be used from all seven agro-climatic zones for selection and crop improvement. Five ISSR markers produced 53 bands in total across 20 samples, with an average frequency of 10.6 bands per primer. The ISSR-PCR analysis revealed a high level of polymorphism (94.34%). Primer UBC 811 has the highest PIC value (0.428), indicating that it is the most informative marker for assessing genetic diversity in M. *arundinacea*. The genotypes from the wet zone and dry zone were categorized individually based on the dendrogram created using UPGMA cluster analysis. The study found genetic variety in M. *arundinacea* based on their varied agro ecological zones, and the current findings will be useful in future crop improvement efforts in M. *arundinacea*.

Keywords: Arrowroot, *Maranta arundinacea*, Plant Morphology, Principal Component Analysis, Traits

1. Introduction

Food shortage has emerged as a problem in many developing countries due to population increase, urbanization, climate change, and environmental degradation, among other factors [1]. According to the global food security index, Sri Lanka faces "volatility of agricultural production" as a challenge, which places the country in the "moderate performance" category. Availability of natural resources can

lead to the diversification of agricultural production with underutilized food crops, which promotes the substitution of staple foods with underutilized food sources.

Although rice is Sri Lanka's staple food, root and tuber crops continue to play an important role in food security as the main course, side dish, or ingredients in food products. They are highly adaptable to climatic variations, produce optimal yields with minimal inputs and management procedures, are less susceptible to pest infestations, and produce higher yields per unit area. As a result, those low-cost, high-nutrient products are an excellent option for improving food security in under-developed countries. Root and tuber crops are in danger of extinction due to a lack of knowledge about their cultivation and consumption, although they nevertheless play a role in rural communities' traditional meals [2]. Research has been focused on the use of root and tuber crops, which have significant potential in terms of feeding Sri Lanka's fast-growing population [3].

Arrowroot (*Maranta arundinacea*, Family: Marantaceae) is a perennial, herbaceous underutilized tuber crop that is native to south and central America but grown in tropical countries including Sri Lanka [4]. It is well known locally as "*Hulankeeriya*" or "*Aerukka*" and is more widely used in folk and Ayurveda medicine than in food preparations due to unawareness about the plant food source and the time-consuming starch extraction process. Rhizome of the arrowroot plant generates a high quality starch that is easily digestible and gluten-free and can be used as a wheat flour substitute in the food industry [5]. Nonetheless, the use of arrowroot flour in food has been limited to infant foods and porridges as a dietary therapy for urinary and bowel abnormalities.

The "Wet Zone," "Intermediate Zone," and "Dry Zone" are the three major climatic zones in Sri Lanka. "Low Country Wet Zone" (LCWZ), "Mid Country Wet Zone" (MCWZ), "Up Country Wet Zone" (UCWZ), "Low Country Intermediate Zone" (LCIZ), "Mid Country Intermediate Zone" (MCIZ), "Up Country Intermediate Zone" (UCIZ), and "Low Country Dry Zone" (LCDZ) are the subcategories of these zones. The distribution of those agro-climatic zones is depicted in Figure 1. The dry zone receives less than 1750 mm of annual rainfall, whereas the wet zone receives more than 2500 mm. Wet zone rainfall is divided into two seasons: "major rainy season" (November to January) and "minor rainy season" (May to July) [6]. The soil types that make up the Up Country and Low Country are Ultisol and Alfisol, respectively [7].

Arrowroot prefers warm and humid climates with 25°C – 28°C mean temperatures and 1500 mm – 1800 mm mean annual precipitation [8]. Published reports suggest that arrowroot grows well in Sri Lanka's mid- and low-country wet zones [9].

Because to vegetative propagation, M. *arundinacea* has a limited genetic variety [4]. Due to the lack of seed sets, natural hybridization and the creation of hybrid types are problematic [10]. The genetic diversity assessment of M. *arundinacea* germplasm is critical for aiding the improvement of superior cultivars, particularly those with high starch content and yield. Morphological characterization is ineffectual in germplasm characterization because environmental factors influence it [11]. Molecular markers are an important tool for the rapid and accurate assessment of genetic diversity, and they are required for the effective conservation of genetic resources and their focused exploitation in plant breeding [12]. The Inter Simple Sequence Repeat (ISSR) marker is a relatively new ideal genetic marker that is widely used to assess the extent of genetic variation at the inter and intra-specific levels in a variety of crops [13]. ISSR markers are a quick, easy, and effective procedure. ISSR is a dominant marker that is mainly segregated. Previous research has demonstrated that the ISSR marker is more appropriate for analyzing M. *arundinacea* genetic diversity than the RAPD marker [10].

