MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF *AZOLLA* ACCESSIONS IN KENYA †

[CARACTERIZACIÓN MORFOLÓGICA Y MOLECULAR DE ACCESIONES DE *AZOLLA* EN KENIA]

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SUMMARY

Background. *Azolla* Lam., a mosquito fern, is invasive in major rice growing Schemes in Kenya, where it clogs irrigation canals and forms dense mats in paddy fields. However, the species of *Azolla* has not been established. **Objective.** to characterize *Azolla* accessions collected from six major rice Irrigation Schemes in Kenya: Mwea, Ahero, West Kano, Bunyala, Taveta and TARDA. **Methodology.** *Azolla* accessions were collected, grown for 10 days at Mwea Irrigation Agricultural Development Centre (MIAD) and their vegetative traits examined microscopically using 13 Pereira's morphological characters. The vegetative characteristics were evaluated on a binary 0/1 system, pairwise similarity was estimated using Jaccard's coefficient (S1) and a dendrogram generated. Genomic DNA was extracted from each of the accessions, amplified with SCAR primers and amplified products resolved and scored using agarose gels. Polymorphic SCAR markers were identified and correlated to the accessions. **Results.** Nine vegetative characters useful for distinguishing between the two *Azolla* sub-genera (*Euazolla* and *Rhizosperma*) and the seven *Azolla* species were examined. Possession of hook-like, septate glochidia suggested the presence of *Azolla filiculoides* in TARDA1 accession. The presence of pinnate sporophyte with septate rhizome papillae and fronts measuring 2-4 cm with 2-4 cm long roots and lack of anthocyanin suggested the presence of *Azolla nilotica* for TARDA 2 and Taveta 2 accessions. SCAR marker based 490 bp primers that identify with *A. filiculoides* also amplified Mwea and Taveta 1 accessions to give a distinct band. **Implications.** Results suggest the existence of *Azolla nilotica* and *Azolla filiculoides* among the Kenyan accessions. **Conclusion.** *Azolla filiculoides* and *Azolla nilotica* are the two main *Azolla* species characterized in the major Irrigation Schemes in Kenya. Of the two species, *Azolla filiculoides* has infested four of the Kenya irrigation schemes (Mwea, Ahero, Bunyala, Tana River and West Kano), while *Azolla nilotica* exists only in Taveta and TARDA.
Key words: *Azolla;* accession*; c*haracterization*;* morphology; species

RESUMEN

Antecedentes. *Azolla* Lam., helecho mosquito, es invasivo en los principales esquemas de cultivo de arroz en Kenia, donde obstruye los canales de riego y forma esteras densas en los arrozales. Sin embargo, la especie de Azolla no se ha establecido. **Objetivo.** Caracterizar las accesiones de Azolla recolectadas de seis grandes esquemas de riego de arroz en Kenia: Mwea, Ahero, West Kano, Bunyala, Taveta y TARDA. **Metodología.** Se recogieron las accesiones de Azolla, se cultivaron durante 10 días en el Centro de Desarrollo Agrícola de Riego de Mwea (MIAD) y sus rasgos vegetativos se examinaron microscópicamente con 13 caracteres morfológicos de Pereira. Los caracteres vegetativos se evaluaron en un sistema binario 0/1, se estimó la similitud por pares utilizando el coeficiente de Jaccard (S1) y se generó un dendrograma. El ADN genómico se extrajo de cada una de las accesiones, se amplificó con cebadores SCAR y los productos amplificados se resolvieron y puntuaron usando geles de agarosa. Se identificaron marcadores SCAR polimórficos y se correlacionaron con las accesiones. **Resultados**. Se examinaron nueve caracteres vegetativos útiles para distinguir entre los dos subgéneros de Azolla (Euazolla y Rhizosperma) y las siete especies de Azolla. La posesión de glochidia septada en forma de gancho sugirió la presencia de Azolla filiculoides en la entrada

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TARDA1. La presencia de esporofitos pinnados con papilas rizomatosas septadas y frentes de 2-4 cm con raíces largas de 2-4 cm y la falta de antocianinas sugirieron la presencia de *Azolla nilotica* para las accesiones TARDA 2 y Taveta 2. Los iniciadores de 490 pb basados en marcadores SCAR que se identifican con *A. filiculoides* también amplificaron las accesiones Mwea y Taveta 1 para dar una banda distinta. **Implicaciones.** Los resultados sugieren la existencia de *Azolla nilotica* y *Azolla filiculoides* entre las accesiones de Kenia. Conclusión. *Azolla filiculoides* y *Azolla nilotica* son las dos principales especies de Azolla caracterizadas en los principales esquemas de riego en Kenia. De las dos especies, *Azolla filiculoides* ha infestado cuatro de los esquemas de riego de Kenia (Mwea, Ahero, Bunyala, Río Tana y West Kano, mientras que *Azolla nilotica* solo existe en Taveta y TARDA.
Palabras clave: Azolla; accesión; caracterización; morfología; especies

