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Sexual System, Pollen Stainability and Chromosome Number of *Zehneria capillacea* (Shumach) C. Jeffrey

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***Abstract*: Cucurbit germplasm include cucurbit crops and their wild relatives with diverse reproductive systems. *Zehneria capillacea* is an edible wild cucurbit with scarce information on the sex morph and ploidy level. This research was carried out to ascertain the sexual system, pollen fertility and chromosome number of *Z. capillacea* growing in different micro-habitats obtained from four locations in Southern Nigeria. The anthers from plants in their natural habitat were excised at anthesis, the pollen grains dislodged and stained with acetocarmine glycerol jelly while the squash method was used for chromosome preparation from root tips. The result obtained revealed that the breeding system is andromonoecy having staminate and hermaphrodite flowers on the same plant which is an uncommon sex type in cucurbits. The micrographs of pollen stainability provide conclusive evidence that *Z. capillacea* is functionally andromonoecious with the two floral morphs exhibiting equal pollen number and fertility (100%). The pollen grains are aperturate and colporate with three pores and colpi (tricolporate). The study also confirmed that *Z. capillacea* is a diploid (2n = 2x = 22) with a basic chromosome number of 11 (x = 11). Polyploids were not observed in the natural populations across the study locations.**

***Keywords*: Andromonoecious, Diploid, Pollen viability, Tricolporate, *Zehneria capillacea*.**

1. Introduction

Zehneria (Endl.) is a genus of the family Cucurbitaceae, tribe Benincaseae and subtribe Cucumerinae (Jeffrey, 2005). It is a genus of about 35 species distributed from tropical Africa to Australia and the Pacific (Jeffrey, 1990). The family Cucurbitaceae Jussieu is a dicotyledonous family comprising about 800 species in 130 genera (Jeffrey 2005, Jeffrey and De Wilde, 2006). The family is predominantly tropical having 90% of the species in Africa, Madagascar, Central and South America, and Southeast Asia and Malaysia (Jeffrey, 1990). They are among the largest and most diverse plant families including four major economically important crops such as water melon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), melon (*Cucumis melo*) and squashes (*Cucurbita* spp.) and also minor crops and wild species distributed worldwide.

Z. capillacea commonly known as wild cucumber is a slender herb trailing or climbing to 0.3m. The stem is almost glabrous. The leaf blade is triangular, scabrid-punctuate above, smooth beneath except on the nerves, entire or sinuate or sinuate toothed, obtuse and apiculate, 26 -72mm long, 28-93mm broad, petiole 7-9mm long. It is monoecious, with 1-2 male flowers, pedicels filiform, 11-45mm long, receptacle tube 2mm long. The petals are small, white becoming cream when old; Stamens are 3 and shortly inserted. The female flowers are solitary, sometimes co-axillary with male flowers; pedicel filiform, 26-56mm long; ovary fusiform with small basal collars 3-5mm long; 1-2mm across, receptacle tube 2mm long, lobes filiform, 0.5mm long. Petals are 2mm broad. The fruit is on a slender 20-58mm long stalk, globose, red, 6-10mm in diameter. The seeds are elliptic in outline, smooth, bordered with depressed convex disk. The taxon range is west tropical Africa from Sierra Leone to Angola, also in Fernando Po. (Jeffrey, 1990).

Cucurbit genetic resources include cucurbit crops and their wild relatives which are maintained in specified facilities known as genebanks at the local and national level by governments, universities, companies, farmers and others in the private and public sectors either acting alone or networked with other institutions. The documentation and characterization of many collections at national gene banks in developing countries are still inadequate and much of the existing data is not electronically accessible (Weng and Sun, 2012). In Cucurbitaceae, the sexual system is monomorphic and dimorphic. 50% of the species are monoecious and 50% are dioecious with very few species being androdioecious or bisexual (Schaefer and Renner, 2011). Shifts between monoecy and dioecy occur both, within genera e.g., *Bryonia* (Volz and Renner, 2008), *Luffa* (Schaefer and Renner, 2011), *Momordica* (Schaefer and Renner, 2010) and within species e.g. *Ecballium* (Costich, 1995)

Cytogenetic investigations have been conducted on several cucurbits and there is a considerable range in the monoploid (x) chromosome number (Jeffrey, 1990). Most species of the Cucurbitaceae family are naturally diploids and have basic chromosome numbers of 7 (*Cucumis sativus*), 11 (*Citrullus spp.*), 12 (*Benicasa hispida*), 13 (*Luffa spp.*) or 20 (*Cucurbita*

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spp.) and relatively small genome sizes (Arumuganathan and Earle 1991; Tatum *et al*. 2006). Haploid numbers of 11 and 12 are common in most Cucurbitaceae genera (Chung *et al*., 2003). The whole genomes of the major cucurbits such as cucumber (Huang *et al*., 2009), melon (Benjack *et al*., 2010) and watermelon (Xu *et al*., 2009) have been sequenced. The chromosome numbers of minor cucurbits have also been determined. In *Luffa*, all the species were found to be the same (2n = 2x = 26) with basic chromosome number of 13 (x = 13). The species in the genus *Momordica* vary in their chromosome numbers. The monoecious species, *M. charantia* and *M. balsamina* have a diploid chromosome number of 2n = 2x = 22 while the dioecious specie *M. dioica* is 2n = 2x = 28. *Telfairia and Tricosanthes* species also have a diploid number of 2n = 2x = 22 (Sirohi *et al*., 2005).