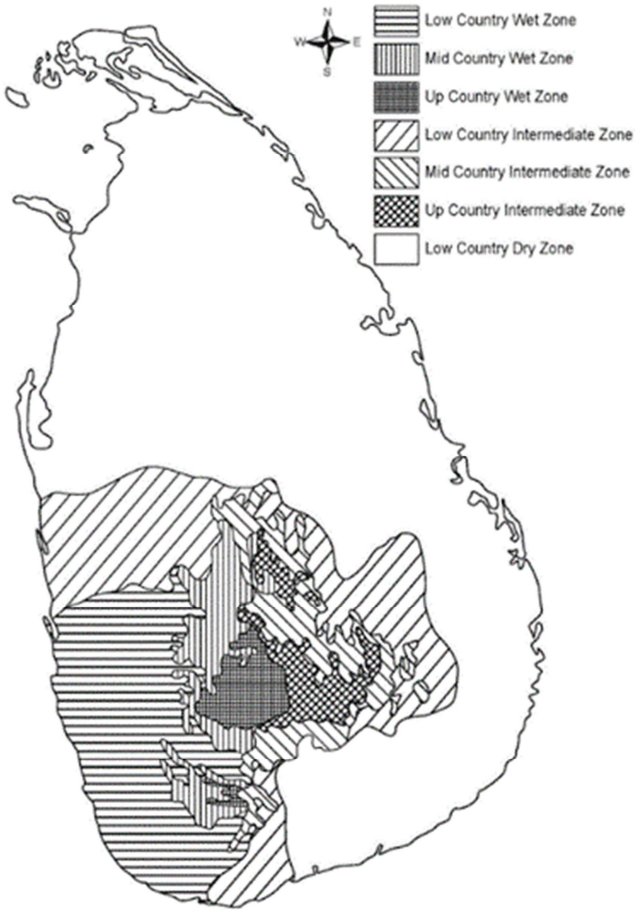


Figure 1. *A map of Sri Lanka's seven agro-climatic zones, with elevation above mean sea level (Low Country<300 m, Mid Country = 300-900 m, and Up Country > 900 m) and annual rainfall zones (Dry<1750 mm, Intermediate = 1750-2500 mm, and Wet > 2500 mm .*

The purpose of this study was to look at the differences in morphological and yield-related characteristics of arrowroot populations from Sri Lanka's seven agro-climatic zones in order to select, improve, and use the plant as a secondary food source. M. *arundinacea* farming in Sri Lanka is still highly traditional and done on a modest scale. M. *arundinacea* and its starch are poorly defined in terms of production and commercialization. As a result, the current study was designed to characterize morphological and molecular diversity among M. *arundinacea* genotypes using morphological parameters

and Inter Simple Sequence Repeat (ISSR) markers, respectively, for future germplasm conservation and subsequent crop improvement.

2. Materials and Method

2.1. Sample Collection

The snowball sampling method was used for sample collection due to the rareness of sample populations of arrowroot. Samples were collected from Deraniyagala (LCWZ), Gannoruwa (MCWZ), Hakgala (UPWZ), Mahara (LCWZ), Matara (LCWZ), Kuliyapitiya (LCIZ), Welimada (UPIZ), Matale (MCIZ) and Ampara (LCDZ), representing the seven agro-climatic zones in Sri Lanka. Plants in the harvesting stage were selected for sample collection. Slight yellowing and wilting of leaves were considered as indices to identify the harvesting stage. Plant populations cultivated without adding fertilizer were selected. For germplasm characterization Twenty *M. arundinacea* genotypes were collected from eleven different locations in Sri Lanka namely Mahara, Divulapitiya, Matara, Kandy, Makandura, Kuliyapitiya, Matale, Ampara, Jaffna, Mullaitivu and Hambantota. These samples were selected to represent a wide agroecological range where *M. arundinacea* was abundantly available.

2.2. Determination of Plant Morphological Traits

A plant morphology descriptor list was developed according to the guidelines provided by the International Plant Genetic Resources Institute [14], Biodiversity International [15], and using the specifications for *Maranta arundinacea* by the Center for Agriculture and Bioscience International [16]. Thirteen quantitative and four qualitative morphological traits were used to evaluate the plant samples. Quantitative traits included, the number of aerial stems per hill, height of aerial stem (to the upper petiole), stem diameter (at 10 cm from the crown), number of leaves per hill, length of first mature leaf from top, width of first mature leaf from the top, total leaf area per hill, total above ground biomass per hill, depth of root system, length of rhizome, number of rhizomes per hill, and total weight of rhizomes per hill. As qualitative traits, the colours of stem, first mature leaf from the top, raw rhizome, and the cooked rhizome were recorded using the Munsell Plant Tissue Color Book [17].