INTRODUCTION

Azolla is a free floating water fern, native to the tropical and temperate paddies (Campbell, 2011; Subedi and Shrestha, 2015; Watanabe *et al*., 1980). Worldwide, it is represented by seven (7) recognized species namely *A. nilotica, A. pinnata, A. filiculoides, A. mexicana, A. rubra, A. microphylla and A.caroliniana* (Pereira, 2011). The species are within the Sub-genera *Rhizosperma* and *Euazolla. Azolla* multiplies fast and fixes nitrogen at a higher rate than legumes (Wagner, 1997), and when incorporated in the soil, it releases about 75% of its nitrogen within 6-8 weeks (Watanabe *et al*., 1980). This makes it important for use in paddies as a bio-fertilizer. However; its fast growth rate makes it a noxious weed. In West Africa, it has been reported as a troublesome weed in paddy fields along Gambia River (Ivens, 1987). In Mwea Irrigation Scheme, it was christened "Acquired immune deficiency syndrome" due to difficulty in its control and an unknown source of invasion. The presence of *Azolla* can also restrict the growth of other aquatic plants including *Salvinia* and *Eichhornia crassipes* (water hyacinth) as it competes for the available nutrients with these plants.

The distribution of *Azolla* within the temperate and tropical paddies is wide. New world *Azolla* species have been introduced in other areas through human activities causing elimination of native species (Carrapico, 2000). Three world species have been classified as invasive; *A. pinnata* R.Br., *A. filiculoides* Lam. and *A. mexicana* Presl. (online data www.cabi.org/isc). *Azolla filiculoides* has previously been reported in Tanzania, South Africa and Kenya (Henderson, 2002). *A. pinnata* sub sp. *africana* are native to Africa, however, *A. pinnata* sub sp. *asiatica* is present in South Africa. Further, A. *cristata* Kaulf. (*A. mexicana* & *A. microphylla* Kaulf) are established in South Africa, Mozambique, Zimbabwe and Ghana (Madeira *et al.*, 2013).

Morphological features have been used for a long time to identify and characterize *Azolla* (Pereira *et al.*, 2011). Saunders and Fowler (1992) successfully used morphological characteristics and identified *Azolla nilotica* Decaisne ex Mett., *A. pinnata, A. microphylla, A. filiculoides, A. rubra* R. Br.and *A. caroliniana* Willd.. The major distinguishing features which were relied upon included; number of float capsules, type of glochidia, branching pattern and leaf trichomes, as described by Saunders (1992) and Zimmerman (1989). Pereira (2011) however presented 13 polymorphic descriptors used to characterize existing *Azolla* species thus; sporophytic shape and arrangement, rhizome indumentum and papillae, dorsal lobe apex, angle and shape, hyaline border cells, symmetry, and papillae, dorsal and ventral stomata. Madeira *et al*. (2013) successfully used morphological characteristics to identify the sub-sections *Rhizosperma* Sadeb. and *Euazolla* Sadeb. and to distinguish the Asian *Azolla pinnata* and *African Azolla pinnata*. Morphological characterization has been relied upon for *Azolla* characterization. This however has some limitations due to variability of species and environmental effects on sporulation of cultured accessions (Abraham *et al.*, 2013). Molecular sequencing and phylogenetics, which exploits the DNA techniques, is hence a complementary precise technique (Caetano-Anolles, 1991). Abraham *et al.*, (2013) consequently developed specific sequence characterized amplified region (SCAR) markers from randomly amplified polymorphic DNA (RAPD) which they used to differentiate amongst *Azolla pinnata* (182 bp), *Azolla rubra* (390 bp), *Azolla filiculoides* (490 bp) and *Azolla micropylla* (709 bp) species. The SCAR markers amplified clear bands that differentiated the four species. RAPD markers have been used to identify exotic *Azolla pinnata* sub species *pinnata* and native *Azolla caroliniana* in Florida. Madeira *et al.* (2013) also used molecular characterization to identify exotic *Azolla pinnata* sub species *pinnata* and native *Azolla caroliniana* in Florida and identified *A. mexicana* and *A.microphylla* as same species. Similarly, Evrard and Van Hove (2004) used molecular phylogenetic to identify *A.mexicana* and *A. micropylla* as one species (Cristata). Pereira *et al*. (2011) also used RAPD markers to differentiate amongst *A. pinnata, A. mexicana, A. nilotica* and *A. rubra*.

Many studies have been conducted on *Azolla* species globally, but little has been done on identification of the *Azolla* species in Kenya. Thus, a scientific gap on characterization of *Azolla* will remain until all species and subspecies in different geographical regions are

studied. In order to harness the specific and full benefits of this fern, there is need to collect and identify all the species existing in major irrigation schemes within Kenyan. This study aimed at characterizing and identifying *Azolla* species found in major irrigation schemes in Kenya using morphological and molecular methods.

