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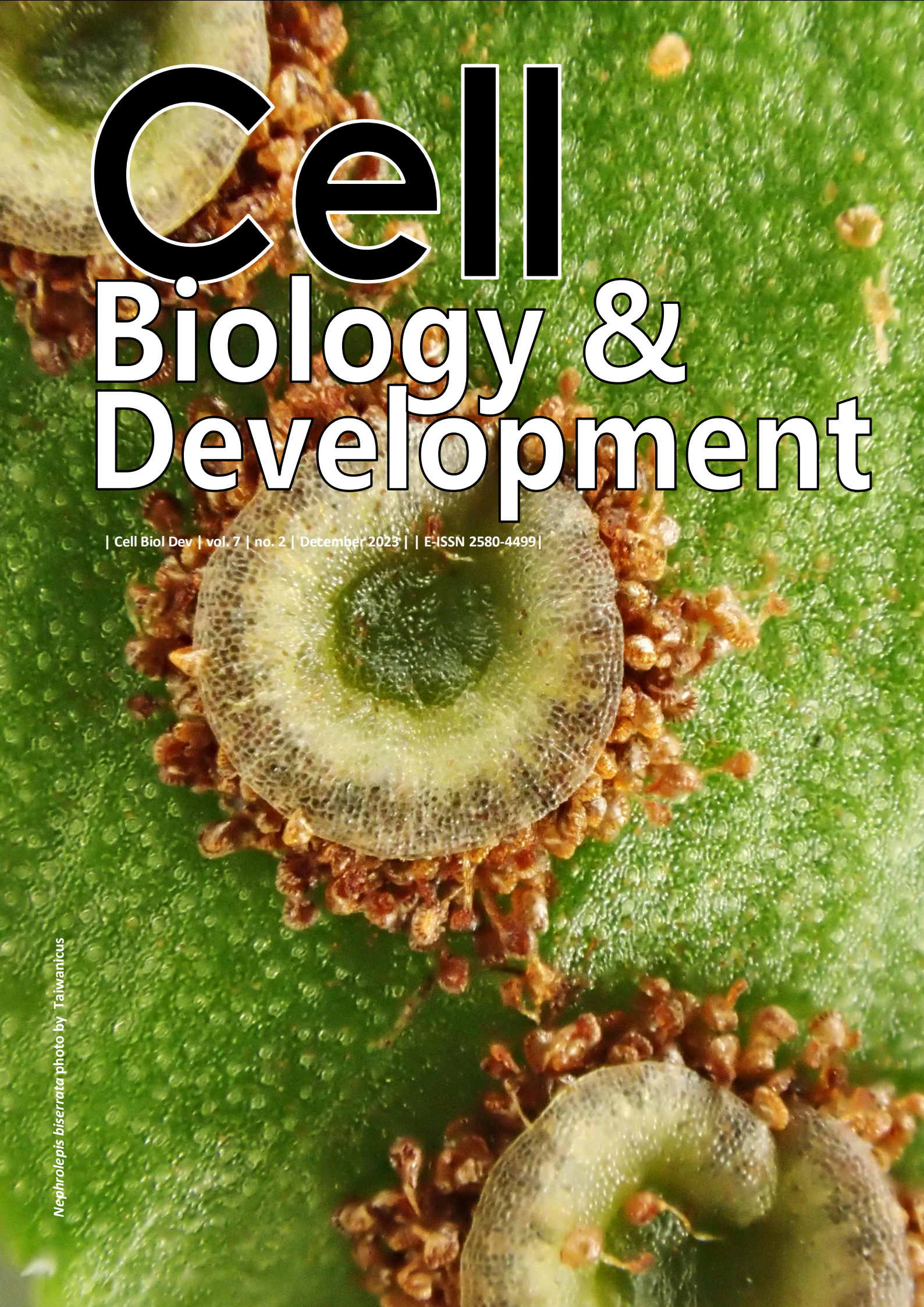

Nephrolepis biserrata photo by Taiwanicus

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The title of the article should be written in compact, clear, and informative sentence, preferably not more than 20 words. Name of author(s) should be completely written, especially for the first and the last name. **Name and institution** address should also be completely written with street name and number (location), postal code, telephone number, facsimile number, and email address. We choose local names in Bahasa Indonesia for universities in Indonesia. The mention of "strata" program, should be avoided. Manuscript written by a group, author for correspondence along with address is required (marked with "♥"). **The title page** (first page) should include title of the article, full name(s), institution(s) and address(es) of the author(s); the corresponding authors detailed postage and e-mail addresses (P), and phone (O) and fax numbers (O).

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Introduction is about 600 words, covering the aims of the research and provide an adequate background, avoiding a detailed literature survey or a summary of the results. **Materials and Methods** should emphasize on the procedures and data analysis. **Results and Discussion** should be written as a series of connecting sentences, however, for a manuscript with long discussion should be divided into subtitles. Thorough discussion represents the causal effect mainly explains why and how the results of the research were taken place, and do not only re-express the mentioned results in the form of sentences. **Concluding** sentence should be given at the end of the discussion. **Acknowledgements** are expressed in a brief; all sources of institutional, private and corporate financial support for the work must be fully acknowledged, and any potential conflicts of interest are noted.

Figures and Tables of a maximum of three pages should be clearly presented. The title of a picture is written down below the picture, while the title of a table is written above the table. Colored figures can only be accepted if the information in the manuscript can lose without those images; the chart is preferred to use black and white images. The author could consign any picture or photo for the front cover, although it does not print in the manuscript. All images property of others should be mentioned the source. Author is suggested referring to Wikipedia for international boundaries and Google Earth for satellite imagery. If not specifically mentioned, it is assumed to refer to these sources. **There is no appendix**, all data or data analysis is incorporated into Results and Discussions. For broad data, it can be displayed on the website as a supplement.

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Journal:

Saharjo BH, Nurhayati AD. 2006. Domination and composition structure change at hemic peat natural regeneration following burning; a case study in Pelalawan, Riau Province. Biodiversitas 7: 154-158. DOI: 10.13057/biodiv/d070213.

The usage of "et al." in long author lists will also be accepted:

Smith J. Jones M Jr. Houghton L et al. 1999. Future of health insurance. N Engl J Med 965: 325-329. DOI: 10.10007/s002149800025.

Book:

Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in the book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds.). Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.). Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from the internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. DOI: 10.1038/msb.2008.24. www.molecularsystembiology.com.

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Short Communication: Comparative foliar epidermal study of some species of pteridophytes in Rivers State University, Nigeria

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Abstract. *Ibiye AI, Green BO, Ajuru MG. 2023. Comparative foliar epidermal study of some species of pteridophytes in Rivers State University, Nigeria. Cell Biol Dev 7: 51-55.* Pteridophytes show many forms and are cosmopolitan in distribution, from sea level to high mountains. They are about 13,500 species of fern and allies distributed throughout the world. Some are edible, while some are ornamental. They are economically important to humans in food, medicine, ornaments, fibers, and cultural usages. Ferns generally are an understudied group of plants, and no work on the foliar epidermal study of the three species in the South-South part of Nigeria has been done. The study aimed to investigating the foliar anatomy of three species of pteridophytes at Rivers State University, Nigeria, to elucidate their taxonomic knowledge using this line of evidence. The standard method for foliar epidermal analysis was employed. Fresh mature leaves were soaked in sodium hypochlorite (5%) for 3-5 minutes to soften the tissues, and the adaxial surface was scraped off with a sharp razor blade until the abaxial surface was reached; equally, the abaxial surface was scraped off to reveal the adaxial surface. The species include: *Nephrolepis biserrata* (Sw.) Schott, *Phymatosorus scolopendria* (Burm.F.) Pic. Serm., and *Microgramma mauritiana* (Wild.) Tardieu. Results of the foliar epidermal anatomical study revealed that the presence of uniseriate non-glandular trichomes on both the abaxial and adaxial surfaces of *N. biserrata* and absent in *P. scolopendria* and *M. mauritiana* is diagnostic of this species. The presence of diacytic stomata in addition to anomocytic type in the abaxial and adaxial surfaces of *N. biserrata* also separates it from *P. scolopendria* which had amphidiacytic stomata on the abaxial surface and *M. mauritiana* lacked stomata on this surface. All three species possessed numerous crystal sand on both surfaces, which is also diagnostic. These characters have been reported to be diagnostic and considered highly significant for solving taxonomic disputes through identification and delimiting among the species, thus broadening the scope of their taxonomic knowledge.

Keywords: Foliar anatomy, *Microgramma mauritiana, Nephrolepis biserrata, Phymatosorus scolopendria,* pteridophytes

INTRODUCTION

Pteridophytes have a great range of forms and are cosmopolitan in their distribution. Altogether, there are about 13,500 species of pteridophytes found throughout the world (Moran 2006). Some are edible, and some are used for ornamental purposes. Economically, they are important to humans in food, medicine, fiber, and cultural utilization (Camus et al. 1991). There is a high intake of *Nephrolepis biserrata* (Sw.) Schott by domestic animals in the tropics. Babayemi et al. (2006) reported that *N. biserrata* can be used as animal feed due to its high nutritional value.

The *N. biserrata* is used for ornamental purposes (Oloyede et al. 2011). Also, the rhizomes are used for treating sores, boils, abscesses and blisters of the skin in Sarawak but in India, it is used for treating respiratory diseases (Christensen (1997). Due to its high nutritive content, It is used as fodder for feeding the African dwarf goats, sheep, and other small ruminants in Nigeria (Nwosu 2002; Babayemi et al. 2006; Oloyede et al. 2008; 2013). In Sabah and Malaysia, the locals use the tip of the young shoots of *N. biserrata* as a vegetable (Kulip et al. 2010). In traditional medicine, *N. biserrata* is recommended for the treatment of various diseases, such as for prevention of miscarriage, fetus development, and different microbial infections, including boils, blisters, sore, and abscesses; it is employed in the treatment of wounds, bleeding, and stomach ache (Piggott 1996: Jiofack et al. 2008; Malan and Neuba 2011).

It is also prescribed as a ground cover on wooded edges and borders and is available in local nurseries (Florida Native Plant Society 2020). In Java and New Guinea, very young, soft, curled-up fronds of *N. biserrata* are eaten either cooked or steamed as a vegetable. The rhizomes are sometimes dried, pounded, prepared, and eaten like *sago* (Darnaedi and Praptosuwiryo 2003). In Micronesia, the fronds are used as cockroach repellent (David 1987). In traditional medicine, *N. biserrata* treats dropsy, swellings, diarrhea, venereal diseases, dysentery, gout, and edema, even as a pain-killer (Burkill 1985).

Fronds of *Phymatosorus scolopendria* (Burm.F.) Pic. Serm. are used in Indo-China to treat boils and filariasis, chronic diarrhea, while the whole fronds are kept on beds to ward off bed bugs (Mannan et al. 2008). In Polynesia, the fronds are grounded and mixed with *Atuna racemosa* Raf. scraps to make perfume. Sometimes, the mashed fronds are wrapped with *Morinda citradolia* L., then cooked and used as a medical bandage for medicinal

treatment. Juice from leaves is used in Fiji to treat stomach pain, boils, and swollen breasts (Snogan et al. 2007). When crushed, the fern issues a scent similar to maile; sometimes, pieces of the fern are interlaced in leis made of the pandanus fruit (Ukui and Elbert 1986).

Whole plants of *Microgramma mauritiana* (Wild.) Tardieu are used for the treatment of pubic lice in humans and the prevention of the lice from being transferred from one person to another. The transfer of these lice is believed to be inflicted by witchcraft (Hutchings 1996; Roux 2003).

The foliar epidermal features of the epidermis have played an important role in taxonomy and for a plant systematist. The epidermis possesses several important diagnostic characteristics that offer valuable clues for taxonomic identification, such as size, shape, distribution of stomata (Oznur and Tugha 2006; Ajuru and Okoli 2012; Bassey et al. 2016), guard and subsidiary cells, as well as presence or absence of trichome and their different types and lengths (Metcalfe and Chalk 1979). The presence or absence of foliar appendages is significant in the delimitation of taxa, especially at the generic and specific levels.

Anatomical characters are highly valued in taxonomy, as supported by several researchers (Metcalf and Chalk 1979; Shaheen et al. 2009; Oloyede et al. 2011). The anatomical features are popularly used for identifying and classifying plant species and indicating relationship patterns and phylogeny (Essiet and Iwok 2014). Ajuru and Okoli (2012) used anatomical characters as one of the parameters in delimiting the different types of melons in the family Cucurbitaceae.

Generally, ferns have been classified into a different taxonomic hierarchy, though there is very poor or little information about the relationships or differences between several ferns in their taxonomic classification. Also, most species of ferns are difficult to identify easily using only the traditional morphological characteristics, which often leads to wrong identification and classification. The ripple effect of these actions could lead to wrong results and under-usage of these plants. Also, it can lead to adulteration in using these plants for medicinal purposes since they are highly sought after for medicinal and pharmaceutical purposes. This study is therefore carried out to provide more features for delimiting these species using foliar epidermal characteristics.