The objective of this study was to confirm the sexual system, pollen fertility and chromosome number of *Z. capillacea* growing in different micro-habitats obtained from four locations in Southern Nigeria.

2. Materials and Methods

Plant Materials: The *Z. capillacea* plants used in this study were collected from four locations, Choba - ZC-PH001 (Lat. 4°54′06.11″N and Long. 6°55′19.52″E), Aluu - ZC-PH002 (Lat. 4°55′55.13″N and Long. 6°56′21.70″E) in Rivers State, Idi-Ose - ZC-IB003 (Lat. 7°29′59.57″N and Long. 3°54′47.35″E) and Jericho - ZC-IB004 (7.3919° N, 3.8631° E) in Ibadan, Oyo State.

Inflorescence and Pollen characters: Anthers from male and hermaphroditic flowers were collected at anthesis from field grown *Z. capillacea* plants to evaluate pollen count, size and fertility in the different inflorescence types. These were fixed in 1:3 (v/v) glacial acetic acid, 95% ethanol, for 3 h and placed in 70% ethanol until required. The pollen grains were dislodged from the stamen and the nuclei were stained in a drop of acetocarmine glycerol jelly (Marks, 1954), squashed and examined after incubation for 5 - 10 min at room temperature. The length of the polar axis and equatorial diameter of 50 randomly selected fertile pollen grains was measured using a light microscope with a micrometer. Pollen fertility was determined as the ratio of deeply stained and fully rounded pollen in a field of view to the total number pollen in the field of view. The values obtained were expressed as percentage pollen fertility. At least 300 pollen grains (random samples) were examined per inflorescence.

A. Cytological Studies

The roots used for chromosomal analysis were obtained from field grown plants. Fresh roots (10 – 25mm long) were excised between 10 – 12 h, pre-treated with 0.002M aqueous solution of 8-hydroxyquinolin for 24 h, fixed in freshly prepared 1:3 (v/v) glacial acetic acid, 95% ethanol for 24 h at room temperature. Thereafter, the roots were stored in 70% ethanol at 4°C, pending hydrolysis and squashing. Root tips about 1mm from the fixed root apex were excised, hydrolyzed in 18% aqueous HCl for 2 min and squashed in a drop of FLP-orcein (2 g of Orcein dissolved in 10 ml of solvent, containing equal parts of formic acid, lactic acid, propionic acid and water), following the method of Ndukwu and Okoli (1992). The materials were squashed directly by tapping with the blunt end of a biro, to cause the cells to spread out properly. Nail vanish was used to seal the edges of the cover glass to prevent quick drying of the mounts. They were observed under a binocular light microscope using the oil immersion objective and photomicrographs were taken.

3. Result

Inflorescence characters: The observed breeding system is andromonoecy having hermaphroditic and staminate flowers on the same plant in the ratio of 10:1 respectively (Table 1). The few male flowers were solitary on the leaf axils or co-axillary with hermaphroditic flowers. The male pedicel was 3–8 mm long. The petals were small, 5 in number, white when young and became creamy when old. The hermaphroditic flowers could be solitary, co-axillary with each other or co-axillary with male flowers. The anther in both flowers was yellow in color.

Pollen characters: The palynological features showed that pollen grains of *Z. capillacea* are tricolporate with 3 pores and 3 colpi, radially symmetrical and rounded in shape (Plate 1). The pollen viability as indicated by well stained and fully rounded pollen ranged from 96 – 100% in both hermaphroditic and male flowers. No variation was observed in the pollen size for all the grains examined in the two floral morphs.

Cytological characters: Chromosome counts of *Z. capillacea* from root tip squashes in different locations confirmed that this species is diploid with 2n = 2x = 22 (Plate 2). White root tips from field grown plants provided the most promising material for chromosome counting rather than roots generated from seeds.