MATERIALS AND METHODS

Study sites

The study was conducted at Mwea Irrigation Scheme in Kenya. *Azolla* accessions used in this study were collected from Mwea, Ahero, West Kano, Bunyala, Tana & Athi River Development Authority (TARDA) and Taveta Irrigation Schemes in Kenya. Within the paddy fields, *Azolla* is a noxious weed, which causes mechanical obstruction, impedes water flow, clogs pumps and decreases light intensity (Yanni *et al*, 1994).

Mwea Irrigation Scheme is located in Kirinyaga County, at an altitude of 1159 metres above the sea level, 0° 37'S and 37° 27'E. The climate is tropical within agro-ecological zones Lower Midland 3 (LM3) and Lower Midland 4 (LM4). Rainfall pattern is bimodal with an annual mean of about 930 mm with 66% reliability. The average temperature is 22 °C. The soils are predominantly vertisols (black cotton soils) and imperfectly drained. Ahero and West Kano Irrigation scheme are located in Kisumu County, within Kano plains, at an altitude of about 1100 masl, with an annual average rainfall of 900 mm. Soils are vertisols, with a mean pH of 6.9**.** Bunyala Irrigation scheme is located in the northern part of Yala swamp, along lower Nzoia area, within agro-ecological zone LM4. The area has an average temperature of 24^{0}C, with an annual rainfall of 900–1000 mm. Soils are predominantly alluvial sediments, dark grey brown friable sandy to clay. Tana River Development Authority scheme is located 210 km north of Mombasa, within agro-ecological zone Coastal lowland 3 (CL3), in the Tana delta flood plains. It has a bimodal rainfall pattern with 800-1000 mm of rainfall per year and an average temperature of 27 ^{0}C. Taveta has several small holder irrigation schemes, located to the south west of Kenya, all within lower midland 4 agro-ecological zone The area receives 350-750 mm of rainfall per year, with an average temperatures of 27 ^{0}C. Soils are predominantly clay loam, with a mean pH of 7.97.

Table 1.1. Morphological descriptors of *Azolla* for cluster I and II, corresponding to sub-genera *Rhizosperma* and *Euazolla*,

Cluster I	**Cluster II**
Deltoid sporophyte	Polygonal sporophyte
Pubescent rhizome	Pubescent rhizome
Sub-round dorsal lobe apex	Round dorsal lobe apex
Acute angle of the dorsal lobe	Obtuse angle of the dorsal lobe
Asymmetrical hyaline borders	Symmetrical/asymmetrical hyaline border
3–4 layers of cells on the hyaline border	2–6 layers of cells on the hyaline border
Sub-cluster Ia	**Sub-cluster Ib**
Sub-pinnate, alternate deltoid branching pattern	
Absence of stomata on the ventral lobe	
Sub cluster IIa	**Sub cluster IIb**
Anisotomous opposite (except *A. rubra*)	Anisotomous opposite (except *A. rubra*)
Bicellular non-prominent dorsal lobe papillae	Multicellular prominent dorsal lobe papillae
Annular stomata with middle longitudinal ridge on the dorsal lobe	Annular stomata witht middle longitudinal ridge on dorsal lobe
Absence of ventral lobe stomata	Absence of ventral lobe stomata
Symmetrical hyaline border	Asymmetrical hyaline border

Source: Adopted from Pereira et al. (2011), modified based on findings from Kenyan accessions.

Table 1.2: Morphological distinctions of *Azolla* species.

Species	Origin	Distinguishing characteristics					
		Sporocarps		Megasporocarp	Microsporocarp	Leaf trichomes	Others
A. nilotica	Central, East Africa	Set of 4 sporocarps,	9 floats	2 tiers , lack of defined collar	Small glochidia	≥ 2 celled	Up to 40 cm, 2 mm thick, leaves on main stem
A. pinnata	Asia, Oceania, Africa	A pair of sporocarps,	9 floats	dense filosum of collar	No hook like tip in glochidia	≥ 2 celled	Less than 5 cm, leaves at base of stem
A. filiculoides	Latin America	A pair of sporocarps,	3 floats	2 floats overlying a glabrous collar	Hook like tip in glochidia, 0-2 septa	Single celled	
A. rubra	Oceania	A pair of sporocarps	3 floats		Hook like tip in glochidia	Less pronounced	
A. cristata							
A. mexicana	Latin America	A pair of sporocarps,	3 floats	filaments on surface of periospore	Hook like tip in glochidia, > 2 septa	double celled	
A.caroliniana	Latin America	A pair of sporocarps, collar	3 floats	perisospore have dense filosum	Hook like tip in glochidia, > 2 septa	double celled	
A.microphylla	Latin America	A pair of sporocarps,	3 floats	glabrous collar and uniform coverage	Hook like tip in glochidia > 2 septa	double celled	

Source: *Evrard and Van Hove (2004)* and Perkins *et al*. (1985).