MATERIALS AND METHODS

Study area

The study area is the Rivers State University (RSU) campus within the Port Harcourt metropolis, Port Harcourt Local Government area in Rivers State, Nigeria. Port Harcourt is an industrialized cosmopolitan city located in the heart of the Niger Delta. The study area, RSU, lies South-South of the Niger Delta within Latitudes 4º31' - 4º 40'N and Longitudes 70º0'-7º10' E. It has an elevation of about 10-15 m above sea level.

Plant collection and identification

The plant materials used for this study were collected from Rivers State University in polythene bags and taken to the Biosystematics laboratory for proper identification and authentication; Prof. B.O. Green, a Plant Taxonomist, identified them. The plants were dried, pressed, and properly mounted to be deposited in the University Herbarium. Voucher specimens were deposited in the Rivers State University Herbarium with the following Herbarium number assigned to them: RSUPb0102, RSUPb0103, and RSUPb0104 for *N. biserrata*, *P. scolopendria*, and *M. mauritiana*, respectively.

Foliar epidermal study

Fresh matured leaves were prepared according to the simplified method described by Okoli (1992). The fresh leaves were soaked in sodium hypochlorite (5%) for 3-5 minutes to soften the tissues and make them easy to scrap. The leaves were placed on a flat surface (tile), and the adaxial surface was scraped off gently with the razor blade until the abaxial surface was reached. Equally, the abaxial surface was scraped off to reveal the adaxial surface.

The transparent epidermal peels were soaked in distilled water to rehydrate the cells, after which they were stained with 1% safranin for 3 minutes and later rinsed again in distilled water. The specimens were mounted with 3 drops of glycerin and a cover slip placed correctly. Slides of both abaxial and adaxial surfaces were prepared. These were examined using a light microscope, and photographs were taken with a micrograph unit.

The qualitative characteristics of the foliar epidermis were observed and taken with a light microscope. Measurements of epidermal cells, trichomes, and stomatal lengths and widths were taken. All measurements were taken at x10 objective. Stomata Index (SI) was calculated using the formula:

$$SI = \frac{S}{E+S} \times 100$$

Where: S = Number of stomata per view and E = Number of epidermal cells per view (Metcalf and Chalk 1979). Basic terminologies used in the description of stomata were those of Metcalf and Chalk (1979).

RESULTS AND DISCUSSION

The foliar epidermal characteristics of the three ferns studied are presented in Figure 1 and Table 1.

Abaxial surfaces

Nephrolepis biserrata

Epidermal cells were irregularly shaped with thick and wavy anticlinal walls, epidermal cells were 63-66 per field view. The stomatal type was diacytic and anomocytic, with elliptical to oblong guard cells filled with chloroplasts. Stomata were about 18 per field view. Trichome types were non-glandular, multicellular, uniseriate trichomes present, and numerous.

Phymatosorus scolopendria

Epidermal cells are irregularly shaped with thick wavy to sinuous anticlinal walls. Epidermal cells were 205-210 number per field view. Crystal sand was present. The stomatal type was aphidiacytic with elliptic to oblong guard cells filled with chloroplasts. The stomata were 84-87 per field view. Trichomes were not present.

Microgramma mauritiana

Epidermal cells were irregularly shaped with thick wavy to sinuous anticlinal walls. Epidermal cells were 183-185 in number per field view. There was numerous crystal sand. The stomatal type was anomocytic and diacytic with elliptical to oblong guard cells filled with chloroplasts; the stomata were 23-25 per field view. There were no trichomes.

Adaxial surfaces

Nephrolepis biserrata

Epidermal cells were irregularly shaped with thick, wavy, sinuous anticlinal walls. Epidermal cells per field view were 47-53. The stomatal type was anomocytic and diacytic, bigger but fewer than those in the abaxial surface. No stomata per field view = 38. Trichomes, non-glandular, multicellular, uniseriate, and numerous guard cells were filled with chloroplasts. There was crystal sand in the foliar epidermal layer.

Phymatosorus scolopendria

Epidermal cells were irregularly shaped with less wavy anticlinal walls. Epidermal cells per field view = 146-153. The stomatal type was anomocytic and diacytic, with oblong to elliptic-shaped guard cells filled with chloroplasts. Stomata were 88-90 per field view. There were no trichomes. There were numerous crystals in the form of crystal sand.

Microgramma mauritiana

Epidermal cells were highly irregularly shaped with thick, wavy, and highly sinuous anticlinal walls. Epidermal cells were 188-193 in number per field view. There was numerous crystal sand. There were no stomata; also, trichomes were not found.

Discussion

Epidermal anatomical characters have been regarded as important in the classification of vascular plants, and these characteristics are known to provide additional features, which along with other characters, are usually of taxonomic value in the classification and identification of plants.

This study provides a comprehensive micro-morphology of the three ferns studied. The epidermal cell shape of the three species was irregularly shaped with thick wavy anticlinal walls, as reported by Oloyede et al. (2011). The stomatal type patterns of the studied species represented useful diagnostic characteristics, as Oloyede et al. (2011) reported. Variations in types, arrangements, and distribution of stomata are characters that are taxonomically important at the generic level of classification, as reported by Oloyede et al. (2011).

Table 1. Foliar epidermal characters of three fern species studied

Abaxial surface	***Nephrolepis biserrata***	***Phymatosorus scolopendria***	***Microgramma mauritiana***
Epidermal	63-66 per field view	205-210 per field view	188-193 per field view
Cell shape	Irregularly shaped	Irregularly shaped	Irregularly shaped
Epidermal cell wall	Thick and wavy anticlinal wall	Thick, wavy, and highly sinuous anticlinal walls.	Thick, wavy, and highly sinuous anticlinal walls
Stomatal type	Diacytic and anomocytic	Amphidiacytic	Stomata absent
Stomatal distance	18 per field view	84-87 per field view	Stomata absent
Stomatal index	22	29	Stomata absent
Shape of guard cells	Elliptical to oblong	Elliptic to oblong	Guard cells absent
Trichome	Non-glandular	Trichomes absent	Trichomes absent
Trichome type	Uniseriate trichomes	Trichomes absent	Trichomes absent
Adaxial	*N. biserrata*	*P. scolopendria*	*M. mauritiana*
Epidermal	47-53 per field view	146-153 per field view	183-185 per field view
Cell shape	Irregularly shaped	Irregularly shaped	Irregularly shaped
Epidermal Cell wall	Thick and wavy to sinuous anticlinal walls	Less wavy anticlinal walls	Thick wavy to sinuous anticlinal walls
Stomatal type	Anomocytic and diacytic and bigger size than the abaxial	Anomocytic and diacytic	Anomocytic and diacytic
Stomatal distance	38 per field view	88-90 per field view	23-25 per field view
Stomatal index	43	37	12
Shape of guard cells	Elliptical to oblong	Oblong to elliptical	Elliptical to oblong
Trichome	Non-glandular	No trichomes	No trichomes
Trichome type	Uniseriate trichomes	Not present	Not present
Crystals	Crystal sands found in foliar epidermal layer	Presence of numerous crystal sands in form of crystal sand	Presence of numerous crystal sand

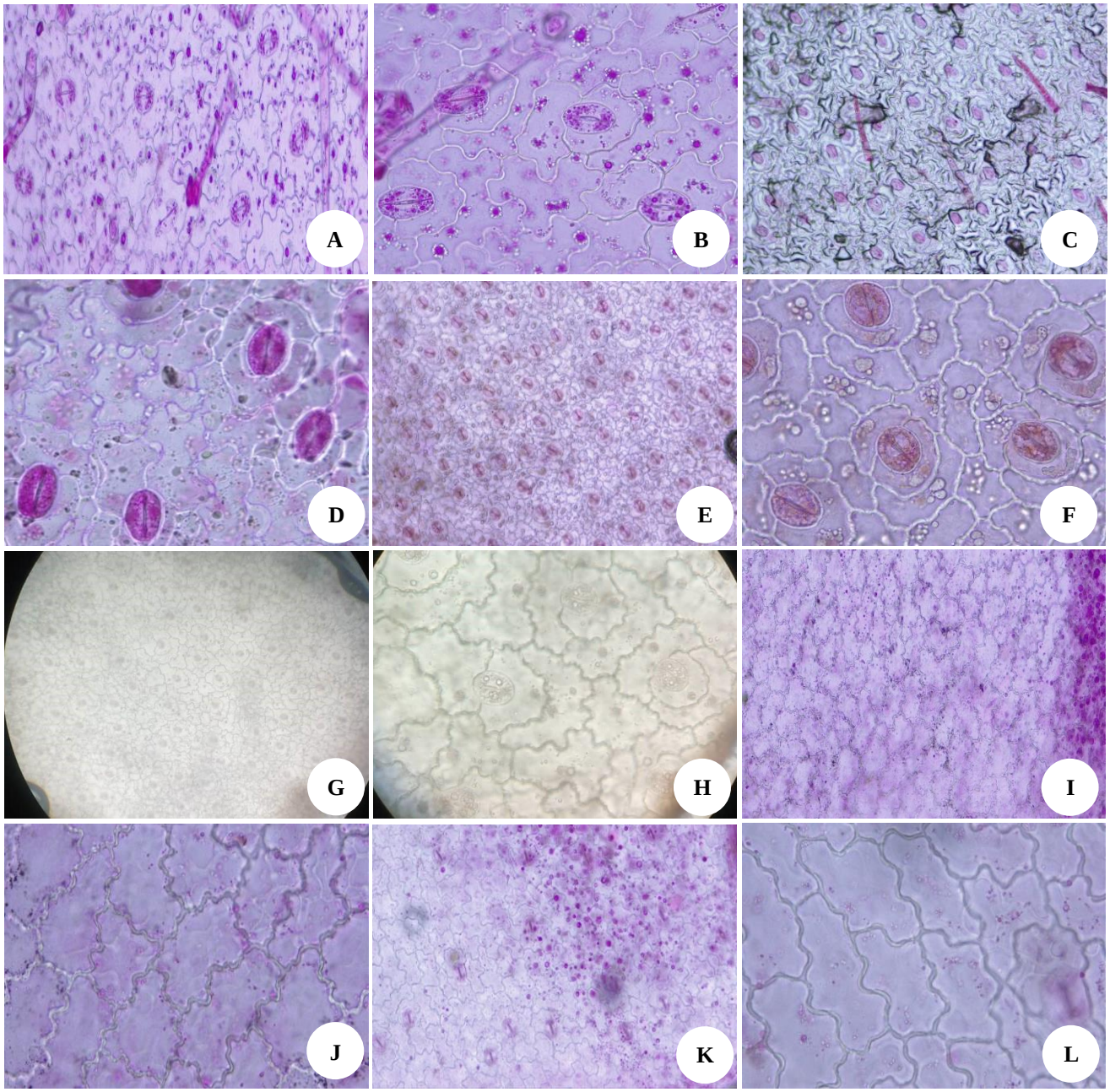

Figure 1. A-L. Epidermal characters of the ferns studied. A. Abaxial of *Nephrolepis biserrata* x100, B. Abaxial of *N. biserrata* x400, C. Adaxial of *N. biserrata* x100, D. Adaxial of *N. biserrata* x400, E. Adaxial of *Phymatosorus scolopendria* x100, F. Adaxial of *P. scolopendria* x400 G. Abaxial of *P. scolopendria* x100, H. Abaxial of *P. scolopendria* x400, I. Adaxial of *Microgramma mauritiana* x100, J. Adaxial of *M. mauritiana* x400, K. Abaxial of *M. mauritiana* x100, L. Abaxial of *M. mauritiana* x400

Stomata are microscopic openings on plant leaves surfaces that allow for easy passage of water vapor, carbon dioxide, and oxygen. Different stomatal types were observed and studied. These are anomocytic, Diacytic, and amphidiacytic. The stomatal type on the abaxial surface of *N. biserrata* was diacytic and anomocytic, but Oloyede et al. (2011) reported diacytic and anisocytic stomata in this same plant in Osun State, Nigeria; the variation in this report maybe as a result of environmental condition. The Stomatal type in *P. scolopendria* was amphidiacytic, while *M. mauritiana* had no stomata on the abaxial surface. On the adaxial surface, the three fern species all had anomocytic and diacytic stomata, which can be used to delimit the species for taxonomic purposes.

The stomatal index varied from one species to another. The stomatal index for the abaxial surfaces of *P. scolopendria* was twenty-nine (29), followed by *N. biserrata* twenty-two (22), while *M. mauritiana* had no stomatal index. Adedeji and Jewoola (2008) reported that the stomatal index is constant for any given, and the value is more uniform on the abaxial surface than the abaxial surface except in an isobilateral leaf. The adaxial stomatal

index for *N. biserrata* was forty-three (43) as the highest, *P. scolopendria* thirty-seven (37), and *M. mauritiana* twelve (12) as the lowest (Table 1). This result conforms to the findings by Essiet and Iwok (2014) that the stomatal index is independent of the environment or size of the leaf surface and, thus, serves as a reliable tool for identification.