2.3. Arrowroot Germplasm Characterization

The modified CTAB (Cetyl-trimethyl ammonium bromide) approach was used to extract genomic DNA from fresh and immature leaves of M. *arundinacea* [18]. The extracted DNA samples were kept at 4°C. In this investigation, five ISSR primers (UBC 811, UBC 818, UBC 825, UBC 817, UBC 827) were chosen since they had yielded positive findings in earlier studies on M. *arundinacea* [10].

2.4. PCR Amplification

The extracted DNA was subjected to PCR. The reaction mixture consisted of 3 µl of 5x buffer, 1.2 µl of dNTPs (2.5 mM of each dNTP), 1.0 µl of each primer (20 µM), 1.5 µl of $MgCl_2$ (25 mM), 0.25 µl Taq DNA polymerase (5 U/µl) and 2 µl of genomic DNA. The total reaction volume was made up to 15 µl using sterile distilled water. The amplification was carried out in a Thermal Cycler (Alpha Cycler PCRmax, USA) with initial denaturation at 94°C for 10 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 55°C - 56°C for 1 minute, extension at 72°C for 1 minute and final extension at 72°C for 10 minutes [10]. The annealing temperature was optimized for each primer.

2.5. Analysis of PCR Products

The PCR products were analyzed in a 2% agarose gel containing SYBER SAFE staining (Invitrogen) and visualized using the Gel Documentation system (Vilber BioPrint, USA). A 1 kb DNA ladder was used to compare the molecular weight of each amplified PCR product.

2.6. Experimental Design and Statistical Analysis

Five plants were selected from each geographic location for the analysis. Data were analyzed using Analysis of Variance (ANOVA), principal component analysis and cluster analysis by Minitab statistical software (Version 19). The distinct and reproducible amplified bands produced by ISSR markers were scored as present (1) or absent (0) by observing stained gels. Genetic distance and genetic identity were analyzed using POPGENE (version 1.32). Based on the genetic similarity matrix, a dendrogram was generated using the unweighted pair group method with arithmetic means analysis (UPGMA). The polymorphism information content (PIC) was calculated for each primer [11].

3. Results and Discussion

3.1. Morphological Traits of Arrowroot

Variations in the morphological characteristics of arrowroot from seven agro-climatic zones of Sri Lanka are presented in Table 1. The number of aerial stems per hill, height of aerial stem, width of the first mature leaf, total above ground biomass per hill, depth of the root system, number of rhizomes per hill, the total weight of rhizomes per hill, length and width of rhizome were significantly different among the plant samples from seven agro-climatic zones. The number of aerial stems per hill was in the range of 3.4 - 8.0, and the depth of the root system varied from 19.8 cm - 29.64 cm. Plant populations did not differ significantly in terms of total leaf area or the total number of leaves per hill. Arrowroot is a low-light tolerant plant and the total number of leaves is unaffected by light intensity [19]. Although the nine distinct sites had varying light intensities, the total number of leaves and total leaf area per hill were not statistically different. Under low light conditions, the plant grows taller with broader leaves as shown by the higher aerial stem lengths of the plants from Matara, Ampara and Kuliyapitiya sites which were shaded home gardens. Plants from Matara and Ampara had the

highest total biomass above ground, which could be attributed to their higher aerial stem height. On the other hand, the lowest stem height was recorded by plants from Deraniyagala which was a site exposed to sunlight. The lowest leaf width was also recorded by plants from Deraniyagala while the highest leaf width was observed in plants from Gannoruwa. The notable variation in the width of the first mature leaf from top could be related to different environmental conditions [20].

The rhizome length ranged from 14.92 - to 24.48 cm (Table 1). According to a previous study [21], length of the rhizome ranges between 10 cm – 25 cm. Total leaf area per hill and total above ground biomass per hill had a strong positive correlation ($r = 0.802$; $P<0.05$), and the number of leaves per hill had a strong positive correlation with total leaf area per hill ($r = 0.871$; $P<0.05$). There were two moderate positive correlations between the number of rhizomes per hill and the weight of rhizomes per hill ($r = 0.605$; $P<0.05$) and the number of leaves per hill with total above ground biomass ($r = 0.69$; $P<0.05$). The total weight of rhizomes per hill correlated only with the number of rhizomes per hill ($r = 0.605$; $P<0.05$) and it was a moderate correlation.