Sampling

Azolla accessions were collected from Mwea, Ahero, West Kano, Bunyala, Taveta and Tana River Development Authority (TARDA) Irrigation Schemes in Kenya, during the long and short rains in 2016. These Irrigation Schemes were selected for the study because they are the major irrigated paddy rice production areas in Kenya. In the deep outlet of Lake Jipe and excavated ponds in TARDA scheme, a giant *Azolla* accession was noted and collected. The sampling areas were denoted as Mwea, Ahero, West Kano, Bunyala, Taveta 1, Taveta 2, TARDA 1 and TARDA 2. Three samples of *Azolla* biomass (each 100 g) were collected from each of the irrigation canals of these points using transparent plastic bags (9 x 16 cm), in two successive seasons (long and short rains of 2016 for tissue N, P and K and for morphological and molecular characterization.

Morphological characterization of *Azolla* accessions

Eight *Azolla* samples collected from the six Irrigation Schemes were grown in plastic containers (8.4 x 10^{-3} m^3) for 30 days in 4 liters of canal water from the Mwea Irrigation Scheme drain. Fresh sporophytes of *Azolla* plants were obtained and left to drip dry for 10 minutes. They were then mounted on a binocular stereomicroscope (Olympus, UK) and a light microscope (Olympus BX60 at x 400) coupled to a Leica DP50 camera (Leica Microsystems, Germany) at x 400, examined and images acquired. The fronds of sporophytes were sliced to expose the internal sections. Massula were isolated from the microspores and the glochidia examined. Key vegetative traits namely; types of leaf trichomes, glochidia, megasporocarp, microsporocarp, and floats were visualized, photographed and images were matched to Pereira et *al*. (2011) and Evrard and Van Hove (2004) shown in Table 1.1 and 1.2.

Molecular identification using sequence characterized amplified regions (SCAR) markers

Seven *Azolla* accessions from six major irrigation schemes namely Mwea, Ahero, Bunyala, Tana River Development Authority and Taveta Irrigation Schemes, were characterized using SCAR markers developed by Abraham *et.al*. (2013). Genomic DNA was extracted from accessions of *Azolla* sporophyte, according to the procedure by Lin Rong, *et al.* (2001). The amplified PCR products were resolved by electrophoresis on 1.0% agarose gel in 1 × TAE buffer. Banding of PCR was done on gels and visualized by 0.5 μg/mL ethidium bromide staining and the images visualized under gel documentation system.

Data analysis

Morphological data was analyzed using NTSYS-pc Exeter Software version 2.1 (Setauket, USA). The vegetative characters were evaluated in a 0/1 binary system, presence or absence of features based on Pereira et al, (2011) descriptors. Pairwise similarity was estimated using the Jaccard coefficient (Sj). Jaccard similarity coefficient and unweighted pair-group method with arithmetic mean (UPGMA) for the cluster analysis were performed by Sequential Agglomerative Hierarchical Nested (SAHN) method, where *Azolla* specimens were grouped according to their similarity. A dendrogram was generated using 13 polymorphic morphological descriptors namely sporophytic shape, polygonal branching pattern, deltoid branching pattern, rhizome indumentum, rhizome papillae, dorsal lobe apex, apex dorsal lobe angle, dorsal lobe shape, hyaline border symmetry, number of cells of hyaline border, dorsal lobe papillae, dorsal lobe stomata, dorsal leaf lobe stomata type and ventral lobe stomata. Polymorphic SCAR markers were identified based on clear resolved bands and matched to the respective accessions.

Table 1.3: Primers sequence for-specific SCAR loci used in the experiment.

***Azolla* species**	**RAPD primer**	**Sequence of SCAR primer**	**Product size and accession number**
A. rubra	S-series	gcctaagtccaagcttactcatctta	390
		atttaggcttgggccacagatagaag	JQ435715
A. pinnata	,,	caataccttgttcagtgttcgtagg	182
		tggcaatgaccatgaagtagaata	JQ43571516
A. filiculoides	,,	agatggttagaagtgacagcatatctt	490
		ttctatagctactcgaccatgagaagt	JQ435717
		gacatatccacctatcgtctctgtgtc	709
A. microphylla	,,	agacaacttcgatagtcacagttc	JQ435718

Source; Abraham *et al*, (2013).

RESULTS

Morphological traits

The morphological traits of the respective accessions namely, leaf trichomes, rhizome indumenta, hyaline borders, stomata and glochidia, are shown in Figures 1.2-1.8. In general, two sporophytic shapes were observed: Polygonal (2-dimentional) and deltoid (entire leaf shape triangular). The Polygonal shape showed resultant unequal sized branching from the main axis (anisotomous), while deltoid shape had leaves with feather- like arrangement on both sides of the axis (sub-pinnate). The dorsal lobes were hairy (pubescent) and either unicellular or multi-septate, with stomata that had middle longitudinal lamina (anormocytic) in all cases. Mwea, Ahero, West Kano, Bunyala, TARDA 1 and Taveta 1 accessions had polygonal and anisotomous sporophytic shape, with obtuse dorsal angle, sub-round dorsal apex and obovate dorsal lobe shape (Fig1.1a, 1.2b). Taveta 2 and TARDA 2 accessions however had deltoid and sub-pinnate sporophytic shapes with an acute dorsal lobe angles (Fig 1.1 c and 1.1d).

