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Infraspecific Variations and Similarities among Onion (*Allium cepa* L.) Varieties Using Floral and Palynological Markers

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Authors' contributions

This work was carried out in collaboration between all authors. Authors DAZ and FBJS designed the study. Authors DAZ, UAY and IM did the field work. Author DAZ performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DAZ and EK managed the analyses of the study. Authors DAZ and AA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Efficient utilization of plant genetic resources for nutrition and crop improvement requires systematic understanding of the important traits. Despite the potentials of onions to enhance food security and economic livelihoods, systematic information about it by farmers in sub-Saharan Africa is scanty. Eight onion varieties namely Ex-Dutsi, Ex-Huguma, Ex-Kudan, Ex-Kura, Ex-Kwadon, Ex-Romi, Wabe and Wuyan Bijimi were cultivated under field experiment using a randomized complete block design with five replications and their pollen grains collected, acetolysed and fixed during flowering to compare their palynological features. Results obtained showed low infraspecific variations in the eight varieties. Two types of pollen aperture (mococolpoate and inaperturate),

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spherical shape with microreticulate sculpture were observed in all the varieties using light microscope. Variety 'Wuyan-bijimi and Ex-Romi recorded the highest percentage occurrence of monocolpoate pollen (92%) but with the lowest percentage occurrence of inaperturate pollen (8%). DMRT of monocolpate density indicated that Waɓe was significantly different ($p \leq 0.05$), it had 56.60±2.52 (mm^2) while the lowest density was recorded by Ex-Dutsi (15.40±2.18 mm^2). Varieties Ex-Dutsi and Ex-Kura not significantly different but otherwise with other varities by the peduncle diameter. Numbers of anther and peduncle length are not good diagnostic characters among the varieties. There was no significant difference recorded in the pollen polar length, equatorial diameter, and exine thickness among the varieties. Multivariate analysis showed three principal components contributing 21.53% of observed variability, while cluster analysis yielded two groups (Ex-Kwadon, Ex-Kura, Ex-Romi and Wuyan-bijimi in cluster 1 while Ex-Dutsi, Ex-Huguma, Ex-Kudan and Waɓe formed cluster 2) at 89.7% similarity coefficient. Varieties in the different clusters obtained can be exploited for desired nutritional traits.

Keywords: Systematics; diversity; flower; pollen; characterisation; Gombe.

1. INTRODUCTION

Onion (*Allium cepa* L.) of the family Alliaceae is an important vegetable crop in most parts of the world, particularly the varieties that are grown for bulbs [1]. It is one of the most important edible vegetable crops used for seasoning not only in Nigeria but all over the world [2]. In terms of global weight of vegetables produced, at nearly 28 million tonnes per annum, only tomatoes and cabbages exceed bulb onions in importance [3].

Rich in natural resources and agro-ecological diversity, Nigeria has a lot of potential in food markets. According to the FAO's estimated data using trading partners database, in 2010 Nigeria exported 60 metric tonnes of dried onions that were worth US$17,000 [4]. In 2012 alone, an estimate of about 240, 000 tons of green onions and 1, 350, 000 tons of dry onions were produced in Nigeria, with the country ranking sixth amongst the top ten producers of green onions in the world. However, Nigeria had its peak production of green onions in 2001, when it turned out 277, 912 tons of the vegetable, the highest output since 1960, when the country had an output of 71, 000 tons. On the other hand, dry onions output peaked in 2008, with about 1, 365, 670 tons, compared to an output of 350, 000 tons produced in 1960. Organosulfur compounds in onions have cardioprotective effects [5]. Onions possess essential antioxidant, anti-inflammatory, and antimicrobial properties [6]. [7] states chemical properties of onions may treat and prevent cardiovascular diseases, cancer. In addition, onion is known for its anti-bacterial, anti-viral, anti-allergenic and anti-inflammatory potential [8,9]. It also contains some important vitamins (A, B and B2) and minerals (Ca, P, Fe, Cu and Zn) in addition to some soluble sugars and nicotinic acid [10].

The nomenclature of onion even at the family level is confusing as many authors referred to onion as belonging to the family Alliaceae [1] Amarylliaceae [3] and Liliaceae [11]. At the species level, [12] divided onions into three different taxa namely: *Allium cepa, Allium aggregattum,* and *Allium prolilum*. A preliminary study conducted in the study area revealed that farmers are technically inefficient with the identification of high yielding varieties. Consequently, farmers tend to cultivate onion varieties that produce green leaves with small or no bulb. [12] established that successful onion production depends mainly on the selection of varieties that are adapted to different conditions imposed by specific environment. In Gombe State, Kano red (Kan ban) and Waɓe is the most popular variety grown by framers, both for home use and source of income. Therefore, the introduction of new varieties represents an important axe to enhance production by increasing the number of cultivars available for growers, which is not only an advantage for them but also for markets and processing industries.