Trichomes were found both on the abaxial and adaxial surfaces of *N. biserrata*, and it conforms to the findings of Oloyede et al. (2011) that the trichomes of *N. biserrata* are multicellular non-glandular, uniseriate, and numerous while absent on the abaxial and adaxial of *P. scolopendria* and *M. mauritiana.* This is due to the nature of the leaf surface of the fern species. Trichome types in plants are very useful for delimitating and identifying plants, even in the present study. Trichomes function in the reduction of the rate of transpiration in plants they occur. They are also used for protection against insect infestation.

In conclusion, the three species studied show close interrelationships in their foliar anatomical features, which can be used to identify, delimit and classify them. The foliar epidermal characters of significance in the delimitation of the studied species include type, shape, and number of stomata, epidermal cell shape, number, presence and absence of trichomes and trichome type on both abaxial and adaxial surfaces as well as the epidermal cell wall pattern. It is, therefore, indicated that the foliar anatomical similarities displayed among these three species are why they are grouped into the same family, while their differences are also why they are separated into different genera. Further evaluation using other taxonomic markers, such as phylogenetic and molecular properties, is recommended to provide more diagnostic characteristics.

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Effects of cutting source and IBA concentration on shooting and rooting ability of *Pouteria adolfi-friederici* stem cuttings at polypropagator

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Abstract. *Bahru T, Derero A. 2023. Effects of cutting source and IBA concentration on shooting and rooting ability of* Pouteria adolfi-friederici *stem cuttings at polypropagator. Cell Biol Dev 7: 56-66. Pouteria adolfi-friederici* (Engl.) Baehni is an indigenous tree species extensively exploited for high-quality timber. Limited availability of seeds, intensive seed predation and recalcitrant seeds are bottlenecks for growing the tree species. Therefore, the present study aimed to evaluate the effect of cutting source and Indole-3-Butyric Acid (IBA) concentration on vegetative propagation of the species at a non-mist polypropagator. A total of 160 stem cuttings comprising four treatments (seedling cuttings + 0.2% IBA dose, seedling cuttings + 0.4% IBA dose, branch cuttings + 0.2% IBA dose and branch cuttings + 0.4% IBA dose) established using a completely randomized design for 180 days. The survival rate, rooting response, number of leaves/or buds, root number, and root length per rooted cuttings of seedling cuttings treated with 0.2% were significantly ($p<0.01$) the highest. Consequently, 70% of shooting and rooting success and higher mean root length (1.97+1.92 cm) were induced by seedling cuttings treated with 0.2% IBA concentration. In conclusion, seedling cuttings treated with 0.2% IBA concentration are more promising for vegetative propagation at a non-mist polypropagator. Hence, establishing mother stocks in nurseries and their proper management to serve as sources of juvenile stems will be essential for the successful macro-propagation of the species besides growing the species with seeds.

Keywords: IBA, *Kerero*, polypropagator, propagation, rooting

INTRODUCTION

The *Pouteria adolfi-friederici* (Engl.) Baehni is an indigenous multipurpose tree species belonging to the family Sapotaceae (Friis 2003). It is a very tall tree with a height of up to 45-50 m tall (Friis 2003) with an apparent straight bole nearly 16 m long (Maundu and Tengnäs 2005; Bekele 2007). The tree has a breast height diameter of up to 4 m (Fichtl and Adi 1994). According to Friis et al. (2011), it is one of the predominant characteristic species of Moist Evergreen Afromontane-Forest (MAF) in the vegetation types of Ethiopia. Therefore, the species is widely distributed in Shewa, Arsi, Welega, Illubabor, Keffa, Gamo Gofa, Sidamo and Bale floristic regions, Ethiopia (Friis 2003; Bekele 2007) within an altitudinal range of 1,350-2,450 m above sea level (Friis 2003). However, *P. adolfi-friederici* is extensively overexploited for its high-quality timber production in Ethiopia. Of course, the species is listed as Least Concern by the IUCN Red List of Threatened Species (IUCN 2020).

On the other hand, the limited availability of seeds and seedlings is a major practical problem for this species propagation. In addition, collecting, packing, and loading processed seeds or fruits, transportation, handling, and processing are additional practical problems to obtain fresh and mature seeds for further propagation and genetic conservation through seed storage. This is because the temperature and the moisture content affect the seed viability and germination potential. Consequently, fallen seeds or fruits are collected under mother trees, which are not fresh, well-matured, or easily attacked by insects or infected by disease. Fallen seeds or fruits, in turn, are eaten by seed predators (e.g., birds, rodents, smaller animals), decayed or rapidly germinated due to higher moisture availability. Seedlings and saplings are further easily free-grazed by herbivores, and there is little probability of being a mature tree. At the same time, there is a shift of highly suitable habitats for *P. adolfi-friederici* from the northern and central parts to the southern parts of Ethiopia (Tadesse et al. 2022).

Thus, to address the practical problems faced by *P. adolfi-friederici* species, vegetative propagation using stem cuttings might be a promising option to be investigated to reverse the current trend of species depletion and subsequent extinction. This is because vegetative propagation is an effective solution to a wide range of tropical tree species for mass propagation and plantation establishment within a tree improvement program (Negash 2010). This can be achieved within a short rotation period, especially for slow-growing plant species, for large-scale plantation expansion and harvesting of the intended end products. At the same time, due to its great economic contribution and the possibility of growing the species on-farm, it has been suggested as a potential candidate species for tree domestication under the agroforestry system or around homesteads. Similarly, an intraspecific morphological variation was identified using three main morphometric parameters (stem height, BDH and bole

length) among the five populations in different natural forests of southwest Ethiopia (Seid and Mengesha, 2022). Such future research direction further helps conserve the species, establish seed orchards, improve its genetic and breeding base, improve productivity (fast growth and high timber quality and yield) and plantation expansion and development. In this respect, successful vegetative propagation using a low-technology non-mist polypropagator is one of the recent advances in the development of plant propagation for a wide range of plant species using leafy stem cuttings.

Nevertheless, various key factors contributed to the method's inappropriate application and limited practicality. Various sources reported that the shooting and rooting response of stem cuttings are influenced by sources of stem cuttings and the application of plant growth hormones for several plant species (Hartmann and Kester 1983; Negash 2010), particularly *P. adolfi-friederici* species (Derero et al. 2019). Of course, an earlier study conducted by Derero et al. (2019) confirmed that an encouraging result was obtained for the application of Indole-3-Butyric Acid (IBA) hormone on cutting sources (seedling and branch cuttings) in promoting shoot and root induction compared to the control treatment (without the application of IBA hormone). However, far earlier studies on various tree species reported that stem cuttings responded differently to shooting or rooting response or root number or root length due to different concentrations of IBA hormone (Leakey et al. 1982; Tchoundjeu and Leakey 2000, 2001; Tchoundjeu et al. 2002; Negash 2003; Tiwari and Das 2010; Asl et al. 2012; Kebede et al. 2013; Sevik and Guney 2013; de Souza et al. 2014; Elhaak et al. 2015; Junior et al. 2017; Phuyal et al. 2018; Pigatto et al. 2018; Tilahun et al. 2019; Vallejos-Torres et al. 2020; Olaniyi et al. 2021; Vallejos-Torres et al. 2021a, b; Khandaker et al. 2022; Zamora et al. 2022). Despite this, stem cuttings treated with different concentrations (doses) of IBA hormone have not yet been investigated. Further research is needed to select and apply the optimum IBA dose to improve the shooting and rooting success of *P. adolfi-friederici* stem cuttings. With this understanding, the present study aimed at investigating suitable cutting sources with the application of optimum IBA dose for the successful shooting and rooting ability of *P. adolfi-friederici* leafy stem cuttings at non-mist polypropagator.

MATERIALS AND METHODS

Description of the study site

This experimental study was conducted at Central Ethiopia Forestry Development Center (CEFDC), Ethiopian Forestry Development (EFD), Addis Ababa, Ethiopia. It is situated at Gurd Shola, Bole Sub-District. The experimental site (Nursery) is located in the Highland (*Dega*) Agro-ecology at 2,368 masl, between 37°04'E Longitude and 09°96'N Latitude. Addis Ababa has a mean annual rainfall of 1,000 mm and a mean monthly temperature of 20°C.

Description of the overall research

This research was conducted at a non-mist polypropagator in the nursery site with two factorial experiments. These were sources of stem cuttings and application of different hormone concentrations, each factor with two levels or treatments. Leafy young stem cuttings (hereafter referred to as stem cuttings) were derived from about a 1-year-old raised seedlings (hereafter referred to as seedling cuttings) and young branches of matured mother trees (hereafter referred to as branch cuttings). On the other hand, Indole-3-Butyric Acid (IBA) hormone with two concentrations (doses), i.e., 0.2 and 0.4%, was applied for better initiation of shoots and roots on stem cuttings. This hormone is selected since IBA is one of the most effective and widely used auxins, with low toxicity, mobility, and high chemical stability (Hartmann and Kester 1983). At the same time, between 0.2 and 0.4% IBA doses were considered since far more previous studies (Hartmann and Kester 1983; Negash 2010; Derero et al. 2019; Zamora et al. 2022) found out that these doses had successful results in vegetative propagation of a wide range of plant species. Thus, these doses were selected and applied to be more efficient in time, energy, and resources. After that, prepared stem cuttings were established at a non-mist polypropagator, and the research was conducted for 180 days. Finally, survived stem cuttings with developed buds, leaves and roots were transplanted to polyethylene pots at the nursery, and the survival count was supervised further for 120 days.

Polypropagator design and construction

A low-technology non-mist polypropagator used for the vegetative propagation of *P. adolfi-friederici* stem cuttings in this study was constructed from wooden frames (see Figure 1.D), following the construction design of Leakey et al. (1990), which was applied for tropical trees. An earlier study on *P. adolfi-friederici* stem cuttings conducted by Derero et al. (2019) also applied the same propagator construction design. Likewise, vegetative propagation on *Khaya ivorensis* A.Chev. (Tchoundjeu and Leakey 2000), *Lovoa trichilioides* Harms (Tchoundjeu and Leakey 2001), *Prunus africana* (Hook.fil.) Kalkman (Tchoundjeu et al. 2002), *Podocarpus falcatus* A.Cunn. ex Parl., 1868 (Negash 2003), *Juniperus procera* Hochst. ex Endl., *P. falcatus* and *Olea europaea* L. (Negash 2010), *P. africana* and *Syzygium guineense* (Willd.) DC. (Kebede et al. 2013) and *Picralima nitida* (Stapf) T.Durand & H.Durand (Olaniyi et al. 2021) were carried out using this design.

Preparation of stem cuttings

In this experiment, two sources of *P. adolfi-friederici* stem cuttings were applied for the macro-propagation experiment in the non-mist polypropagator. These were seedling cuttings and branch cuttings. About 1-year-old seedling cuttings were collected from naturally regenerated seedlings around and adjacent to mother trees. By contrast, branch cuttings were taken from young branches of mother trees. The size of stem cuttings ranged from 0.2-0.6 mm in diameter, with at least four nodes considered. Matured mother trees in good physical condition and physically

normal appearance were considered during sample collections (Figure 1.A). Following this, the shoot tip of about 3-5 cm was removed from stem cuttings to get matured stem cuttings. After that, the top 2-3 leaves were maintained on the stem cuttings, and the remaining were removed. In this regard, leaf area also considerably affects the rooting response of stem cuttings (Tchoundjeu et al. 2002). The leaf area considerably affects photosynthesis through light interception and transpiration rate for optimizing water loss. Thus, following the recommendation from various sources, leaves were trimmed to 50 cm^2 to reduce leaf areas and hence the transpiration rate (Leakey et al. 1982; Tchoundjeu and Leakey 2000, 2001; Tchoundjeu et al. 2002). Following this, all stem cuttings were prepared in such a way and safely put in the icebox and brought from the field to the experimental site (Figure 1.B). During transportation, stem cuttings were checked to maintain leaves on stems and ensure moisture did not dry out. During establishing stem cuttings in the non-mist polypropagator, each stem cutting (with 2-3 trimmed leaves per stem cutting) was planted in the rooting (sand) medium. Accordingly, 10 cm of the size of the stem cutting (two nodes) was maintained below the sand level, while about 20 cm was kept above the sand level.