Table 1. *Variation in morphological traits of arrowroot from seven agro-climatic zones of Sri Lanka.*

Morphological Trait	Location				
	DR	MH	GN	MT	WL
No. of aerial stems per hill	8.00 ± 2.35^{a}	3.40 ± 0.55^{b}	5.00 ± 2.35^{ab}	5.00 ± 3.08 ^{ab}	4.20 ± 1.30^{b}
Depth of root system (cm)	29.64 ± 3.27^{a}	24.50 ± 4.64^{ab}	25.40 ± 3.65^{ab}	25.74 ± 4.88^{ab}	19.80 ± 4.97^{b}
Height of aerial stem (cm)	53.72 ± 6.88^{b}	103.00 ± 19.24^{a}	89.10 ± 18.57^{ab}	106.40 ± 30.80^{a}	96.80 ± 25.00^{a}
Aerial stem diameter (cm)	1.60 ± 0.41^{a}	1.64 ± 0.54^{a}	1.74 ± 0.23^{a}	1.38 ± 0.39^{a}	1.76 ± 0.64^{a}
No. of leaves per hill	31.40 ± 14.29^{a}	31.60 ± 15.52^{a}	44.20 ± 17.82^{a}	40.80 ± 28.90^{a}	44.00 ± 25.60^{a}
Length of first mature leaf from the top (cm)	27.16 ± 2.32^{a}	33.50 ± 2.45^{a}	33.00 ± 2.57^{a}	28.94 ± 7.05^{a}	32.20 ± 2.80^{a}
Width of first mature leaf from top (cm)	7.34 ± 0.55^{c}	8.74 ± 1.54^{bc}	11.06 ± 1.31^{a}	9.20 ± 1.22^{abc}	9.28 ± 0.85^{abc}
Total leaf area per hill (cm^2)	3108.00 ± 1700.00^{a}	3002.00 ± 1413.00^{a}	6962.00 ± 3403.00^{a}	6108.00 ± 3294.00^{a}	5874.00 ± 2888.00^{a}
Total above-ground biomass per hill (g)	281.00 ± 124.40^{ab}	157.40 ± 32.90^{b}	603.00 ± 231.00^{a}	606.00 ± 339.00^{a}	585.00 ± 341.00^{ab}
No. of rhizomes per hill	11.40 ± 2.97^{a}	8.60 ± 0.89^{ab}	7.00 ± 1.58^{ab}	5.20 ± 2.39^{ab}	9.00 ± 4.85^{ab}
Length of rhizome (cm)	22.96 ± 2.06^{ab}	14.92 ± 2.19^{c}	15.56 ± 2.28^{c}	24.48 ± 3.8^{a}	16.40 ± 2.14^{c}
Width of rhizome (cm)	2.40 ± 0.22^{ab}	2.14 ± 0.19^{b}	2.46 ± 0.36^{ab}	2.32 ± 0.27^{ab}	2.46 ± 0.36^{ab}
Total weight of rhizomes per hill (g)	463.80 ± 217.60^{a}	254.10 ± 119.40^{ab}	176.40 ± 59.80^{b}	254.10 ± 119.40^{ab}	237.80 ± 113.60^{ab}

Table 1. *Continued.*

Morphological Trait	Location			
	HK	AM	ML	KL
No. of aerial stems per hill	3.40 ± 0.55^{b}	3.00± 1.15^{a}	3.66 ± 0.57^{b}	4.80 ± 0.84^{ab}
Depth of root system (cm)	23.40 ± 2.70^{ab}	21.50± 8.06^{ab}	18.33 ± 6.11^{b}	25.70 ± 4.82^{ab}
Height of aerial stem (cm)	70.40 ± 7.83^{ab}	114.75 ± 15.44^{a}	88.70 ± 33.50^{ab}	102.20 ± 16.59^{a}
Aerial stem diameter (cm)	1.48 ± 0.34^{a}	1.45 ± 0.42^{a}	2.00 ± 0.00^{a}	1.96 ± 0.40^{a}
No. of leaves per hill	39.20 ± 9.91^{a}	21.50 ± 9.57^{a}	16.33 ± 2.52^{a}	18.00 ± 6.28^{a}
Length of first mature leaf from the top (cm)	32.06 ± 2.33^{a}	32.38 ± 5.19^{a}	32.33 ± 2.08^{a}	33.50 ± 2.06^{a}
Width of first mature leaf from top (cm)	9.80 ± 0.55^{ab}	9.37 ± 0.94^{abc}	10.33 ± 0.57^{ab}	0.60 ± 0.69^{ab}
Total leaf area per hill (cm^2)	4109.00 ± 680.00^{a}	2521.00 ± 561.00^{a}	2148.80 ± 134.10^{a}	2884.00 ± 1276.00^{a}
Total above-ground biomass per hill (g)	594.80 ± 132.40^{a}	427.50 ± 115.60^{ab}	258.30 ± 38.20^{ab}	540.00 ± 114.00^{ab}
No. of rhizomes per hill	8.60 ± 6.43^{ab}	3.50 ± 0.57^{b}	3.33 ± 0.57^{b}	3.80 ± 1.79^{b}
Length of rhizome (cm)	18.30 ± 2.23^{bc}	17.90 ± 1.77^{bc}	17.3 ± 1.60^{bc}	22.70 ± 4.92^{ab}
Width of rhizome (cm)	2.32 ± 0.28^{ab}	2.02 ± 0.25^{b}	2.10 ± 0.17^{ab}	2.80 ± 0.47^{a}
Total weight of rhizomes per hill (g)	424.80 ± 201.90^{ab}	214.20 ± 40.40^{ab}	114.33 ± 11.02^{b}	163.50 ± 97.10^{b}