Leaf trichomes

Mwea, Ahero, West Kano, Bunyala and TARDA1 and Taveta1 accessions had bicellular non-prominent trichomes (Fig 1.2a and 1.2b) while Taveta 2 and TARDA 2 accessions had prominent multi-septate leaf trichomes (Fig 1.2c and 1.2d).

Rhizome papillae

All accessions had pubescent rhizome indumentum (Fig 1.4 a- 1.4 g). Taveta 2 & TARDA 2 accessions were multi-septate (Fig 1.3b). Others were uni-septate (1.3 a).

Figure 1.1 a: Fronds for West Kano accession x400

Figure 1.1 b: Fronds for Bunyala accession x400

Figure 1.1 c: Fronds for TARDA 1 accession X400

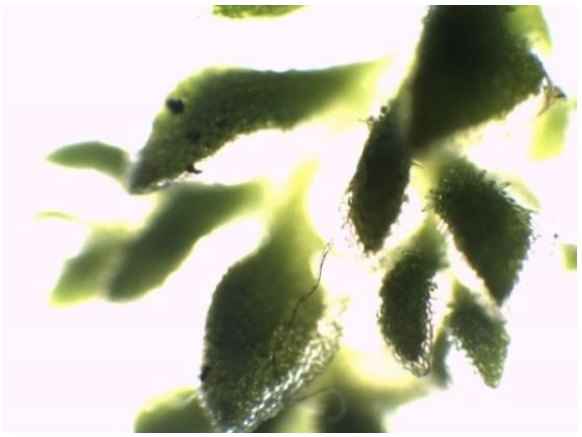


Figure 1.1 d: Fronds for Taveta 2 accession X400

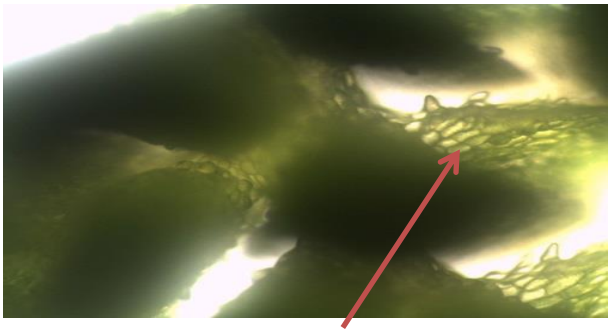


Figure 1.2 a: Leaf trichomes for Mwea accession x400

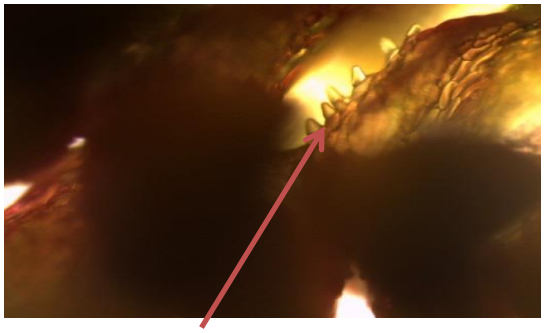


Figure 1.2 b: Leaf trichomes for Ahero accession x400

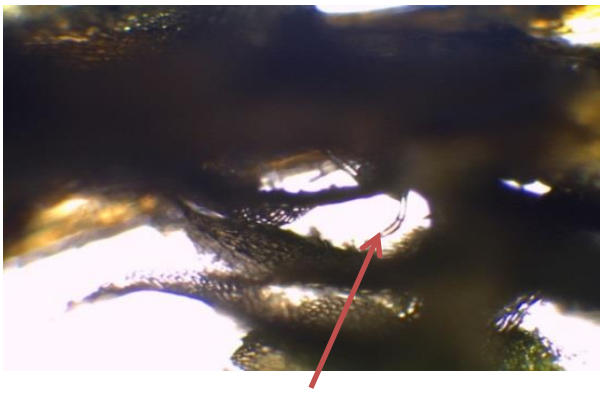


Figure 1.2 c: Leaf trichomes, for Taveta 2 accession x400

Figure 1.2 d: Leaf trichomes, for TARDA 2 x400

Hyaline border cells

The accessions from Mwea, Ahero, West Kano, Bunyala, TARDA 1, TARDA 2, Taveta 1 and Taveta 2 had a hyaline border with 3-4 layers of cells (Fig 3.5 a-i). The hyaline borders for Ahero, West Kano, Bunyala, Taveta 2 and TARDA 2 accessions were symmetrical compared to the asymmetrical ones of Mwea, TARDA 1 and Taveta 1 accessions.