Preparation of growth hormones

Various studies reported that IBA is better for rooting stem cuttings than α-Naphthalene Acetic Acid (NAA) (Leakey et al. 1982; Hartmann and Kester 1983; Kassahun and Mekonnen 2012; Osman et al. 2013; Kebede et al. 2013; Phuyal et al. 2018; Sahoo et al. 2021). Indole-3-butyric acid is the most reliable in stimulating cuttings in rooting in many plant species and is non-toxic to plants over a wide concentration range (Hartmann and Kester 1983). Consequently, the IBA hormone is preferred and mostly applied in other propagation studies instead of other hormones (NAA, IAA). Therefore, IBA was selected in this particular study over other auxins to be successful in our result and to be more efficient in resource use. Furthermore, the previous preliminary finding by Derero et al. (2019) on *P. adolfi-friederici* stem cuttings showed that 0.4% IBA was effective for initiating roots and shoots compared to the control treatment (treatment without hormone application). At the same time, the same study on *P. adolfi-friederici* (Derero et al. 2019) further suggested that different concentrations of IBA should be considered to enhance shooting and rooting response. Hence, in this experiment, the levels of IBA concentrations (0.2% and 0.4%) were evaluated without the control treatment. However, 0.4% dose was considered the control from the two levels. A wide range of previous studies also reported that hormone-treated stem cuttings had higher rooting ability than the control treatment, i.e., treatment without application of hormone (Leakey et al. 1982; Hartmann and Kester 1983; Leakey et al. 1990; Kebede et al. 2013). With this understanding and experience, IBA was selected and applied at two concentrations (0.2 and 0.4%) since these concentrations are the most effective for shooting and rooting of various tropical tree species (Hartmann and Kester 1983; Negash 2010; Kebede et al. 2013).

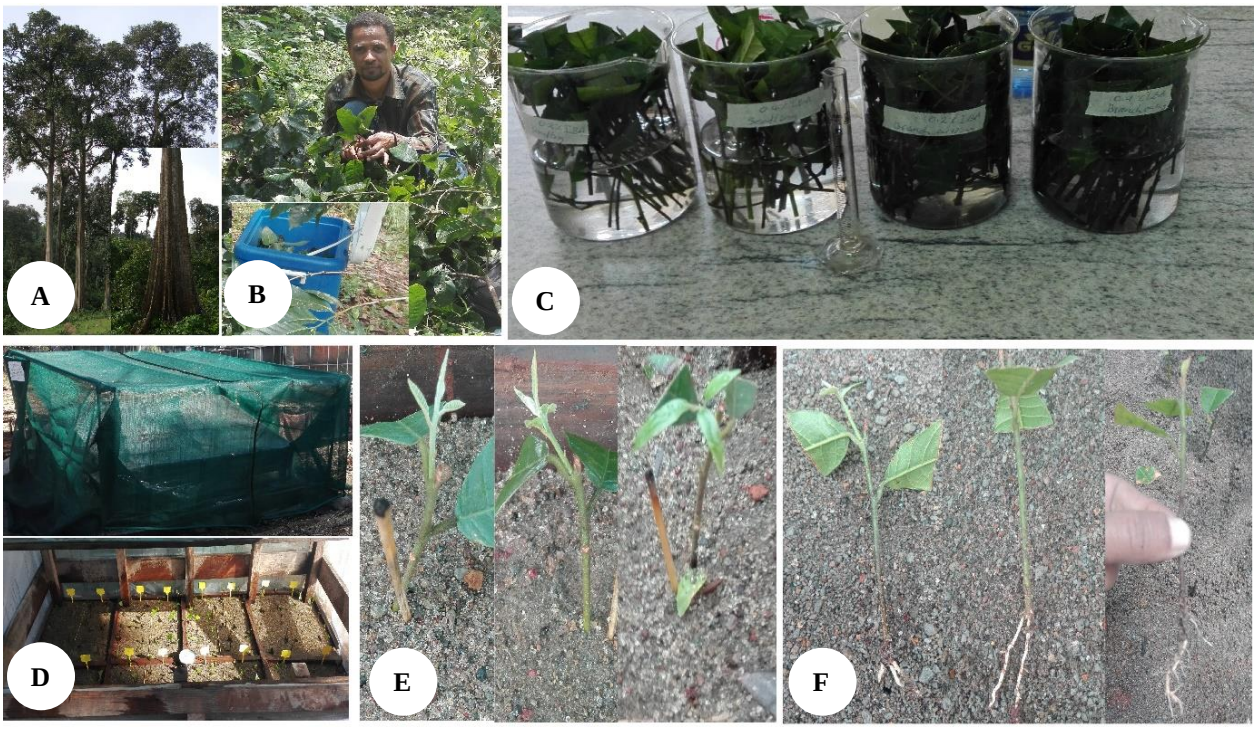

Figure 1. Vegetative propagation process of *Pouteria adolfi-friederici* stem cuttings at non-mist polypropagator: A. Selected mother tree for collection of stem cuttings; B. Preparation of enough stem cuttings with 2-3 leaves placed in the icebox; C. Stem cuttings dipped at 0.2% and 0.4% IBA concentrations for 24 hours; D. Established & tagged stem cuttings on sand medium at non-mist polypropagator and covered with shade-net; E. Shooting success of stem cuttings on sand medium; and F. Rooting success of stem cuttings on sand medium

Hence, the levels were dissolved and prepared with 96% ethanol, following the method on *S. guineense* and *P. africana* tree species by Kebede et al. (2013). Accordingly, 100 mg IBA will be diluted in 100 mL distilled water to prepare an IBA stock solution, and 1 mL 95% ethanol alcohol will be added to dissolve the hormone (Smith 2000; Trigiano and Gray 2000). After that, from the IBA stock solution, 20 mg 100 mL^{-1} and 40 mg 100 mL^{-1} solution were prepared to make 0.2% and 0.4% IBA solution, respectively (Trigiano and Gray 2000). Eventually, each cutting will be dipped into the respective IBA solution (either 0.2% or 0.4%) for 24 hours (Figure 1.C) and planted in the rooting medium (Figure 1.E), following the method by Hartmann and Kester (1983) and Derero et al. (2019).

Experimental design and treatment combinations

In this experiment, four treatment combinations (2 sources of stem cuttings and 2 IBA hormone concentrations at 0.2% and 0.4%) having 4 replicates for each treatment were applied. According to Hartmann and Kester (1983) and Hartmann et al. (2014) measurements, 0.2% IBA hormone equals 2,000 ppm or 2 g L^{-1} or 2,000 mg L^{-1} IBA concentration. However, this experiment did not include the control treatment (treatment without applying hormones). This is because stem cuttings had a better rooting response than the control treatment in the previous experiment application of IBA at 0.4% concentration (Derero et al. 2019). With this, the experiment was planned to evaluate the effect of different IBA concentrations (doses) on the shooting and rooting success of *P. adolfi-friederici* stem cuttings. Using Completely Randomized Design (CRD), 10 stem cuttings (repetitions) were prepared from each treatment to give a total of 160 (n=160) stem cuttings (10 stem cuttings x 2 stem cutting sources x 2 hormone concentrations x 4 replicates). All stem cuttings were dipped in the respective IBA solution, i.e., 0.2 or 0.4%, for 24 hours and established in a well-prepared non-mist polypropagator. The polypropagator was divided into 8 boxes (sections), and each box was again divided into two sub-sections. As a result, seedling cuttings and branch cuttings treated with the same IBA hormone (0.2 or 0.4%) were placed within the same box under sub-sections. With this, treated seedling cuttings and branch cuttings were tagged separately and planted in each sub-section.

On the contrary, stem cuttings treated with different concentrations of IBA hormone (0.2 or 0.4%) were established with different boxes separately to avoid contamination. Finally, stem cuttings were established on sand as a rooting medium at non-mist polypropagator (Figure 1.E). After that, watering every day early in the morning and late in the afternoon was conducted throughout the experimental period (180 days). Regular supervision was conducted to manage the relative humidity, daily temperature, and overall experimental conditions. Daily supervision was carried out since the establishment of stem cuttings in the non-mist polypropagator. Side by side, data on survivability, shooting and rooting ability, number of buds and leaves per stem cutting, number of roots per stem cutting and root length per stem cutting were collected. Following this, newly-rooted stem cuttings (Figure 1.F) having at least one root longer than 1 cm (Tchoundjeu and Leakey 2001) were uprooted, counted, marked to avoid double counting, and maintained in the rooting medium. The remaining unrooted or rooted stem cuttings of less than 1 cm were still maintained in the rooting medium. At the end of 180 days, all the survived stem cuttings were uprooted separately, and the root length and the total number of roots from each stem cutting were recorded. Eventually, all stem cuttings with developed shoots and roots were transplanted to pots for further survival study. Accordingly, seedling polyethylene pots (20 cm height and 16 cm diameter size) were pre-prepared with the required 2:1:1:1 soil mix ratio (2 forest soil: 1 local soil: 1 manure: 1 sand), and the pots were kept at nursery under shade. The stem cuttings were further supervised for an additional 120 days after being transplanted into nursery pots, and the cuttings' survival rates were recorded. Finally, statistical analyses on observed parameters, including survival rate, shooting response, rooting response, number of leaves and buds per cutting, number of roots per cutting and root length per cutting, were further performed using both descriptive (percentage, table, and graph) and quantitative statistics using SPSS version 27 software.

RESULTS AND DISCUSSION

The propagator environment

In this experiment, the test result confirmed that both temperature and relative humidity recorded in the propagator significantly ($p<0.001$) varied across months and daily duration. However, temperature and relative humidity significantly ($p<0.01$) but negatively correlated for the vegetative propagation of stem cuttings. A mean temperature and mean relative humidity of 22.4+4.75^{o}c and 75.5+6.50%, respectively, with 12 hours natural light and 12 hours dark was recorded within 180 days at non-mist polypropagator kept under 60% shade-net (Figures 2.A and 2.B). The mean minimum and maximum daily temperatures were recorded at 16.5+2.76^{o}c at 08:30 a.m. and 25.1+2.72^{o}c at 02:30 p.m., respectively. In the same way, the mean maximum (24.6+3.32^{o}c) temperature was recorded in May, while the mean minimum temperature (20.7+5.21^{o}c) was observed in December. By contrast, the highest mean daily relative humidity (78.8+2.81%) was recorded at 08:30 a.m., whereas the lowest mean daily relative humidity (72.5+8.11%) was recorded at 02:30 p.m. The highest (78.2+3.76%) and the lowest (72.2+4.73%) mean monthly relative humidity was recorded in April and May, respectively.

Survival rate of stem cuttings

In our finding, the mean survival rate differed significantly between cutting sources ($F=59.91$; $p<0.001$) and IBA doses ($F=4.15$; $p=0.043$), as indicated in Table 1. In the same way, a significant ($F=8.13$; $p<0.01$) interaction effect on survival rate was also observed between cutting sources and IBA doses. A 2-tailed Pearson correlation analysis further confirmed that the survivability was

significant ($p<0.01$) and positively associated with other recorded parameters (Table 2). Out of 160 stem cuttings planted in the non-mist polypropagator, 50 (31.3%) stem cuttings survived at the end of 180 days (Figure 3). However, 40% survived at the end of 30 days and gradually declined. During this time, 1-6 leaves and 1-5 buds in each stem cutting were developed.

Nevertheless, some developed leaves and buds on stem cuttings were gradually wilted and died out during the experimental period. The developed buds also grew to the leaves or wilted and eventually died. Some other stem cuttings survived for a few months or the entire experimental period without initiation of buds and leaves or even adventitious roots. A few stem cuttings also developed adventitious roots without initiation of buds and leaves, or stem cuttings developed buds and leaves without roots. Figure 4 shows the developed adventitious roots on some stem cuttings.

By the age of 180 days, 30 (60%) stem cuttings survived by applying 0.2% IBA concentration, while 0.4% IBA level contributed to a 40% survival rate. However, stem cuttings treated with both IBA doses (0.2 and 0.4%) showed a similar declining trend (except seedling cuttings treated with 0.2% IBA dose) in survival rate across the entire experimental period (30 to 180 days). On the other hand, stem cuttings treated with 0.2% IBA level contributed to the survival of 28 (56%) seedling cuttings compared to seedling cuttings treated with 0.4% IBA dose, which accounted for 32% (16 seedling cuttings) at the age of 180 days. By contrast, branch cuttings treated with 0.4% IBA dose had a better survival count (8% or 4 branch cuttings) than 0.2% IBA application (4%) in the same period.

In conclusion, the present result indicates that seedling cuttings treated with 0.2% IBA concentration considerably increased the survival rate compared to those treated with 0.4% IBA or branch cuttings treated with 0.2% or 0.4% IBA dose.