Values are Mean ± SD; n = 5. The same superscript letter in each row represents values not significantly different from each other at p = 0.05.
DR – Deraniyagala, MH – Mahara, GN – Gannoruwa, MT – Matara, WL – Welimada, HK – Hakgala, KL – Kuliyapitiya, ML – Matale, AM - Ampara

Colour attributes of arrowroot plants are given in Table 2. Colour of the aerial stem resulted in two colour coordinates from the yellow-green colour category. Colour of first mature leaf from the top was same for all plant samples and it was dark green colour. Occasionally, purplish-green colour could be observed in the pulvinus of well-grown stems. The raw rhizome was white, whereas the cooked rhizome was slightly darker. It can be read as a lower chroma value in Munsell colour chart for plant tissues [17].

Table 2. *Colour attributes of arrowroot plant.*

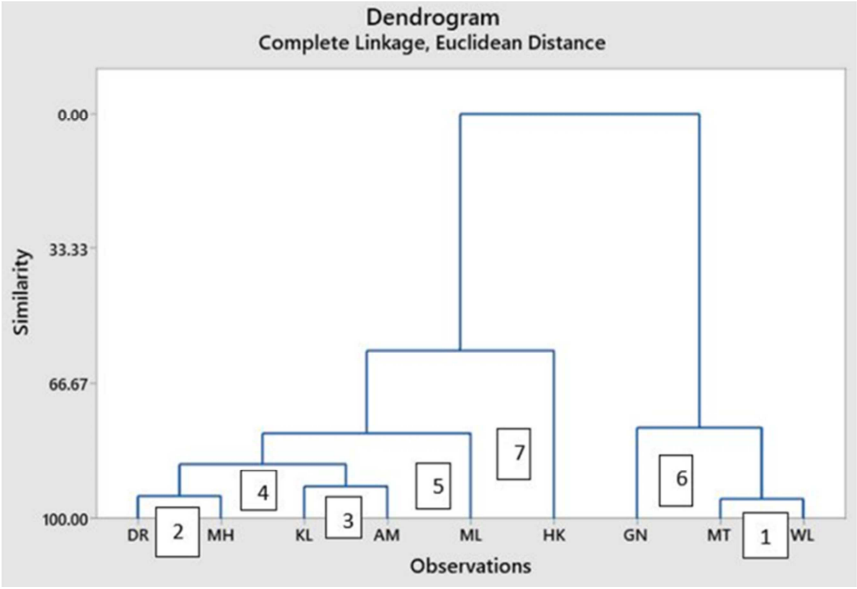
Part of the plant	*Colour Coordinates (Munsell Plant Tissue Colour)
Aerial stem	5 GY 6/6, 5 GY 7/6
First mature leaf from the top	7.5 GY ¾
Raw rhizome	5 Y 8/4
Cooked rhizome	5 Y 8/2

*There was no variation in colour of plants from nine geographical locations.

3.2. Cluster Analysis for Arrowroot from Different Locations

Similarity levels of arrowroot plant populations from seven agro-climatic zones are presented in Figure 2. Matara and Welimada (95.11 %), Deraniyagala and Mahara (94.43 %) and Kuliyapitiya and Ampara (92.04 %) were the three main clusters with highest similarity levels. The second and third clusters formed a sub-cluster with a 78.82 % similarity level. The newly formed sub-cluster develop another sub-cluster with Matale and the first cluster developed a new sub-cluster with Gannoruwa (77.41 %).

Deraniyagala and Mahara (second cluster) are similar in ten out of thirteen plant morphological traits and those two sites belong to the low country wet zone (LCWZ). The advantageous factor is that all arrowroot plant samples have higher morphological trait similarity levels. Furthermore, there are no significant deviations in morphological characters of arrowroot with respect to agro-climatic zones. This plant is now cultivated in the wet zone, although its agro-ecology is equally suited to cultivation in the intermediate and dry zones, as the plant's yield does not vary substantially among the seven agro-climatic zones.