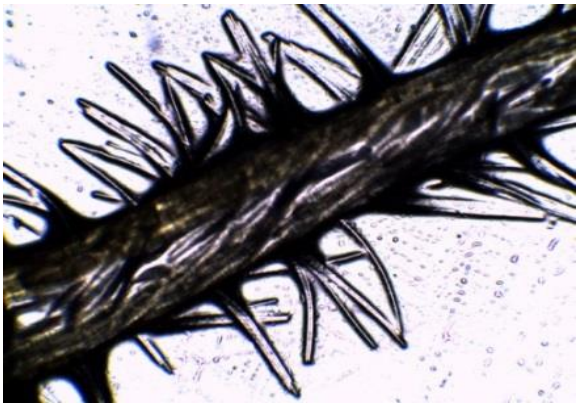


Figure 1.3 a: Rhizome papillae for Bunyala x400

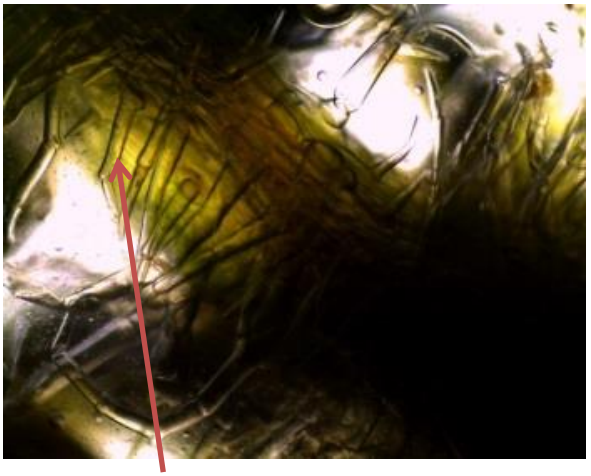


Figure 13b: Rhizome papillae for TARDA 2 accession

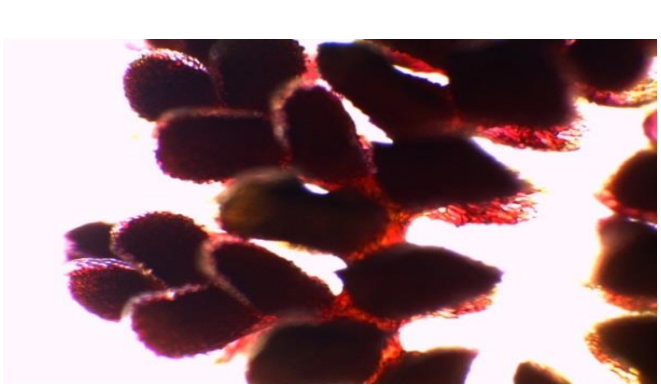


Figure 1.4a: Hyaline border for West Kano accession x400 **Figure1.4 b:** Hyaline border for TARDA 1 accession x400

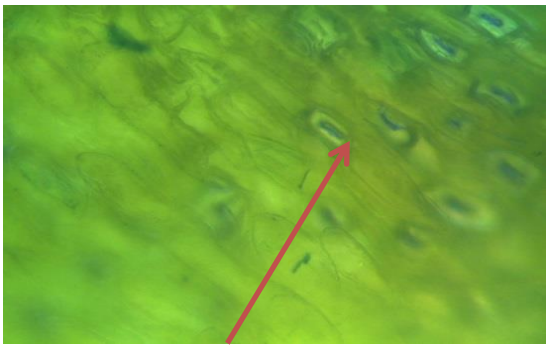
Figure 1.4 c: Hyaline border for TARDA 2 accession x400

Dorsal stomata

All the accessions from Mwea, Ahero, West Kano, Bunyala, TARDA, Taveta 1 and Taveta 2 had anomocytic annular stomata with middle longitudinal ridge (Fig 1.6a but no ventral stomata).

Glochidia

Sporulation was only noticed in TARDA accession. The sporocarps had prominent hook-like septate (2-3 septa) glochidia (Fig 1.6).



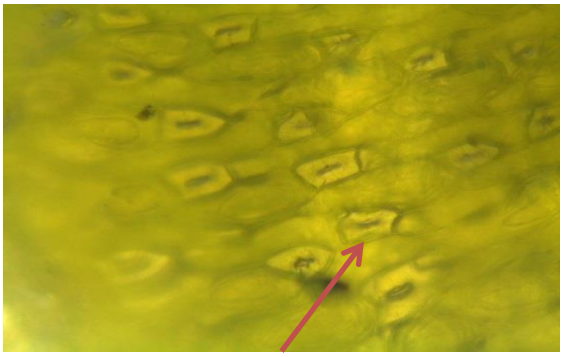


Figure 1.5a: Dorsal stomata for Mwea accession x400 **Figure 1.5 b**: Dorsal stomata for Ahero accession x400