Shooting response of stem cuttings

In this study, the analyses of variance further showed that sources of stem cuttings significantly ($F=34.25$; $p<0.001$) affected the shooting success of *P. adolfi-friederici* stem cuttings at the non-mist polypropagator. Application of IBA concentrations and the interaction between both factors (cutting sources and IBA doses), however, did not affect ($p<0.05$) the shooting response of stem cuttings. However, the shooting response was significant ($p<0.01$) and positively correlated with other recorded parameters (Table 2). In this regard, *P. adolfi-friederici* stem cuttings responded differently to applying IBA concentrations at the non-mist polypropagator. The rate of shooting response (initiation and development of buds and leaves) was not observed in the first week of planting at the non-mist polypropagator. However, the first initiation of buds was observed during the second week of planting on 6 stem cuttings, which accounted for 9.8%. This was followed by the third and fourth weeks after establishing 8 stem cuttings (13.1%) and 6 stem cuttings (9.8%). Of these, 83.3, 75 and 83.3% of seedling cuttings treated with 0.2% IBA application were initiated buds during the 2nd, third, and fourth weeks, respectively. Overall, 16 stem cuttings (28.6%) out of the total survived cuttings showed shooting response at 120 days. This was followed by 28.1, 25.9, and 12.7% shooting success at the end of 30, 90, and 150 days after planting. Of these, stem cuttings treated with 0.2% IBA dose induced 27.5% of the shooting response compared to 0.4% IBA application (17.5% shooting ability) at the age of 30 days (Figure 4). Application of 0.2% IBA concentration on stem cuttings further induced a 22.5% shooting response in seedling cuttings, while 0.4% IBA dose induced 12.5% seedling cuttings in a given period. Similarly, branch cuttings treated with 0.2% or 0.4% IBA concentrations had lower shooting percentages (5% each) than seedling cuttings. Conversely, out of the total seedling cuttings (i.e., 40 samples) treated with 0.2% IBA dose, 70% showed rooting success throughout the period (Figure 5). By contrast, applying 0.4% IBA dose on seedling cuttings induced a 47.5% shooting response, while branch cuttings treated with 0.2% and 0.4% IBA concentrations contributed 32.5% in the same period and samples. Almost in all the treatments, the shooting response was steeply reduced across the entire vegetative propagation period at the non-mist polypropagator. In conclusion, our finding confirmed that seedling cuttings treated with 0.2% IBA concentration substantially improved shooting response as opposed to seedling cuttings treated with 0.4% IBA dose or branch cuttings treated with 0.2% or 0.4% IBA dose.

Number of leaves and buds on stem cuttings

Overall, the mean total number of leaves and buds developed across the entire duration significantly varied between cutting sources ($F=37.17$; $p<0.001$), IBA doses ($F=4.01$; $p<0.05$) and their interaction effect between them ($F=7.29$; $p<0.01$) as indicated in Table 1. Despite this fact, a correlation analysis affirmed that the total number of leaves and buds was significant ($p<0.01$) and positively related to other dependent variables parameters (Table 2). A similar increasing trend was observed among all the treatments despite the variation in the total number of leaves and buds developed between 30 to 180 days after establishment at non-mist polypropagator (Figure 6). The highest number of leaves and buds was recorded by seedling cuttings treated with 0.2% IBA concentration, which accounted for 18-111 total numbers. This was followed by seedling cuttings treated by 0.4% IBA level with 9-59 total numbers of leaves and or buds. In contrast, branch cuttings treated with 0.2% or 0.4% IBA concentrations had relatively lower (2-18) total numbers of leaves and buds. In conclusion, our finding approved that seedling cuttings treated with 0.2% IBA concentration significantly enhanced the total numbers of leaves and buds as opposed to seedling cuttings treated with 0.4% IBA dose or branch cuttings treated with 0.2% or 0.4% IBA dose.

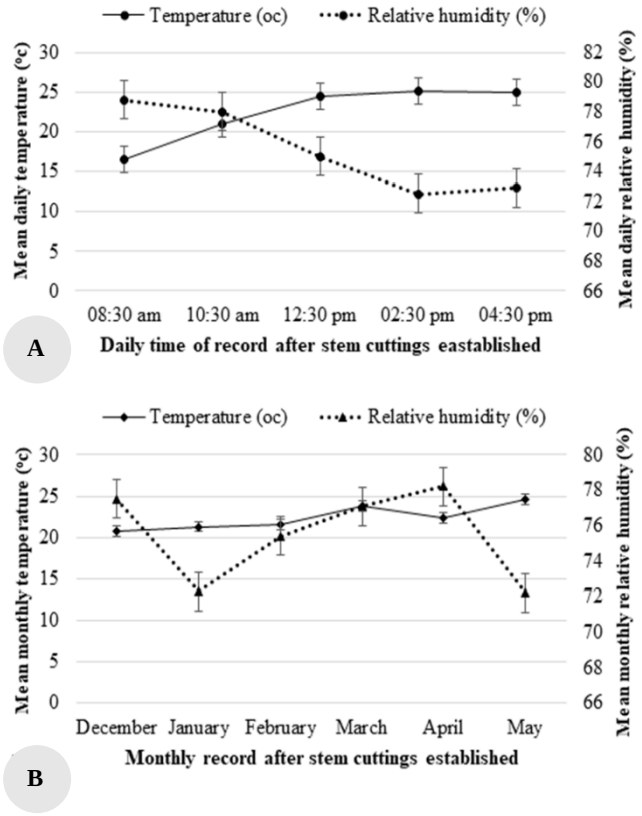

Figure 2. Mean daily (A) and mean monthly (B) temperature and relative humidity record of *Pouteria adolfi-friederici* stem cuttings after established at the non-mist polypropagator

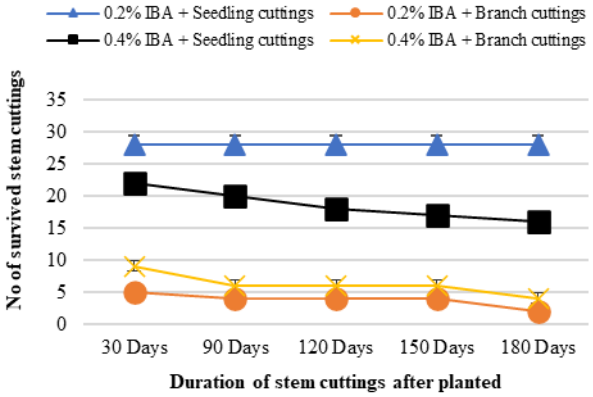

Figure 3. Survival ability of *P. adolfi-friederici* stem cuttings at non-mist polypropagator during the entire vegetative propagation period. Seedling cuttings were 1-year-old raised seedlings at the nursery, while branch cuttings were young branches of matured mother trees collected from mother trees at the field

Figure 4. Rooting ability of *P. adolfi-friederici* stem cuttings at non-mist polypropagator. Rooting success of stem cuttings in A, B, and C indicated stem cuttings treated with 0.2% IBA concentrations for 24 hours, while stem cuttings in D & E showed stem cuttings dipped in 0.4% IBA levels for 24 hours

Table 1. Effects of cutting sources, IBA concentrations and their interaction on vegetative propagation of *Pouteria adolfi-friederici* stem cuttings at non-mist polypropagator

Source of variation	Survival rate	Shooting response	Rooting response	No of leaves &/or buds/cutting	No roots/cutting	Root length/cutting
Cutting sources	59.91***	34.25***	55.91***	37.17***	43.34***	49.88***
IBA concentration	4.15*	1.20^{ns}	3.31^{ns}	4.01*	16.24***	7.42**
Cutting sources*IBA concentration	8.13**	3.35^{ns}	6.90**	7.29**	17.75***	9.48**
R^2	0.316	0.199	0.298	0.237	0.331	0.300

Note: $F^{p\text{-value}}$; significance levels were *** -p<0.001; ** -P<0.01; * -P<0.05; ^{ns} -non significant

Table 2. Pearson correlation analysis of recorded parameters on vegetative propagation of *Pouteria adolfi-friederici* stem cuttings at the non-mist polypropagator

	Survival rate	Shooting response	Rooting response	No of leaves & buds/cutting	No roots/cutting	Root length/cutting
Survival rate						
Shooting response	0.834**					
Rooting response	0.956**	0.820**				
No of leaves & buds/cutting	0.774**	0.813**	0.740**			
No roots/cutting	0.661**	0.506**	0.671**	0.532**		
Root length/cutting	0.771**	0.625**	0.783**	0.609**	0.927**	

Note: **correlation is significant at the 0.01 level (2-tailed)

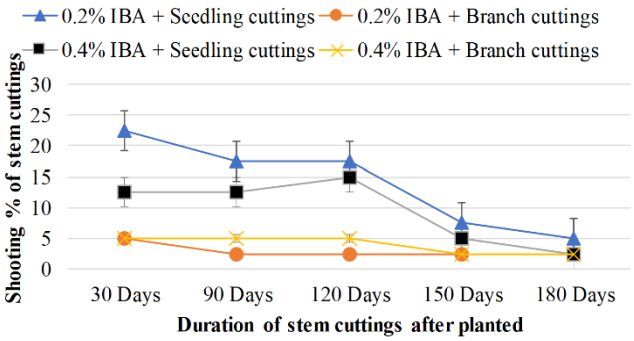

Figure 5. Shooting response of *Pouteria adolfi-friederici* stem cuttings at non-mist polypropagator during the entire vegetative propagation period

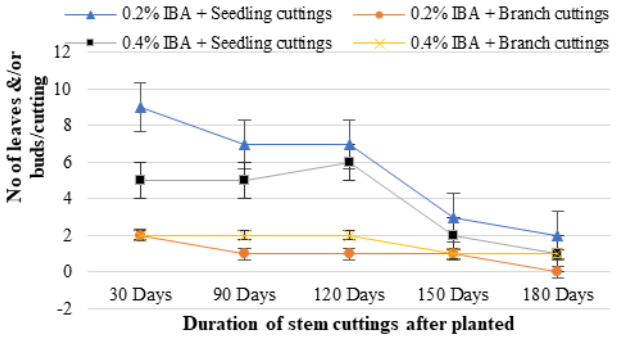

Figure 6. Number of leaves and buds on *Pouteria adolfi-friederici* stem cuttings at non-mist polypropagator during the entire vegetative propagation period

Rooting response of stem cuttings

The present finding revealed that the rooting percentage of *P. adolfi-friederici* stem cuttings strongly varied by cutting sources ($F=55.91$; $p<0.001$), although the application of different concentrations of IBA was insignificant ($F=3.31$; $p<0.05$). On the other hand, the interaction effect also showed a significant difference ($F=6.90$; $p<0.01$) between cutting sources and IBA concentrations. Adventitious roots sprouted and developed on stem cuttings despite its too-late response (120 days after planting) compared to the shooting response. Of the total seedling cuttings (i.e., 40 samples), 70% and 40% rooting success were induced by 0.2% and 0.4% IBA concentrations across the entire propagation period (Figure 7). On the contrary, only a 15% rooting response in branch cuttings was observed by 0.2% and 0.4% IBA concentrations in a given period and samples. Conversely, 35.7% of the rooting response was observed out of the survived cuttings at the end of 120 days after establishment. This was followed by a 36.4% rooting response of *P. adolfi-friederici* stem cuttings at 150 days of planting. However, 47 stem cuttings (94%) out of the survived cuttings showed rooting response in the entire propagation period. Furthermore, a 2-talied Pearson correlation analysis confirmed a significant ($p<0.01$) and positive association between rooting response and other recorded variables (Table 2). In conclusion, this result affirmed that seedling cuttings treated with 0.2% IBA concentration significantly promoted rooting success compared to seedling cuttings treated with 0.4% IBA dose or branch cuttings treated with 0.2% or 0.4% IBA dose.

Mean root number per rooted cutting

The mean root number per rooted cutting was strongly influenced by the sources of stem cuttings ($F=43.34$; $p<0.001$), application of IBA doses ($F=16.24$; $p<0.001$) as well as their interaction effect between them ($F=17.75$; $p<0.001$) as shown in Table 1. The highest mean root number per rooted cutting (7.45+8.19 cm) was recorded in seedling cuttings treated with 0.2% IBA dose (Figure 8). The corresponding mean root number of rooted cuttings applied at 0.4% IBA dose for seedling cuttings and branch cuttings was (1.80+2.78 cm) and (0.18+0.59 cm), respectively. On the contrary, several roots per rooted cuttings were counted for branch cuttings (0.05+0.22 mean root number) treated with 0.2% IBA concentration. The test result also reported that strong significant differences ($p<0.001$) were observed for cutting sources, IBA concentrations and the interaction effect between both factors on recorded mean root number per rooted cutting. At the same time, a significant ($p<0.01$) and positive correlation between the mean root number and other variables was observed (refer to Table 2). The present finding supported that those treated with 0.2% IBA concentration considerably increased mean root number per rooted cutting than seedling cuttings treated with 0.4% IBA or branch cuttings treated with 0.2% or 0.4% IBA doses. Furthermore, the multiple comparisons using the Post Hoc test showed that seedling cuttings treated with 0.2% IBA dose significantly varied ($p<0.001$) in the mean root number per rooted cutting from seedling cuttings treated with 0.4% IBA dose, branch cuttings treated with 0.2% and 0.4% IBA dose.