Pollen and floral morphological traits are widely accepted in plant species delimitation [13], variety protection, registration, and inscription and patenting as descriptors that can show both identity and distinctness [1]. Studying pollen morphology is of advantage because it can help taxonomic investigations and pollen diversity in different environments affect plant identification [14]. Scientists such as [15,16]. [17,18] and [19]. [20] studied the pollen morphological structures of some Asparagus and *Allium* species from Turkey. Pollen flora of Pakistan [21]. [22] studied pollen morphology of some *Allium* species belonging to *Allium* and *Codonoprasum* sections. [23] and [24] studied pollen morphology of some species of *Allium* from [25] studied pollen

features for interspecific crossing between *A. cepa* and *A. roylei* hybrids. There are no reports of pollen features of the onion varieties in Nigeria.

The objective of this study was to consider the infraspecific variations and similarities among onion (*A. cepa*) varieties using floral and palynological markers with the view of characterization and delimitation of these taxa.

2. METHODS

2.1 Nursery Managements

Seeds of Ex-Dutsi, Ex-Gombe, Ex-Huguma, Ex-Kudan, Ex-Kura, Ex-Kwadon, Ex-Romi and Wuyan Bijimi at a rate of 1.0 kg ha-1 with 75% germination were broadcasted on 1 x 3 m well prepared seedbed for each variety. To produce healthy seedlings, nursery management practices such as mulching, watering, application of fertilizers, chemicals and weeding were applied as per recommendation for raising seedling of the crop [26]. Seedlings were managed in nursery until transplanted for 6 weeks.

2.2 Planting and Field Management Procedures

Seedlings were transplanted to the experimental plots by carefully uprooting from nursery bed after an hour of irrigation. During transplanting only healthy, vigorous and uniform seedlings were transplanted and gap filling was done after a week after transplanting. The experimental field was selected where rice was grown with uniform application of fertilizers in the previous cropping season. Before transplanting seedlings, the experimental field was hand ploughed and harrowed. Large clods were broken down to bring the soil to fine gradient, and then a total of 120 plots were prepared in which 15 replication plots were allocated for each variety. Major and minor water, ridges and rows were marked on the farm.

Four-day irrigation interval was maintained for the 1st four weeks and then it was extended five to seven days interval until two weeks to harvest, when irrigation was stopped completely [26,27] and [22] recommended onion farm treatments such as application of several types of fertilizers, fungicides, insecticides, herbicides and pesticides were employed [28]. The weeding was done with hand hoe and by hand-pulling whenever necessary throughout the experimental period to keep the crop free from weeds, for better soil aeration and to break the crust.

2.3 Sample Preparations and Characterisation of Pollen Morphology

Pollens were separated from anthers and smeared on microscope slides. The preparations were stained with 1% safranin, immersed in a few drops of ethanol for 5 minutes to remove waxy substance from the pollens and 50% glycerol and observed under a light microscope [29]. Pollen characters were recorded as described by [13].

Qualitative pollen grain characters such as pollen type, shape, presence or absence of colpi, pores, spines, sculpturing and ornamentation were studied. Quantitative characters include pollen polar diameter, equatorial diameter, exine thickness and floral features such as floral peduncle length, anther density per anther, flower head diameter and anther number per flower were investigated. Using 35 fields of view at x40 objective as quadrat, the numbers of pollens were noted to determine the frequency of the different pollen types. Frequency of each pollen type was expressed as percentage occurrence of such pollen types based on all occurrences using this formula: $\text{Frequency} = \frac{P}{Y} X\ 100$; where p = the occurrence of each pollen type in the field of view; y = total occurrences of all pollen types. The density of pollen types was determined as the number of pollen types per square millimetre (mm^2). Specimens were photographed under oil immersion using Am scope microscopic camera (MU1000, FMA050) and Kodak digital camera (Kodak Easy Share C913). Measurements were made using an ocular micrometer. Pollen description and identifications followed those of [30].

2.4 Statistical Analysis

The data were subjected to analysis of variance (ANOVA) and Duncan Multiple Range Test (DRMT) using R version 3.2 procedures and least significant difference (LSD) test was used to separate means at 5% probability level.