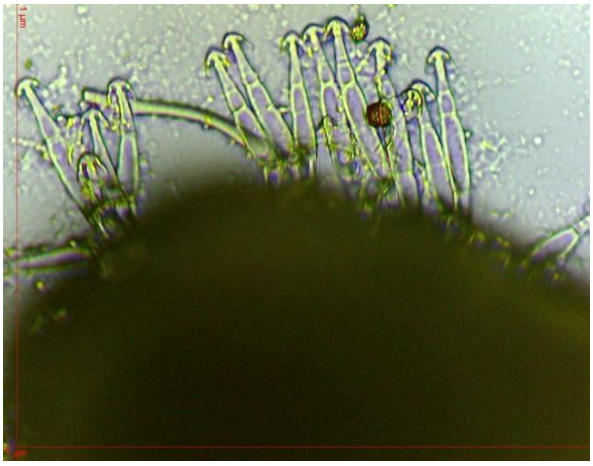


Figure 1.6: Prominent hook-like glochidia, TARDA 1 accession x 400

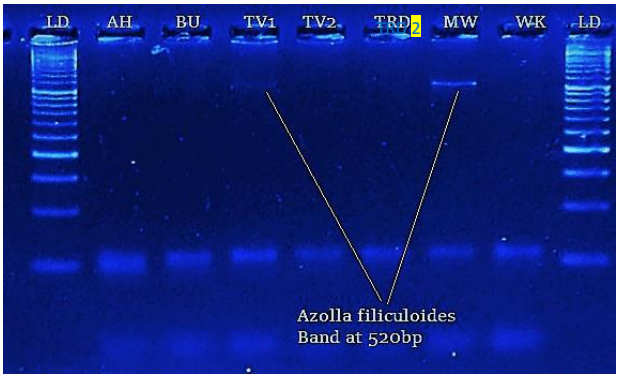


Figure 1.8: Amplification profile of 8 *Azolla* accessions using SCAR markers based on *A. rubra (390 bp) A. pinnata (bp 182 bp), A. filiculoides (bp 490 bp)* and *A. microphylla* (709 bp).

Cluster analysis

A dendrogram generated using 13 morphological descriptors based on unweighted Pair-group method with arithmetic mean (UPGMA) clearly showed the phenotypic relationship amongst the accessions. Cluster analysis revealed two major clusters (Cluster I and Cluster II) for the accessions from the major Irrigation Schemes. Cluster I had two accessions while cluster II had two sub-clusters; IIa and IIb. The sub-clusters IIa had Bunyala, West Kano and Ahero accessions while sub cluster IIb had Mwea, Taveta and TARDA 1 accessions. Except for hyaline border symmetry, the sub-clusters showed complete homogeneity of vegetative characteristics (Sj1.0) and hence no morphological diversity. The two clusters corresponded to sections *Rhizosperma* and *Azolla*. Using 13 polymorphic vegetative characters and UPGMA for the cluster analysis.

Molecular characterization

Genomic DNA of the *Azolla* accession were amplified using sequence characterized amplified regions (SCAR) markers developed by Abraham *et al*., (2013). The sequence of the primers used to amplify the genomic DNA from *A. rubra, A. pinnata, A. filiculoides and A. microphylla*, is shown in Table 1.3 and the amplification profile of the SCAR markers is in Fig 1.8. The primer based on *A. filiculoides* (490 bp) showed distinct bands for Mwea and Taveta 1 accessions and not Ahero, West Kano and Bunyala, which had shown morphological similarity with *A. filiculoides. P*rimers based on *A. rubra, A. pinnata* and *A.microphylla* did not amplify for any accession.

DISCUSSION

The findings of the study showed that of the 13 polymorphic vegetative characters identified by Pereira (2011), 9 were polymorphic for the *Azolla* accessions in Kenya. These vegetative characteristics distinguished the seven *Azolla* accessions into two clusters (I and II). Cluster I possessed a deltoid sporophyte with sub-pinnate branching pattern, a multi-septate pubescent rhizome indumentum, an elliptical dorsal lobe shape with acute apical angle and prominently multi-septate leaf trichrome. These distinguished cluster I as the Sub-genera *Rhizosperma.* Cluster II had polygonal sporophyte with anisotomous branching pattern, pubescent, unicellular rhizome indumentum, sub round dorsal lobe apex and asymmetric hyaline border. These characteristics distinguished cluster II as sub-genus *Euazolla*. Sub genus *Rhizosperma* is indigenous to Africa but the occurrence of sub-genus *Euazolla* in Kenya suggests that New world species of *Azolla* are invasive in the country. Cluster II was further sub-clustered into IIa and IIb differing only on the symmetry of the hyaline border. However, in the sub-clusters, the Jaccards coefficient showed complete homogeneity of vegetative characteristics which suggests similarity of species within each of the clusters and sub-clusters. TARDA 1 possessed prominent hook-like 0-2 septa in glochidia, with single-celled leaf trichomes, which are distinguishing characteristics of *A. filiculoides*. The absence of glochidia in all other accessions could be attributed to environmental factors. According to Abraham *et al.* (2013), environmental factors affect sporulation of *Azolla*. The sub-clusters II therefore bore close resemblance to *A. filiculoides,* whose invasive nature and presence in East Africa had been reported by Henderson (2002).