Mean root length per rooted cutting

The test statistics in this analysis confirmed that root length per cutting was significantly affected by cutting sources ($F=49.88$; $p<0.001$), IBA concentrations (7.42; $p<0.01$) and the interaction between the two factors (9.48; $p<0.01$) on rooted stem cuttings. In our finding, the longest mean root length (1.97+1.92 cm) was measured for seedling cuttings treated with 0.2% IBA concentration compared to branch cuttings (0.04+0.24 cm) treated with the same dose (Figure 9). The longest and the shortest root length was also 2.34 and 1.59 cm, respectively, for seedling cuttings treated with 0.2% IBA dose. The corresponding mean root length attained was 1.24 and 0.49 cm for seedling cuttings treated with 0.4% IBA dose. By contrast, the shortest mean root length per rooted cutting was recorded from branch cuttings (0.04+0.24 cm) treated with 0.2% IBA concentration, followed by branch cuttings (0.11+0.40 cm) treated with 0.4% IBA doses. In conclusion, our result confirmed that seedling cuttings treated with 0.2% IBA concentration significantly promoted mean root length per rooted cutting as opposed to seedling cuttings treated with 0.4% IBA dose or branch cuttings treated with 0.2% or 0.4% IBA dose. In the same manner, the Post Hoc test also showed that except for the

comparison between seedling cuttings treated with 0.4% IBA dose and branch cuttings treated with 0.4% IBA dose, all treatments significantly differed ($p<0.05$) in the mean root length per rooted cutting.

Survival rate of stem cuttings at nursery

At the end of 120 days, the survivability of *P. adolfi-friederici* stem cuttings transplanted on polyethylene pots at the nursery was recorded. Out of the total transplanted stem cuttings, a 48.9% survival rate of stem cuttings was recorded at the age of 120 days of nursery lifespan. Of these, 69.6% of seedling cuttings, compared to branch cuttings, successfully showed higher survival potential. During this stage, the researcher's regular observation of stem cuttings indicated that seedling cuttings had good morphological characteristics (number of buds and leaves, size of leaves, number of branches, number of roots and fibrous roots and size of roots) over a few branch cuttings. Seedling cuttings were grown uniformly and showed limited morphological variation during the entire nursery's lifespan. In contrast, most of the branch cuttings showed stunted growth or wilted and failed to survive and eventually died, while only a few cuttings were able to survive well during this period.

Discussion

The low-technology non-mist polypropagator is one of the most promising options to propagate *P. adolfi-friederici* stem cuttings using the vegetative propagation method. This system provides suitable propagation conditions (optimum temperature, relative humidity and suitable propagation substrate) in maintaining a favorable environment for successful survival rate, shooting and rooting ability, and the required number and length of roots on stem cuttings. Our analysis showed that the propagator's temperature and relative humidity significantly ($p<0.001$) varied across months and daily duration. In this regard, the shooting and rooting response of *P. adolfi-friederici* stem cuttings were observed in the entire propagation period (180 days), when the temperature in the propagator was maintained from 20 to 25ºC. The influence of temperature in the propagator on the shooting and rooting ability of stem cuttings was consistent with other findings reported previously by Leakey et al. (1982) and Derero et al. (2019). Other studies further discussed and confirmed our findings (Leakey et al. 1982; Sevik and Guney 2013; Caplan et al. 2018; Derero et al. 2019). Even the higher temperature (28-30ºc) reported by Shekhawat and Manokari (2016), 26-34ºc by Zamora et al. (2022) and 28-35ºc by Vallejos-Torres et al. (2020 and 2021a,b) was maintained for suitable shoot and root induction at a relative humidity of about 80-90% (Shekhawat and Manokari 2016) or above 80% (Zamora et al. 2022). This is because the higher temperature observed during the propagation period can be compensated by keeping a higher percentage of relative humidity. This, in turn, helps to compensate for the faster loss of water from stem cuttings through evapotranspiration and thereby maintains the process of photosynthesis as opposed to the slower absorption of water by the initially incipient roots. Similarly, relative humidity (75.5%) maintained in the propagator was also in line with other findings in earlier studies despite a slightly higher than 75% relative humidity (Vallejos-Torres et al. 2020 and 2021a, b), around 80% (Pigatto et al. 2018), 85% (Leakey et al. 1982; Kebede et al. 2013) and approximately 100% (Junior et al. 2017). In contrast to these and the present findings, the relative humidity was maintained at a wider range (60-95%) since the establishment of the cannabis stem cuttings up to the end of the experiment (Caplan et al. 2018).

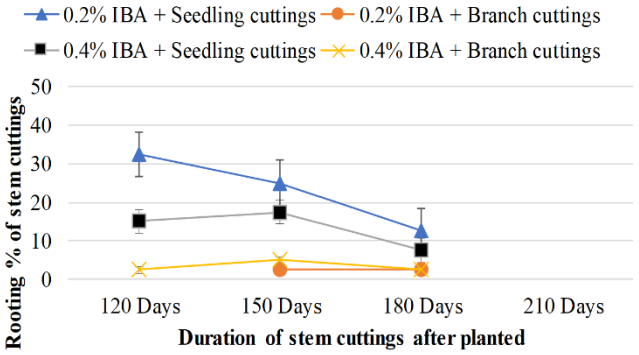

Figure 7. Rooting response of *Pouteria adolfi-friederici* stem cuttings at non-mist polypropagator during the entire vegetative propagation period

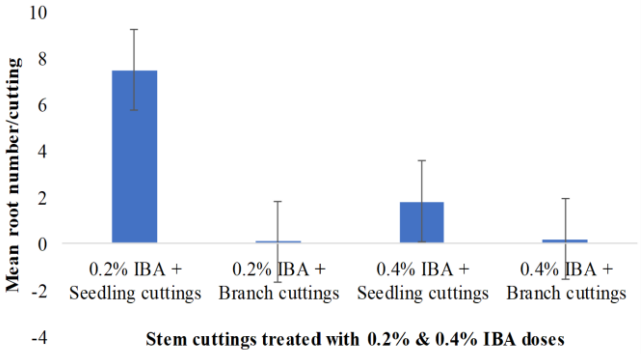

Figure 8. Mean root number per rooted cutting of *Pouteria adolfi-friederici* at non-mist polypropagator during the entire vegetative propagation period

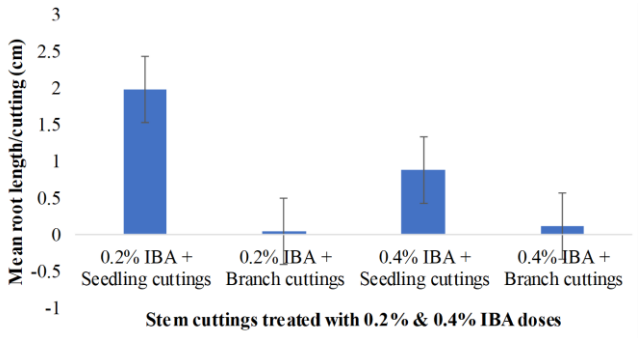

Figure 9. Mean root length per rooted cutting of *Pouteria adolfi-friederici* at non-mist polypropagator during the entire vegetative propagation period

On the other hand, seedling cuttings retained most of the leaves compared to branch cuttings that shed their leaves during the entire shooting period, which probably suggests higher survivability, bud initiation, shooting, and rooting response of seedling cuttings. This is because most of the leaves retained on seedling cuttings might contribute to a higher rate of photosynthesis instead of water stress by enhancing the rate of evapotranspiration. By contrast, branch cuttings reduced water stress by minimizing evapotranspiration rate by shedding most of their leaves despite reducing the photosynthesis rate on leaves. This is because the present experiment was conducted during the hot and dry months (December to May), so the higher temperature outside the non-mist polypropagator contributed to moisture stress and hence higher evapotranspiration inside the propagator. Nevertheless, such an effect does not seem to be influenced by the shedding of most leaves from branch cuttings compared to retained leaves on seedling cuttings. This is because much effort was taken to maintain the temperature and relative humidity to the required level at the non-mist polypropagator by installing a 60% shade net. At the same time, regular supervision and watering of stem cuttings early in the morning and late in the afternoon throughout the entire propagation period was carried out. In conclusion, the present finding proposes that the temperature and relative humidity are optimum for the propagation of the species at the non-mist polypropagator.

The results of the present study suggested promising options for a simple, effective, and rapid vegetative propagation protocol for *P. adolfi-friederici* stem cuttings. This, in turn, is an important step towards conservation and genetic improvement of the species and promoting small- and large-scale plantation development under plantation forest or agroforestry systems. This is because, nowadays, the species is confronted with various major practical problems, including fresh and mature seed provision, poor seed viability and storage, and limited means of propagation. In line with this, there is a decline of suitable habitats associated with climate change and, thereby, a subsequent shift of highly suitable habitats of *P. adolfi-friederici* from the northern and central parts to the southern parts of Ethiopia (Tadesse et al. 2022). However, an intraspecific morphological variation among the different *P. adolfi-friederici* populations in different natural forests of southwest Ethiopia (Seid and Mengesha 2022) create a good opportunity for its genetic resource conservation and improvement options. Our finding showed that cutting source ($p<0.001$), application of IBA concentration ($p<0.05$ except shooting and rooting response) and the interaction between the source of stem cuttings and IBA concentration ($p<0.01$ except for shooting response) considerably influenced survival rate, shooting and rooting success, number of leaves and buds, root number and root length of stem cuttings throughout the propagation period, i.e., 180 days. Initially, higher survived stem cuttings (31.3%) in our study were recorded compared to 13.8% by Derero et al. (2019). In turn, the first initiation of buds was observed during the second and third weeks, followed by the rooting response at the age of 120 days in this study, while bud initiation after the sixth week and rooting response on 195 days were observed in the previous research (Derero et al. 2019). At the same time, 70% shooting and rooting success with 0.2% IBA dose in the present study was 5 times higher than 13.8% shooting and rooting response with 0.4% IBA dose in the former investigation (Derero et al. 2019). All these findings suggest that the application of optimum IBA concentration (0.2%) on selected suitable cutting source (seedling cuttings) in our investigation resulted in earlier, faster and higher survivability, bud initiation, shooting and rooting response, greater number of leaves and roots and longer roots on *P. adolfi-friederici* stem cuttings as opposed to seedling cuttings treated with 0.4%. Consistent with the present study, several investigators have also reported that optimal IBA concentration considerably contributed to the successful survival rate, shooting and rooting response of stem cuttings (Leakey et al. 1982; Kebede et al. 2013). Furthermore, our findings confirmed that *P. adolfi-friederici* stem cuttings considerably vary in response to survival rate, shooting, and rooting success due to various concentrations of IBA and cutting sources. During vegetative propagation of *P. adolfi-friederici* at polypropagator, stem cuttings responded differently to different levels of IBA hormone (0.2 and 0.4%). The experimental result in the present study indicated that seedling cuttings treated with 0.2% IBA concentration had far better survival rate, shooting, and rooting response than seedling cuttings treated with 0.4% IBA concentration. That probably suggests that less IBA concentration (0.2% IBA), instead of a higher concentration (0.4%), easily stimulates and triggers young plant parts, i.e., seedling cuttings; the response was inhibited at more IBA doses (0.4% IBA). Our results are also consistent with those of many other tree species, *P. falcatus* (Negash 2003), *Bougainvillea* sp. (Asl et al. 2012), *Melissa officinalis* L. (Sevik and Guney 2013), *Prosopis alba* Griseb. (de Souza et al. 2014), *Rosmarinus officinalis* L. (Elhaak et al. 2015), *Couroupita guianensis* Aubl. (Shekhawat and Manokari 2016), *Ficus benjamina* L. (Topacoglu et al. 2016), *Theobroma cacao* L. (Junior et al. 2017), *Stevia rebaudiana* (Bertoni) Bertoni (Pigatto et al. 2018), *Coffea arabica* L. (Vallejos-Torres et al. 2020), *P. nitida* (Olaniyi et al. 2021), *Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg. (Vallejos-Torres et al. 2021a), *Manilkara bidentata* (A.DC.) A.Chev. (Vallejos-Torres et al. 2021b), *Syzygium samarangense* (Blume) Merr. & L.M.Perry (Khandaker et al. 2022) and *T. cacao* (Zamora et al. 2022), in which stem cuttings treated with lower IBA concentration had higher root numbers, root length and rooting response than higher IBA concentration. Even applying other plant growth hormones (BAP or NAA) instead of IBA further triggered and enhanced better shooting and rooting ability and increased root length and numbers at lower concentrations compared to higher concentrations. Unlike the present study and the aforementioned earlier studies, successful rooting ability, root number and root length in *Embelia tsjeriam-cottam* (Roem. & Schult.) A.DC. (Tiwari and Das 2010), *Zanthoxylum armatum* DC. (Phuyal et al. 2018) and

Araucaria heterophylla (Salisb.) Franco (Tilahun et al. 2019) stem cuttings were observed as cuttings treated with higher IBA concentration as opposed to lower concentration. Likewise, seedling cuttings treated with 0.4% IBA concentration further triggered better shooting and rooting responses than branch cuttings treated with 0.2% and 0.4% IBA concentrations. This might be further associated with younger plant parts responding better than matured (older) parts to applying growth hormone (IBA hormone). Of course, this trend was reversed in branch cuttings treated with 0.4% IBA dose as opposed to branch cuttings treated with 0.2% IBA concentration. This probably suggests that matured stem cuttings (in this case, branch cuttings) required more doses (0.4% IBA) to induce better shooting and rooting response compared to fewer doses (0.2% IBA). Several other comparative studies (Leakey et al. 1982; Tchoundjeu and Leakey 2000, 2001; Tchoundjeu et al. 2002; Negash 2003; Kebede et al. 2013) on different concentrations of growth hormone have also demonstrated that optimum IBA concentration prompts rooting response, while supra-optimal (overdoses) IBA concentration inhibits the rooting ability. On the other hand, faster and higher survival rate, shooting and rooting response in seedling cuttings compared to branch cuttings on *P. adolfi-friederici* stem cuttings both in our finding and the previous study (Derero et al. 2019) further confirmed that the selection of suitable cutting source a great contribution to successful vegetative propagation. Consequently, higher survival rate and rooting response in seedling cuttings compared to branch cuttings in *P. adolfi-friederici* tree species, which is in line with the apical cuttings as opposed to middle or basal cuttings of *K. ivorensis* (Tchoundjeu and Leakey 2000), *L. trichilioides* (Tchoundjeu and Leakey 2001), *P. falcatus* (Negash 2003), *S. rebaudiana* (Kassahun and Mekonnen 2012), *Cannabis sativa* (Caplan et al. 2018), *A. heterophylla* (Tilahun et al. 2019), suggests young plant parts promote better propagation than mature plant parts.