3. RESULTS AND DISCUSSION

Table 1 and Figs. 1 – 7 show the results obtained from this study. The results generally showed low infraspecific variations in the eight varieties. Two types of pollen aperture – mococolpate and inaperturate (Fig. 5 and Table 1), spherical shape and microreticulate sculpture were observed in all the varieties (Table 1). Variety 'Wuyan-bijimi and Ex-Romi recorded the highest percentage occurrence of monocolpate pollen (92%) but with the lowest percentage occurrence of inaperturate pollen (8%) (Fig. 5). DMRT of monocolpate density (Table 1) indicated that Wabe was significantly different ($p \leq 0.05$) in that it had 56.60±2.52 (mm^2) while the lowest density was recorded by Ex-Dutsi (15.40±2.18 mm^2). Varieties Ex-Dutsi and Ex-Kura are not significantly different but otherwise with other varieties by the peduncle diameter. Numbers of anther and peduncle length are not good diagnostic characters among the varieties. There was no significant difference recorded in the pollen polar length (11 – 12 μm^2), equatorial diameter (6 – 7 μm^2), and exine thickness (0.9 – 1.2 μm^2) among the varieties (Fig. 6). Multivariate analysis showed three principal components contributing 21.53% of observed variability, while cluster analysis yielded two groups (Wabe, Ex-Kura, Ex-Romi and Wuyan-bijimi in cluster 1 while Ex-Dutsi, Ex-Huguma, Ex-Kudan and Ex-Kwadon formed cluster 2) at 89.7% similarity coefficient (Fig. 7).

Table 1. Pollen types, shapes, surfaces and pollen densities

Variety	Pollen type	Pollen shape	Pollen sculpture	Pollen density (mm^2)
Ex-Dutsi	Monocolpate	Spherical	Microreticulate	15.40±2.18^{d}
	Inaperturate	Spherical	Microreticulate	2.80±0.73^{f}
Ex-Huguma	Monocolpate	Spherical	Microreticulate	22.60±2.93^{bc}
	Inaperturate	Spherical	Microreticulate	3.40±0.87^{f}
Ex-Kudan	Monocolpate	Spherical	Microreticulate	21.80±1.36^{c}
	Inaperturate	Spherical	Microreticulate	2.40±0.51^{f}
Ex-Kura	Monocolpate	Spherical	Microreticulate	19.60±2.25^{cd}
	Inaperturate	Spherical	Microreticulate	2.00±0.32^{f}
Ex-Romi	Monocolpate	Spherical	Microreticulate	22.80±4.85^{bc}
	Inaperturate	Spherical	Microreticulate	1.80±0.37^{g}
Wuyan Bijimi	Monocolpate	Spherical	Microreticulate	19.20±2.67^{a}
	Inaperturate	Spherical	Microreticulate	1.60±0.51^{g}
Ex-Kwadon	Monocolpate	Spherical	Microreticulate	23.80±2.87^{b}
	Inaperturate	Spherical	Microreticulate	3.80±1.20^{f}
Wabe	Monocolpate	Spherical	Microreticulate	56.60±2.52^{a}
	Inaperturate	Spherical	Microreticulate	9.80±0.80^{e}

Legend: Pollen densities with the same superscript letter indicate that they are not significantly different at 5% level of confidence