DR – Deraniyagala, GN – Gannoruwa, WL – Welimada, MT- Matara, HK – Hakgala, MH – Mahara, KL – Kuliyapitiya, ML – Matale, AM - Ampara

Figure 2. *Cluster analysis for morphological traits of arrowroot from nine different locations.*

3.3. Principal Component Analysis

Results of Principal Component Analysis (PCA) are presented in Table 3. Principal Component Analysis [22] represents a set of variables as a smaller number of hypothetical variables and it was carried out to select the best plant morphological traits of arrowroot for selection, crop improvement and utilization. Lead plant morphological traits can be sorted out by their similarity in inheritance and expression [23]. There were four identical principal components, with four of the thirteen morphological traits falling under component 1, one under component 2, four under component 3, and three under component 4. The number of plants per hill had no positive impact on factor extraction. A total of 67.60 % of the variation was provided by the characters under analysis (Table 4).

Table 3. *Principal component analysis of arrowroot.*

Morphological Traits	PC1	PC2	PC3	PC4
No. of aerial stems per hill	0.397	0.015	0.173	0.070
Depth of the root system	0.249	0.206	0.255	0.302
Height of aerial stem	-0.037	-0.238	-0.443	0.225
Aerial Stem diameter	-0.175	-0.101	0.616	0.101
No. of leaves per hill	0.421	-0.305	-0.008	-0.195
Length of first mature leaf from the top	-0.144	-0.396	0.381	-0.188
Width of the first mature leaf from the top	-0.184	-0.407	0.198	-0.006
Total leaf area per hill	0.407	-0.369	-0.058	-0.060
Total above ground biomass per hill	0.333	-0.403	-0.099	0.136
No. of rhizomes per hill	0.280	0.222	0.122	-0.482
Length of rhizome	0.242	0.181	-0.025	0.478
Width of rhizome	0.145	0.026	0.317	0.438
Total weight of rhizomes	0.286	0.306	0.127	-0.308

Table 4. *Principal component analysis of arrowroot – Eigen values and cumulative variance.*

Principal Component	Eigen value	Percentage of the total variance	Cumulative Eigen value	Cumulative percentage of variance
1	3.3879	26.10	3.3879	26.10
2	2.4877	19.10	5.8756	45.20
3	1.6481	12.70	7.5237	57.90
4	1.2634	9.7	8.7871	67.60

Arrowroot plant morphological traits under each principal component are presented in Table 5. The maximum factor loading for principal component 1 was observed in total leaf area per hill, the number of rhizomes per hill, total above ground biomass per hill, and the number of leaves per hill. For principal component 2, total weight of rhizomes per hill was the main determinant of loading. The maximum factor loading for principal component 3 was found in the depth of the root system, length of the first mature leaf from the top, width of the first mature leaf from the top, and aerial stem diameter. Principal component 4 had the highest factor loading of the length of the rhizome, width of the rhizome, and the height of the stem.

According to the results, the leading plant morphological traits for arrowroot include the number of rhizomes per hill, total weight of rhizomes per hill, length of rhizome, width of rhizome, total above ground biomass per hill, width of first mature leaf from the top, and aerial stem height. The number of primary fingers, leaf breadth, primary finger length, rhizome diameter, and plant height have been previously identified as lead plant morphological traits for arrowroot [23].

3.4. Results of Arrowroot Germplasm Characterization in Sri Lanka

3.4.1. ISSR Analysis

With an average frequency of 10.6 bands per primer, five ISSR markers produced a total of 53 amplified fragments from 20 M. *arundinacea* genotypes. UBC 811 produced the greatest number of amplified bands (Figure 4), while UBC 818 produced the least number of bands. 50 of the 53 bands were polymorphic, with a polymorphism proportion of 94.34% on average. UBC 811, UBC 818, and UBC 825 produced the highest percentage of polymorphism, whereas UBC 817 produced the lowest rate. The amplified products ranged in size from 100 bp to 3 kb. The range of polymorphic information content (PIC) values was 0.271 to 0.428, with an average of 0.363. The primer with the highest PIC value is UBC 811, showing its usefulness in genetic diversity assessment studies with M. *arundinacea* (Table 5).