Table 1

DESCRIPTORS	PLANTS			
	ZC-PH001	ZC-PH002	ZC-IB003	ZC-IB004
Inflorescence characters				
Days to first flowering	43.6 ± 0.38	43.5 ± 0.34	43.7 ± 0.34	43.6 ± 0.31
Earliness of ♂ flowers	Early	Early	Early	Early
Earliness of ♀ flowers	Early	Early	Early	Early
Sex type	♀/♂	♀/♂	♀/♂	♀/♂
Ratio ♀/♂flowers	10:1	10:1	10:1	10:1
No of petals	5	5	5	5
Cytological characters				
Chromosome number	22	22	22	22
Ploidy level	2x	2x	2x	2x

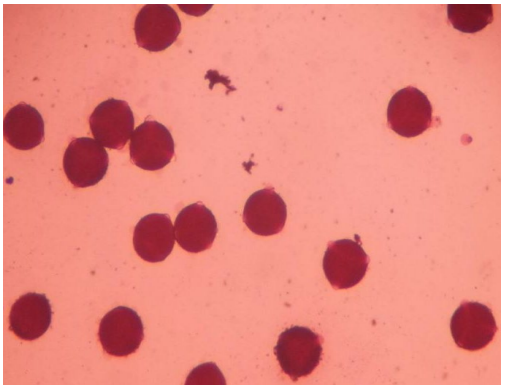

Fig. 1. Plate 1: Stained tricolporate pollen grains of *Z. capillacea* (400x)

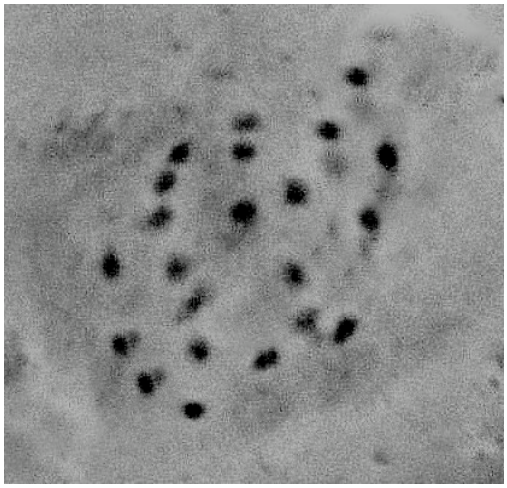

Fig. 2. Plate 2: Mitotic chromosome of *Z. capillacea* (1000x)

4. Discussion

All the pollen grains examined were 100% fertile, spherical in shape with reticulate surface and similar polar and equatorial dimensions (28µm x 29µm). There was no variation observed in the number of colpi and germinal pores. The pollen grains of *Z. capillacea* in both hermaphrodite and male flowers were fully stained with germinal pores indicating that both sexes have fertile grains filled with cytosolic substance. The staining of pollen has been used for determining pollen fertility and stainable pollen which is a consequent of starchy pollen is an indicator of viable pollen (Tome *et al*., 2007; Zaman, 2006). The result provides conclusive evidence that *Z. capillacea* is functionally andromonoecious with the hermaphrodite flowers having both male and female functions. The result agrees with Akimoto *et al*., (1999) who reported functional androdioecy in *Schizopepon bryoniaefolius* (Cucurbitaceae) with a low frequency occurrence of male flowers. In *Decaspermum parviflorum* the pollen grains of apparent hermaphrodites lack cytoplasm and are sterile (Kevan and Lack, 1985) while in *Saurauia veraguensis* (Haber and Bawa, 1984) and *Solanum* species the grains lack apertures (Anderson and Symon, 1989).

There was no difference in pollen morphology between the two sexual morphs. The grains of both morphs were spherical in shape, radially symmetrical, tricolporate, and approximately 29µm in diameter. The pollen morphology observed in this species is in conformity with the results obtained by Van Der Ham and Pruesapan (2006) who reported medium sized pollen (29 – 52 µm) with 3-colporate aperture system in six *Zehneria* species (*Z. bodinieri*, *Z. grayana*, *Z. hookeriana*, *Z. mucronata*, *Z. pisifera* and *Z. repanda*). Pollen grains with 3-colpi were also reported in the genus *Fevillea* (Lima *et. al*., 2010; Van der Ham *et al*., 2010), *Schizopepon bryoniaefolius* (Akimoto *et al*., (1999), *Cucumis sativus* (Diao *et al*., 2010) and in *Cucumis melo* (Fassuliotis and Nelson, 1992) all of the Cucurbitaceae family.

5. Conclusion

Floral morphology revealed that *Z. capillacea* has male and hermaphrodite flowers with 100% fertile pollen as indicated by the pollen stainability tests. The breeding system is therefore andromonoecy having hermaphroditic and male flowers with equal pollen fertility. The results of the pollen stainability provide conclusive evidence that *Z. capillacea* is functionally andromonoecious. The study also confirmed that *Z. capillacea* is a diploid and the chromosome number is 2n = 2x = 22 with no polyploid series in the natural habitats across the study locations.

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