In this study, vegetatively propagated stem cuttings of *P. adolfi-friederici* at a non-mist polypropagator were transplanted to pots after shooting and rooting success for further acclimatization process at nursery for 120 days. This is because successful stem-cutting establishment after transplantation at greenhouses and nurseries is a major bottleneck since seedlings are continuously exposed to a unique microenvironment, including lower relative humidity, high light intensity, scarce nutrient and water availability, disease and insect pest infestation, as well as other stressful conditions. In this stage, seedling cuttings, as opposed to branch cuttings, were healthy, greenish, and grew very well on pots during the 120-day nursery lifespan. More buds and leaves and roots and root systems were successfully developed. Thus, the satisfactory result on the transplantation and acclimatization process of *P. adolfi-friederici* seedling cuttings (69.6% survival rate) at the nursery is an indication of the efficiency of the developed protocol for its vegetative propagation despite a lower survival rate (58.9%). This further contributes to the successful survival rate, growth performance, and adaptation potential of stem cuttings in the field. Negash (2010) also reported the positive response of vegetative propagation of *Hagenia abyssinica* (Bruce) J.F.Gmel. and hence the successful transplantation of stem cuttings at nursery and field establishments. In conclusion, stem cuttings' survival potential, growth performance of stem cuttings at greenhouses and nurseries, and field establishment and adaptation potential are further suggested.

In conclusion, the low-technology non-mist polypropagator is one of the most promising options for propagating *P. adolfi-friederici* stem cuttings using the vegetative propagation method. In our experiment, 70% of shooting and rooting success and higher mean root length (1.97+1.92 cm) were induced by seedling cuttings treated with 0.2% IBA concentration with a mean temperature of 22.4+4.75^{o}c and mean relative humidity of 75.5+6.50% at non-mist polypropagator kept under 60% shade-net. Hence, the results confirmed that the species can be propagated vegetatively by seedling cuttings treated with 0.2% IBA concentration at non-mist polypropagator. Therefore, establishing mother stocks in nurseries and their proper management to serve as sources of juvenile stems will be essential for the successful macro-propagation of the species besides growing the species with seeds.

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Enhancing vegetative and root productions of four turnip genotypes through varied humic acid fertilizer levels

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Abstract. *Ullah H, Kiran M, Haq F, Waseem K, Nadeem MA, Ullah G, Farid A, Aziz T. 2023. Enhancing vegetative and root productions of four turnip genotypes through varied humic acid fertilizer levels. Cell Biol Dev 7: 67-74.* A meticulously designed pot experiment was conducted to investigate the intricate effects of varying Humic Acid (HA) fertilizer levels on the growth patterns of four distinct turnip genotypes. The trial encompassed an array of HA concentrations, from 0 to 120 Kg/ha, to discern their influence on the vegetative and root aspects of the turnip plants. The outcomes of this comprehensive study unveiled an undeniable impact of HA concentrations on virtually every facet of turnip growth and output. Notably, the pinnacle of performance across several critical parameters, including plant height, leaf area, canopy coverage, leaf count per plant, chlorophyll content in leaves, and both the fresh and dry weights of both leaves and roots, was achieved at the HA concentration of 60 kg/ha. Among the four turnip genotypes scrutinized, the Golden Bal genotype reacted favorably to the HA treatments. With a compelling total yield of 11.79 t/ha, the Golden Bal genotype emerged as a leader in productive response. A noteworthy revelation was the intricate interplay between the specific turnip genotypes and the HA concentrations employed. This interplay significantly affected numerous facets of vegetative development and yield-related attributes. The synergy observed between the moderate HA concentration of 60 Kg/ha and the Golden Bal genotype was particularly striking, resulting in a superior manifestation of various traits compared to alternate genotype-HA combinations. In essence, this research has effectively underscored the pivotal role of varying HA fertilizer levels in steering the trajectory of turnip production. The findings of this study bear valuable implications for optimizing agricultural practices to harness the full potential of turnip cultivation while emphasizing the need for a nuanced understanding of genotype-HA interactions in modern agronomic endeavors.

Keywords: Desi Faisalabad, Golden Bal, humic acid, purple top, turnip productivity, White Globe Vikima F1 (Denmark)

INTRODUCTION

Turnips (*Brassica rapa* L. ssp. rapa) are globally cultivated and cherished for their nutritional value and health benefits (Dejanovic et al. 2021). Packed with phytochemicals like glucosinolates, polyphenols, flavonoids, and phenolic acids, turnips possess antioxidant, enzyme-regulating, and apoptosis-controlling properties. Glucosinolates, sulfur-containing compounds, show promise in anticancer research, while polyphenols and flavonoids are known for their antioxidant prowess (Paul et al. 2019). Consuming turnips offers several health advantages, including liver protection against diabetes-related damage, hepatoprotective effects, and robust antioxidant support. These benefits stem from turnips' phytochemical makeup, effectively neutralizing harmful free radicals, guarding against oxidative damage, inflammation, and specific cancers. Turnips also exhibit potential in managing diabetes and regulating blood lipids. Beyond their nutritional and health perks, turnips serve as subjects of study in diverse domains. Genomic analysis of the turnip mosaic potyvirus (TuMV), a significant brassica crop pathogen, has unveiled its historical spread along Silk Road trade routes, aiding crop protection strategies (Kawakubo et al. 2021). Turnips also hold promise in phytoremediation, actively accumulating heavy metals from contaminated soil. Additionally, traditional medicine has utilized turnips to address various conditions, including headaches, chest complaints, rheumatism, and gonorrhea. Moreover, there have been reports of turnip-derived syrup associated with memory enhancement. The availability of nutrients significantly impacts plant growth, production, and the various components of plants (Etesami and Adl 2020). Increased nitrogen levels (N) have enhanced seed production, total dry matter, and harvested index in multiple genotypes of *B. napus* and *B. juncea* (Zou et al. 2020). Moreover, in canola and various other *Brassica* species, phosphorus (P) supplementation has a dual effect, enhancing P absorption and producing remarkable improvements in plant parts (Wang et al. 2021). Organic products, driven by philosophical choices, convictions, commercial needs, or consumer demands, exclude or prohibit conventional agricultural inputs commonly used in modern farming practices. In the context of plant growth, development, and the production of organic compounds, the content of macro- and microelements in the soil

becomes enriched by applying organic fertilizers. Furthermore, utilizing these organic materials promotes human health and is economically advantageous, boosting farmers' income. The literature strongly supports the role of Humic Substances (HS) in promoting plant growth (Canellas and Olivares 2014). In a random-effects meta-analysis, Rose et al. (2014) found that applying HS from external sources resulted in an approximately 22% increase in shoot and root dry weights across various plant species. It is important to emphasize that plant responses to HS are highly dependent on factors such as plant species, developmental stage, application method and rate, HS source, and the prevailing management practices and environmental conditions. Through mitigating the adverse impacts of extreme soil stress, the constituents found in plants and soil play a pivotal role in fostering plant development, enhancing soil fertility and health, increasing plant yield, and improving nutrient availability. Organic manures lies an active group consisting of filvic and humic acids, which play a vital role in soil by facilitating interactions among various elements through chelation and complexation, thereby keeping them in bound forms (Rashad et al. 2022; Hanc et al. 2019). The crucial significance of the concentration of macro and microelements in the soil is enhanced by using organic fertilizers for plant growth, development, and the synthesis of organic compounds (Bhatt et al. 2019). Furthermore, the application of higher rates of organic manure, up to 40 m^3 per fed, resulted in the highest total yield of radish roots, increased levels of crude protein, nitrogen, phosphorus, and potassium, and the most substantial seed output (Gomez et al. 2021). Additionally, the utilization of organic materials poses no harm to human health. Another research demonstrated that applying organic compost enhances soil fertility in turnip plants while mitigating the detrimental effects of chemical fertilizers as the enrichment of soil with macro and microelements through the application of organic fertilizers plays a crucial role in facilitating plant growth, development, and the synthesis of organic compounds (Monfared et al. 2023). This research briefly explains the appropriate levels of HA for the effective growth and yield of four genotypes of turnip.

MATERIALS AND METHOD

During November 2012 to April 2022, four turnip genotypes' root production and vegetative growth were investigated in a pot experiment that explored the effects of various humic acid treatments. The pot size measurments were (Length: 45.72 cm and width: 30.48 cm, respectively). Factor A consisted of seven distinct humic acid treatments (HAT1: 0, HAT2: 20, HAT3: 40, HAT4: 60, HAT5: 80, HAT6: 100 and HAT7: 120 kg/ha, while Factor B comprised four different genotypes (V1: Desi Faisalabad, V2: Vikima F1 (Denmark), V3: Purple white top globe and V4: Golden Bal). Three of four geneotypes, V2, V3 and V4 were hybride varieties. The experiment followed a factorial Completely Randomized Design (CRD) with two components. Each treatment was administered three times in total. Pots were filled with sun-dried soil, and specific levels of Humic Acid (HA) and appropriate NPK dosages of 120:65:100 kg/ha were incorporated into the pots. During November, the tested turnip varieties' seeds were sown in containers. Four seeds were sown within each container in a manner of equal spacing. The pots were regularly irrigated, and the soil was kept adequately moist after seed sowing to ensure proper germination. Additional cultural tasks, such as timely weeding, providing water, spraying, and applying pesticides, were performed as necessary. After harvesting, different parameters were investigated, such as Height of Plant (HP), Canopy Cover Percentage (CCP), Number of Leaves on Plant (NLP), Leaf Area (LA), Chlorophyll Content (CC), Fresh Leaf Weight (FLW), Dry Leaf Mass (DLM), Fresh Root Weight (FRW), Dry Root Mass (DRM) and Total Yield (TY).

Statistical analysis

A thorough statistical analysis was conducted to ascertain the treatment combinations' significance on all the examined parameters. This analysis was conducted using the statistical software "Statistix Version 8.1".

RESULTS AND DISCUSSION

Height of Plant (HP)

The height of turnip plants is impacted by various levels of HA, genotypes, and their interactions, as depicted in Figure 1.A, which offers significant insights. As indicated by the findings in Figure 1.A, there were significant variations among all the humic acid treatments in terms of HP. The recorded values ranged from 22.70 to 17.52 cm. The turnip plant treated with HAT4 exhibited the tallest height of 22.70 cm, tracked by HAT3 (40 kg/ha), HAT7, HAT2, HAT5, and HATT6 with heights of 21.19 cm, 19.63 cm, 18.35 cm, 17.64 cm, and 17.90 cm, respectively. Among the treatments, HAT1 (0 kg/ha) exhibited the lowest HP results at 17.52 cm, and it is worth noting that all of these treatments displayed significant differences. As the Humic Acid (HA) levels increased, HP showed a positive response up to a certain threshold (60 Kg/ha). However, an inverse relationship emerged beyond this threshold, and higher HA levels had a detrimental effect on HP. Additionally, Rose et al. (2014) observed similar outcomes, reporting a 22% increase in plant shoot growth with the application of exogenous HA fertilizers. The HP displayed significant variation among different genotypes, as depicted in Figure 1.A. The height range of the turnip plants varied between 17.85 cm and 20.81 cm. Remarkably, the tallest plants, measuring 20.81 cm, were observed in (V4), followed by V3 (19.63), V2 (19.43), and V1 (17.85 cm) in height. In Figure 1.A, the evaluation of the interaction between turnip genotypes and HA levels revealed significant differences in HP. The observed heights ranged from 23.83 to 14.40 cm. The maximum HP of 23.83 cm was observed for HAT4 V4 , and this treatment displayed contrasting behavior. Similar findings, with HP of 18.67, 18.23, 18.10, and 17.63 cm, respectively,

were observed in HAT5 V3, HAT6 V1, HAT2V2, and HAT5 V4. In contrast, the lowest HP response (14.40 cm) was observed in HAT1 V1, and the interactions exhibited distinct behavior. In terms of HP, all other treatments yielded inconsistent results.