Table 5. *List of primers, sequences, number of bands, polymorphism and polymorphic information content detected by ISSR primers.*

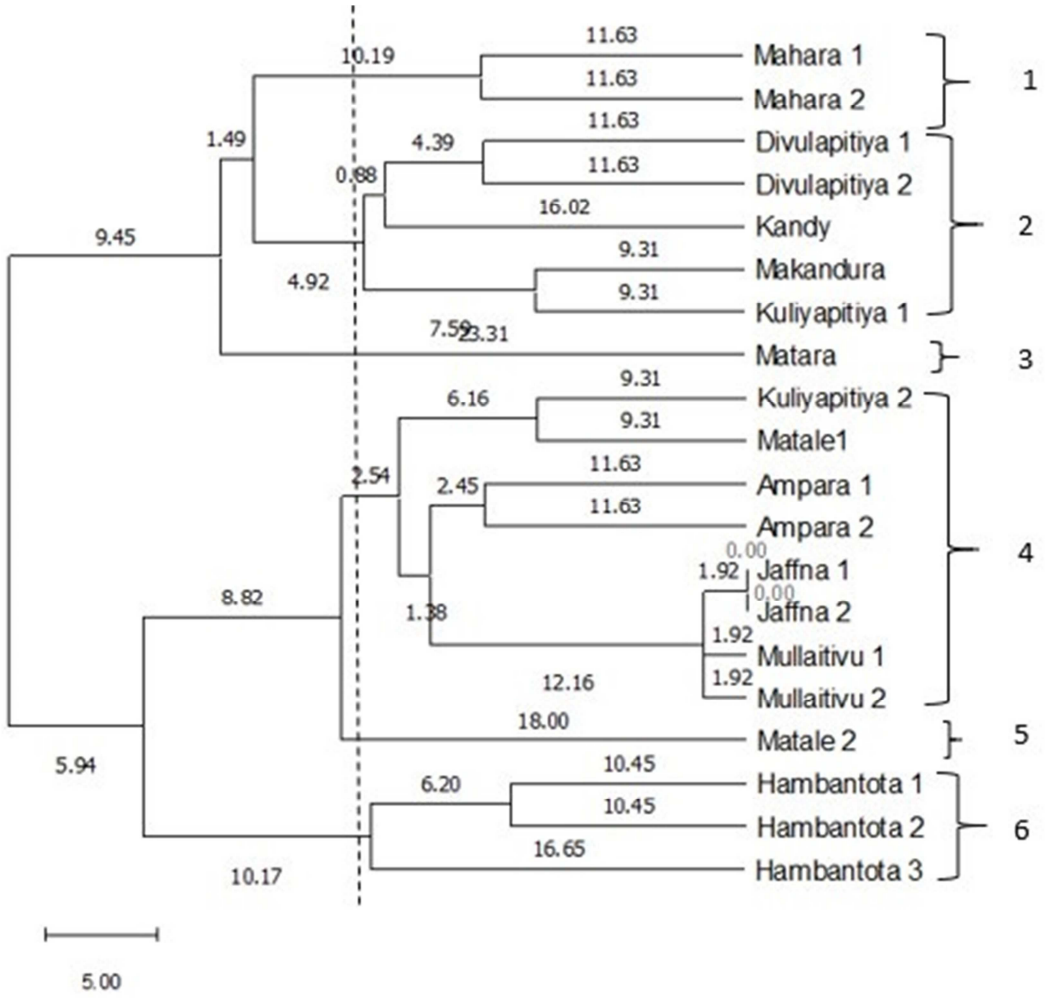
Primer name	Sequence ((5'-3')	Total number of bands observed	Number of Polymorphic bands	Polymorphism%	PIC
UBC 811	$(GA)_8C$	14	14	100	0.428
UBC 818	$(CA)_8G$	8	8	100	0.357
UBC 825	$(AC)_8T$	9	9	100	0.357
UBC 817	$(CA)_8A$	9	7	77.78	0.271
UBC 827	$(AC)_8G$	13	12	92.30	0.404

PIC- Polymorphic Information Content

3.4.2. Genetic Diversity

Based on the genetic distance matrix, the genetic identity ranged from 0.4151 to 1.000 and the genetic distance ranged from 0.0000 to 0.9258. Among the twenty genotypes, the highest genetic similarity was observed between Jaffna 1 and Jaffna 2, the genotypes were from the same location, while the lowest genetic similarity was observed between genotypes of Hambantota 1 and Matara as well as genotypes of Ampara 1 and Kuliyapitiya 1, at a genetic distance of 0.9258. A dendrogram based on the UPGMA cluster analysis classified twenty genotypes into six major clusters, that exhibited distinct separation of each genotype namely cluster 1 consisted of genotypes from Mahara, cluster 2 consisted of genotypes Divulapitiya, Kandy, Makandura, Kuliyapitiya 1, cluster 3 consisted of genotype from Matara, cluster 4 consisted of genotypes from Kuliyapitiya 2, Matale 1, Ampara, Jaffna, Mullaitivu, cluster 5 consisted of genotype from Matale 2 and cluster 6 consisted of genotypes from Hambantota (Figure 3).