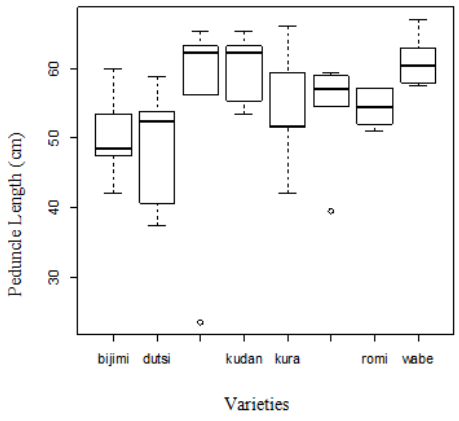

Fig. 1. Peduncle length (cm) among eight varieties of onions

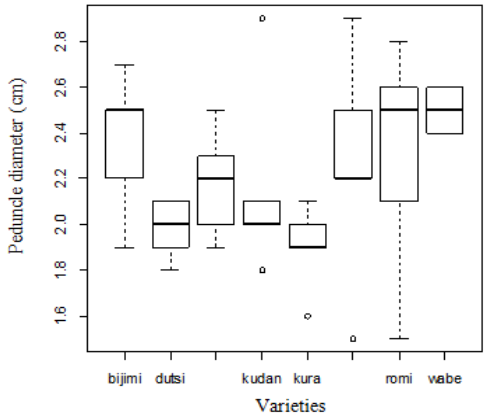

Fig. 2. Peduncle diameter (cm) among the varieties

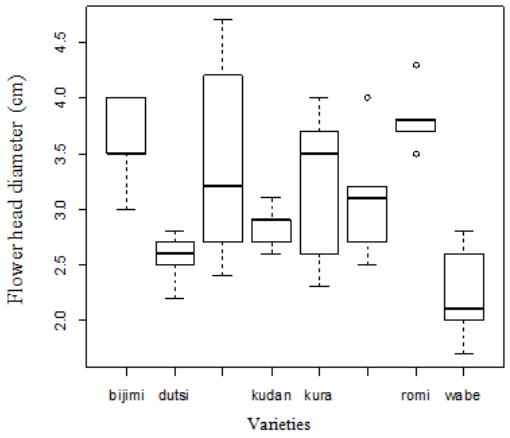

Fig. 3. Flower head diameter among the varieties of onions

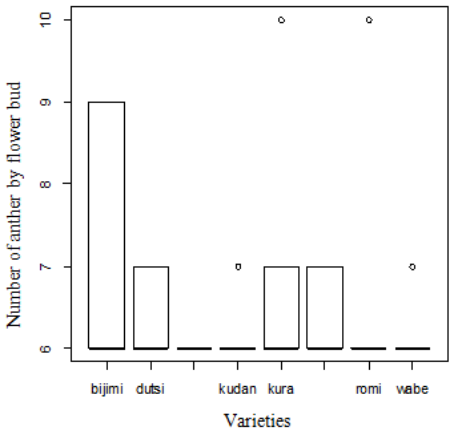

Fig. 4. Number of anthers per flower bud

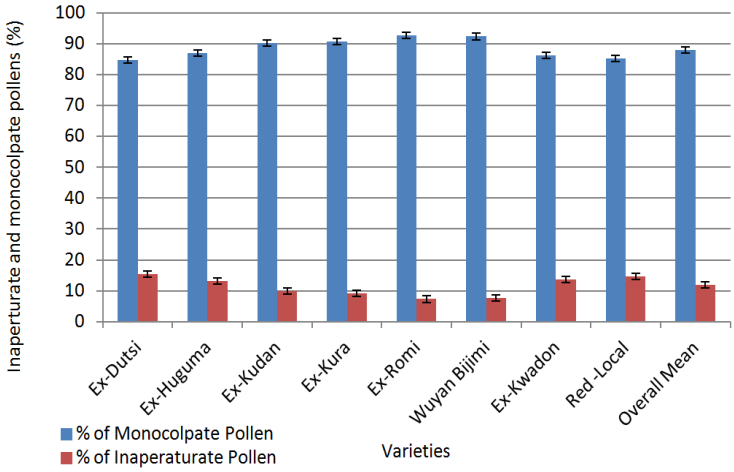

Fig. 5. Low infraspecific variations of percentages occurrence of the two types of pollen apertures in the eight varieties of onions