Table 1. 4: A summary of morphological characteristics of *Azolla* accessions from the six major rice Irrigation Schemes in Kenya, based on polymorphic features.

Accession	sporophytic		Rhizome		Dorsal lobe				Hyaline			stomata presence	
	shape	arrangement	indumentum	papillae	Apex	Angle	Shape	papillae	Border layers	symmetry	papillae	Dorsal lobe	Ventral lobe
Mwea	polygonal	anisotomous	pubescent	present	S/round	obtuse	obovate	unicellular	3-4	asymmetrical	present	present	absent
Ahero	polygonal	anisotomous	pubescent	present	S/round	obtuse	obovate	unicellular	3-4	symmetrical	present	present	absent
W/Kano	polygonal	anisotomous	pubescent	present	S/round	obtuse	obovate	unicellular	3-4	symmetrical	present	present	absent
Bunyala	polygonal	anisotomous	pubescent	present	S/round	obtuse	obovate	unicellular	3-4	symmetrical	present	present	absent
Taveta 1	polygonal	anisotomous	pubescent	present	S/round	obtuse	obovate	unicellular	3-4	symmetrical	present	present	absent
Taveta 2	deltoid	sub-pinnate	pubescent	present	elliptical	Acute	elliptical	multicellular	3-4	asymmetrical	present	present	absent
TARDA 1	polygonal	anisotomous	pubescent	present	S/round	obtuse	obovate	unicellular	3-4	symmetrical	present	present	absent
TARDA 2	deltoid	sub-pinnate	pubescent	present	elliptical	Acute	elliptical	multicellular	3-4	asymmetrical	present	present	absent

The six polymorphic characters namely deltoid sporophyte, sub-pinnate alternate arrangement, with a pubescent and asymmetric hyaline border with 2-6 layers of cells, having stomata on the dorsal with multi-septate papillae, clearly suggest Taveta 2 and TARDA 2 accessions being *A. nilotica*. The identity of *Azolla nilotica* was further verified by the plant length of more than 5 cm (Evrard and van Hove, 2004*)*, and lack of anthocyanin, which is a characteristic of *A. nilotica* (Lumpkins, 1981). *Azolla nilotica* existed in the deep waters of Lake Jipe (Taveta 2) and TARDA areas. According to Birks (2002), *A. nilotica* has low tolerance to high nitrogen and phosphorus levels. These areas had significantly low N and P levels (Table 1.4) thus being conducive for its growth. The giant rhizomes with extensive multi-septate papillae are also suitable morphological adaptations to deep water environments where they were found.

Genomic DNA amplification of Mwea and Taveta 1 accessions using SCAR markers primer 490 (Table 1.3) (that also amplify *A. filiculoides*) showed distinct resolved bands of 490 bp. This shows that the two accessions have similarity with *Azolla filiculoides* at this locus. None of the four primers could identify with Ahero, West Kano and Bunyala accessions. Thus, there is need to develop SCAR markers which are specific to all the local seven Kenya *Azolla* accessions for further analysis of the Ahero, West Kano and Bunyala accessions.

Morphological and molecular characteristics therefore seem to confirm the existence of *Azolla filiculoides* in Mwea and Taveta, and *Azolla nilotica* in Taveta and TARDA Irrigation Schemes in Kenya. This confirms the invasion of *Azolla filiculoides* in East and Central Africa, and this had previously been reported (Henderson, 2002; Hussner, 2010). Lack of *Azolla nilotica* in Ahero, where it was previously reported, may be attributed to drought, high N and elimination by invasive species, *Azolla filiculoides*. The invasive nature of *Azolla filiculoides* and its ability to out compete native species was previously reported (Carrapiko *et al*., 2000; BioNET, fact sheet, 2011**).**

Limitations of the study and recommendations

Characterization of *Azolla nilotica* in this study was based on morphological features, while molecular characterization relied on four SCAR primers that have been used to identify *A. pinnata, A. rubra, A. microphylla* and *A. filiculoides.* There is need to develop more DNA based markers to test similarity of Kenyan accessions to the ones published by Abraham *et al*. (2013). There is also need to undertake sequencing and molecular phylogeny, which may be a more powerful tool of identification based on the limitations noted here.

CONCLUSION

Azolla filiculoides and *Azolla nilotica* are the two main *Azolla* species characterized in the major Irrigation Schemes in Kenya. Of the two species, *Azolla filiculoides* has infested four of the Kenya irrigation schemes (Mwea, Ahero, Bunyala, Tana River and West Kano, while *Azolla nilotica* exists only in Taveta and TARDA.

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Data availability. I declare that this is my own work and that data used in this work is available on request or demand**.**

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