The results from the cluster analysis indicated that genetic variation exists based on their agroecological zones. The first cluster consisted of genotypes from Mahara representing the wet zone. The Divulapitiya and Kandy genotypes from the wet zone and the Makandura, Kuliyapitiya1 genotypes from the intermediate zone were grouped in cluster 2. Similarly, genotypes from Kuliyapitiya 2 and Matale 1 from the intermediate zone were grouped with genotypes from the dry zone in cluster 4. The genotype from Matara formed a distinct cluster (cluster 3) that may be due to its adaptability to the low country wet zone. In the sixth cluster genotypes from Hambantota were grouped separately. Clusters 4 and 6 mainly represent the genotypes collected from the dry zone while Clusters 1, 2 and 3 represent the genotypes collected from the wet zone. The genotypes from the intermediate zone grouped with genotypes from the wet zone and genotypes from the dry zone, and it seemed that they had genetic relatedness to the dry zone and wet zone.



Dendrogram was generated using the unweighted pair group method with arithmetic means (UPGMA)

Figure 3. *Dendrogram showing clustering pattern of M. arundinacea genotypes using ISSR markers.*

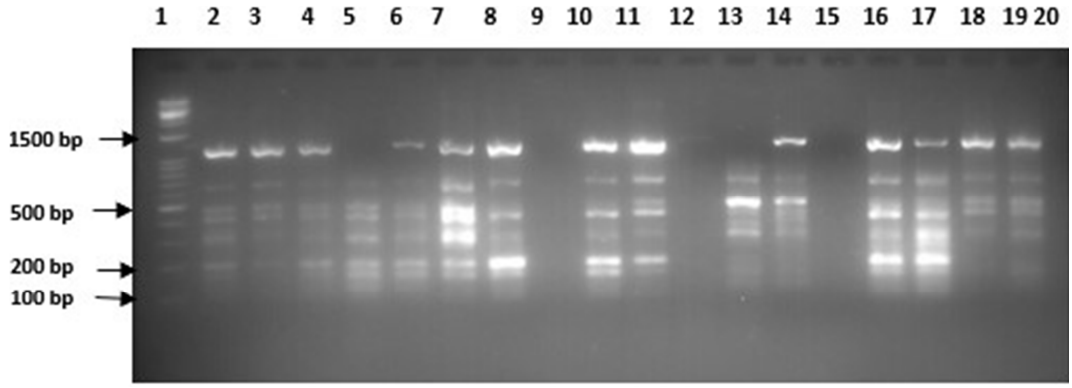


Figure 4. *Agarose gel (2%) image of PCR products of 20 M. arundinacea genotypes generated using UBC 811 ISSR marker.*

Lane 1: 1 kb DNA ladder, Lane 2, 3, 4: Jaffna 1, 2, 3, Lane 5, 6: Mullaitivu 1, 2, Lane 7, 8, 9: Kuliyapitiya 1, 2, 3, Lane10: Makandura, Lane 11, 12, 13: Matale 1, 2, 3, Lane 14, 15, 16: Divulapitiya 1, 2, 3, Lane 17, 18, 19, 20: Ampara 1, 2, 3, 4

4. Conclusions

Plant morphological characterization of arrowroot from the seven agro-climatic zones in Sri Lanka was performed in the current study. Nine of the thirteen morphological traits were found to differ significantly across all plant populations. According to the cluster analysis, the plant populations from seven agro-climatic zones had higher similarity levels, showing that agro-ecology had a minor impact on plant growth and yield parameters. Height of the stem, width of first mature leaf from the top, total biomass above ground per hill,

number of rhizomes per hill, Length of rhizome, width of rhizome, and the total weight of rhizomes were identified by the principal component analysis as lead plant morphological traits for selection, crop improvement, and effective crop utilization through breeding programmes. In the present study, the significant level of genetic diversity among *M. arundinacea* genotypes was revealed by molecular characterization according to different agroecological regions whereas morphological markers failed to differentiate the genotypes comprehensively. The genetic diversity revealed by ISSR markers in the present study would be important in future crop improvement programs in *M. arundinacea*.

Authorship Contribution Statement

Susanga Malki and Sivashoby Sivalingam conducted the research, data gathering, data analyzing, and research article writing. Radhika Gimhani, Amani Wijesinghe and Kamani Ratnayake provided the guidance throughout the research and proof reading of the article.

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