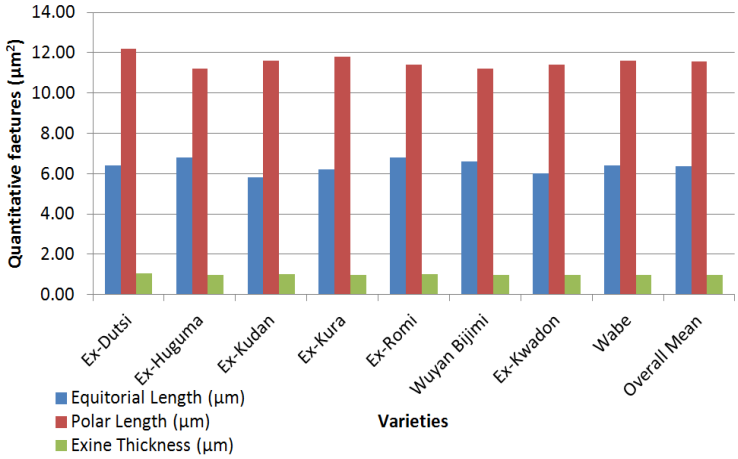

Fig. 6. Quantitative characters of the pollens among the varieties of onions

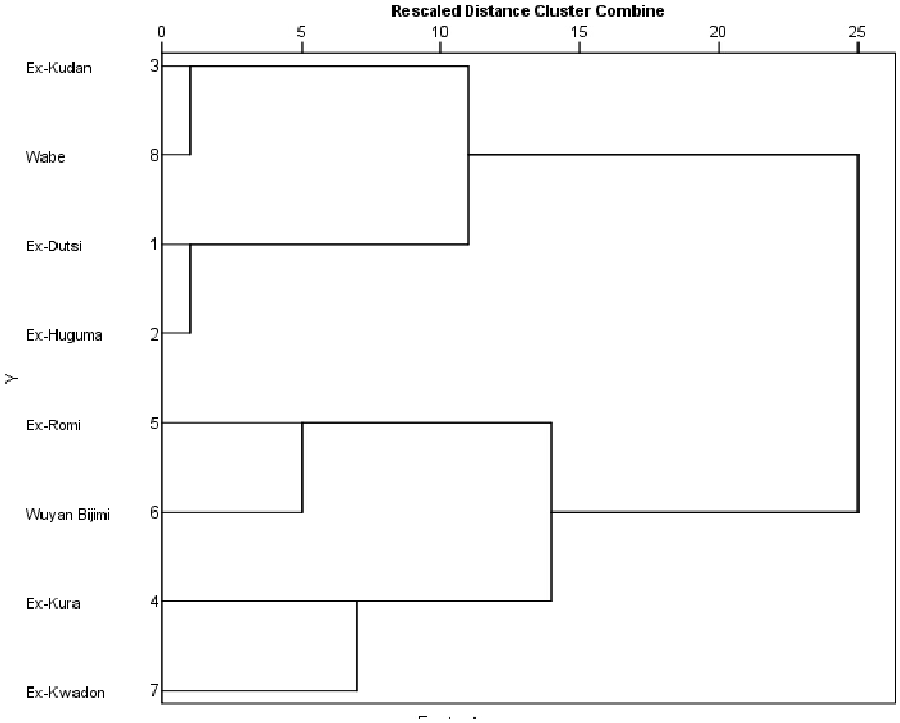

Fig. 7. Dendrogram using average Linkage (Between Groups) clustered the varieties into two distinct taxa

The inflorescence and pollen morphological markers in varieties of onion did not show any diagnostic features for some traits viz., Type of flower (capitulum), flower colour (pale-white) peduncle length (Fig. 1), number of anthers per flower bud (Fig. 4), pollen types, pollen shape, pollen surface and ornamentation (Table 1), polar length, equatorial length, exine thickness (Fig. 5) and pollen ratio. Thus, onion varieties are very difficult to be differentiated based on these traits; an indication that the varieties showed close relationship regarding these characters and these varieties could be said to be stenopalynous group because of these characters. However, varieties evaluated for rest of the traits differed clearly from each other and form very reliable flower and pollen morphological descriptor profile. This has confirmed the results obtained by [14] that size of the polar axis, pollen shapes and sculpture in *Allium ampeloprasum* L. var. *atroviolaceum* did not differ from other species. According to [21], pollen grains in Alliaceae are typically bilateral, heteropolar, Shape mostly elliptic boat shape, monocolpate, exine thicker or thinner than nexine. According to [24], evolutionary pollen surface of 30 species of wild onion showed that onion's pollen is from the single sulcus type. Although [29,28] and [29] stated that monocolpate or sulcate pollen aperture is a typical palynological evidence in most angiosperms, this study revealed an additional type – inaperturate in *Allium cepa*. Based on psilate exine and surface ornamentation (Table 1), subpsilate exine ornamentation was reported by [21] to be found in considerable number of taxa in Alliaceae. Pollen density, frequency of pollen types, length of inflorescence and diameter of flower bud (Table 1 and Figs. 1 – 6) could be of taxonomic significance at intraspecific level. At generic level, [29,30] suggested that there are pollen characters of taxonomic significance in *Allium*. The emergence of inflorescence affects the bulb yield (per. Comm.), according to a season famer, the more the percentage of flowering, the bigger the size of the peduncle and the larger the size of the capitalum on a farm, the lower the bulb yield, and hence poor harvest. Farmers usually sort for seedlings free or at least low occurrence of the floral characters before transplanting. Multivariate analysis showed three principal components contributing 21.53% of observed variability, while cluster analysis yielded two groups (Ex-Kwadon, Ex-Kura, Ex-Romi and Wuyan-bijimi in cluster 1 while Ex-Dutsi, Ex-Huguma, Ex-Kudan and Waɓe formed

cluster 2) at 89.7% similarity coefficient (Fig. 7). Varieties in the different clusters as obtained can be exploited for desired nutritional traits.

4. CONCLUSION

High palynological congruences were observed in the eight varieties of onions studied. Monocolpate and inaperturate pollen apertures, pollen sizes, spherical pollen shape with microreticulate sculpture recorded in this study confirms the typical pollen characters of monocotyledons. Hence, these characters form good diagnostic features of *A. cepa*. Although, these characters may not clearly delimit taxa at infraspecific level, it could be used together with other diagnostic features such as floral morphology which has proven useful in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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