Number of Leaves/Plant (NLP)

The leaf count of turnip plants is influenced by different concentrations of HA, turnip genotypes, and their interactions, as depicted in Figure 1.B.. The data in Figure 1.B clearly demonstrates significant distinctions among all the Humic Acid (HA) treatments concerning the NLP, with values ranging between 10.17 and 9.02. HAT4 exhibited the highest NLP (10.17), followed by HAT2, HAT1, HAT3, HAT5 and HAT6, with 9.75, 9.38, 9.37, 9.12, and 9.05 NLP. All the treatments displayed statistically significant differences except for the smallest result regarding the NLP (9.02), observed in the HAT1. The response plant^{-1} to increasing levels of Humic Acid (HA) was initially positive, leading to taller plant growth until a certain HA level was reached. Beyond that point, further increments in HA had a detrimental effect, resulting in a decrease in the NLP (60 kg/ha). Similar findings were reported by Gutiérrez et al. (2011), who observed an increase in NLP in root crops with higher concentrations of HA. Regarding HP, significant variations were observed for multiple genotypes, as depicted in Figure 1.B. The NLP ranged from 10.58 to 8.01. Remarkably, the highest NLP (10.58) was exhibited by genotype (V4), followed by (V3) with 9.61 NLP, (V2) with 9.45 NLP, and (V1) with 8.01 NLP. Furthermore, Subedi et al. (2018) made an interesting discovery regarding the vegetative development of radish cultivars. In various aspects, including the NPL, Mino Early Long outperformed other radish cultivars, as found by the researchers. Figure 1.B revealed a significant pattern for the NPL concerning the interaction between HA levels and turnip genotypes. Plant 1 The maximum and minimum values of 11.60 and 7.30 of NLP were recorded for V4 to V1. The highest NPL (11.60) was notably observed in the HAT4 V4 treatment, which stood in stark contrast to the others, displaying a substantial divergence in behavior. HAT3 V2, HAT3 V3, and HAT5 V3 displayed remarkably similar results, with 9.86, 9.36, and 8.93 NLP, respectively. On the other hand, the lowest response for the NLP (7.30) was observed in the HAT1 V1 treatment, and the interactions exhibited distinct behavioral patterns. As for plant height, all other treatments yielded inconsistent results.

Leaf Area (LA)

The information presented in Figure 1.C holds significant importance in understanding the impact of different combinations of humic acid treatments and cultivars on turnips' LA (cm^2). The data from Figure 1.C clearly demonstrates substantial variations in LA among all the Humic Acid (HA) treatments. The maximum and minimum values for LA were observed to be ranged from 65.16 to 44.06 cm^2, respectively. The recorded values ranged from 65.16 to 44.06 cm^2. HAT4 exhibited the largest LA, measuring 65.16 cm^2, followed by HAT6 (64.92), HAT5 (64.1), HAT3 (59. 29), HAT7 (55.0), and HAT1 (54.9 cm^2). Conversely, the least LA (54.9 cm^2) was observed in HAT1 HAT2, and these treatments displayed noticeable distinctions. It was observed that increasing the levels of humic acid led to a corresponding increase in LA (cm^2) until a certain threshold (60 kg/ha) was reached. Beyond that point, further increments in humic acid had a negative impact, resulting in a decline in LA. These findings align with previous studies conducted by Ahmad et al. (2013), who also obtained similar results, indicating that higher levels of humic acid contribute to larger leaves in root vegetables.

Based on Figure 1.C, the LA exhibited significant variations among different genotypes. The range of LA was between 58.43 and 48.46 cm^2. Notably, (V4) recorded the highest LA data (58.43 cm^2), followed by V3 (54.18), V2 (51.46), and V1 (48.46 cm^2). In investigating the interaction between turnip genotypes and HA concentrations, Figure 1.C demonstrated prominent patterns concerning LA. The obtained data showed a maximum value of 65.16 cm2 and minimum value of 44.06 cm^2 for LA. Remarkably, within the HAT4 V4 treatment, the maximum LA reached 65.16 cm^2, signifying a profound interaction effect. Additional remarkable findings encompassed measurements of 54.95 cm^2, 53.75 cm^2, and 53.56 cm^2 within the HAT4V2, HAT7V2, and HAT4V3 treatments, respectively. Conversely, the most minimal LA response (44.06 cm^2) was observed in the HAT2V1 treatment, showcasing distinct behavioral patterns in these interactions. It is imperative to highlight that all other treatments yielded incongruous outcomes concerning LA.

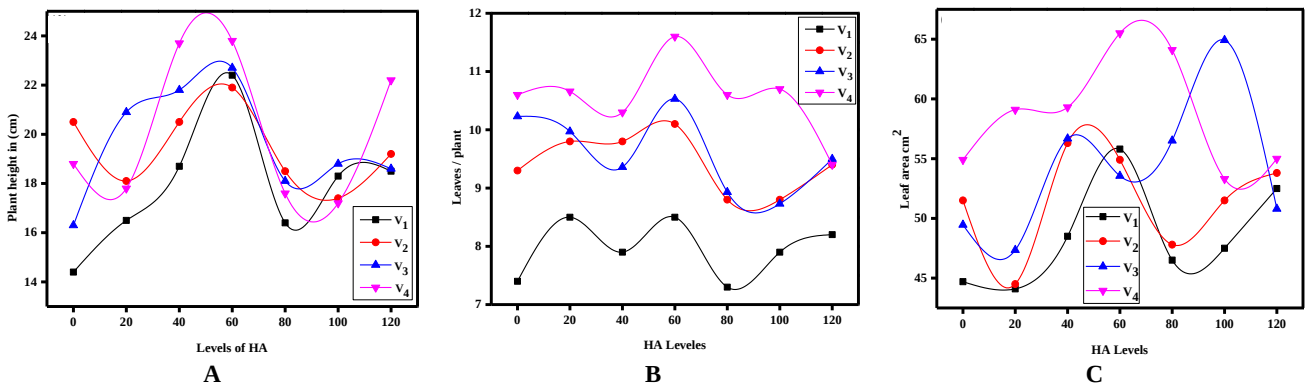

Figure 1. Response of (A) HP, (B) NLP, and (C) LA towards different levels of HA

Canopy Cover (CC) %

Figure 2.A encompasses crucial data regarding the proportion of the turnip plant's CC affected by different concentrations of humic acid, genotypes, and their interactions. The data presented in Figure 2.A revealed that all of the Humic Acid (HA) treatments displayed significantly distinct CC. The recorded values ranged from 63.8 to 17.36 % %. The HAT4 treatment exhibited the highest CC (63.8 %), followed by HAT7, HAT6, HAT3, HAT5 , and HAT2 with (54.60), (54.6), (51.66), (51.6), and (46.96 %). Although HAT1 showed the lowest CC data (34.30 %), it is important to note that these treatments displayed noticeable differences. Observing the response of canopy cover to increasing HA levels, it was observed that the CC increased up to a specific HA concentration (60 Kg/ha), beyond which it began to decline, negatively impacting the CC. Ahmad et al. (2013), Ahmed et al. (2013) and Khan et al. (2018) conducted research that demonstrated the positive influence of humic acid on leaf area and vegetative development in radish. Similarly, Figure 2.A provides significant insights into the behavior of CC among various genotypes. The observed values ranged from 63.8 to 17.36 %. Notably, (V4) displayed the highest CC data (63.8 %), followed by V3 (46.96), V2 (33.83), and V1 (31.23 %). Concerning the interplay between turnip genotypes and HA levels, Figure 2.A disclosed marked patterns regarding the CC. The recorded data ranged between 63.8 and 17.36 %. Notably, the highest CC (63.8 %) was significantly observed in the HAT4V4 treatment, indicating a significant interaction. Comparatively similar results were obtained in HAT1 V2, HAT4 V1, and HAT3 V2, with percentages of 31.66, 31.23, and 30.76 %, respectively. On the other hand, the lowest response in terms of CC (17.36 %) was observed in the HAT1 V1 treatment, and these interactions exhibited substantially distinct behaviors. It is important to note that all other treatments yielded mediocre results regarding CC.

Leaf Chlorophyll Content (LCC)

The data in Figure 2.B is significant in elucidating the ramifications of varying HA levels, genotypes, and their interactions on the LCC of turnips. According to the data in Figure 2.B, all HA treatments displayed substantial variations in LCC from one another. The recorded statistics ranged from 61.80 to 30.0. The highest LCC (61.80) was observed in the HAT4 treatment, while the values for HAT3, HAT2, HAT7, and HAT6, an were 57.76, 57.33, 57.10, and 52.26, respectively. Despite the HAT1 treatment manifesting the lowest LCC values at 30.0, it is imperative to underscore the salient disparities observed among all these treatments. The association amid the escalation of HA levels and LCC unraveled a discernable trend, wherein it initially amplified the chlorophyll content up to a designated HA level of 60 kg/ha. However, beyond that threshold, the LCC began to decline, having a negative impact. This observation aligns with previous studies conducted by Ahmad et al. (2013) which also reported similar findings indicating that humic acid promotes increased development, enhanced leaf area, and elevated LCC of root vegetables. Based on Figure 2.B, the LCC exhibited significant variations among different genotypes. The range of LCC spanned from 61.80 to 30.0. Remarkably, (V4) registered the greatest LCC value of 61.80, succeeded by V3 (51.76), V1 (47.13), and V2 (46.10).

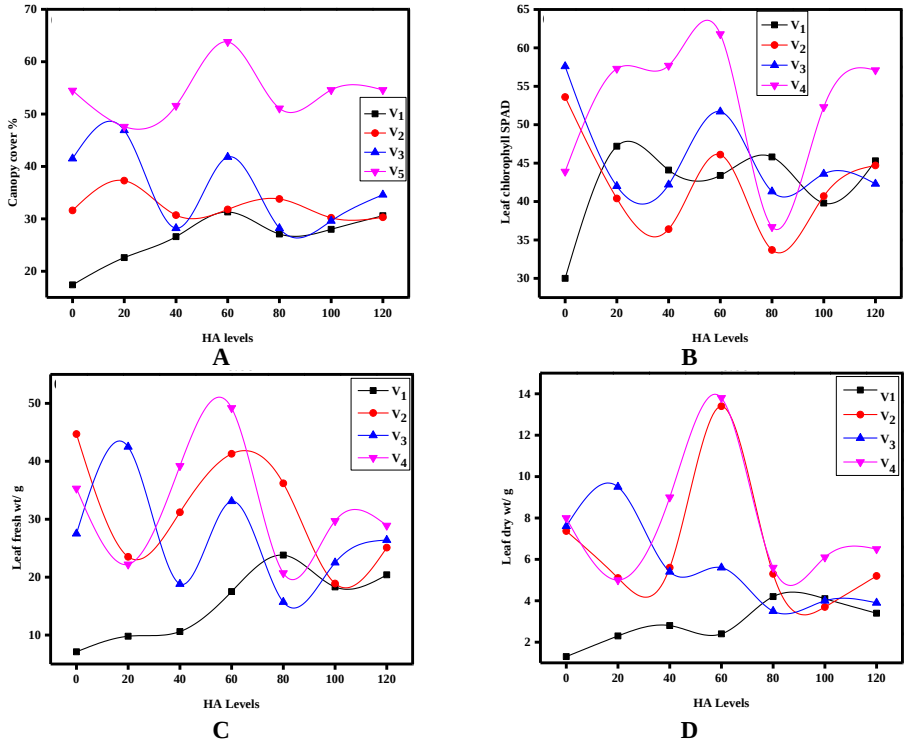

Figure 2. Impact of HA levels on (A) CC %, (B) LC, (C) LFW, and (D) LDW

Dongarwar et al. (2017) described similar findings, stating that the Japanese White variety had the highest observed LCC, followed by the variety Arka Nishant. It is important to note that the amount of chlorophyll in a leaf can vary depending on the leaf's type, size, and genotypic characteristics. Furthermore, a substantial pattern was observed for LCC when analyzing the interplay between HA levels and turnip genotypes, as illustrated in Figure 2.B. The data varied from maximum value of 61.80 to minimum valueof 30.0 for LCC. The highest LCC (61.80) was significantly observed in the HA4 V4 treatment, indicating a significant interaction among the factors. Comparatively, HAT7 V3, HAT3 V3, and HAT2 V3 treatments produced results of 42.23, 42.16, and 42.0, respectively. On the contrary, the HAT1 V1 interaction exhibited the least LCC response at 30.0, and these interactions demonstrated highly distinct behaviors from each other. Notably, all the other treatments resulted in mediocre outcomes in terms of LCC.

Leaf Fresh Weight (LFW)

The data provided in Figure 2.C regarding the number of Turnip LFW (g) and its relationship with various HA Levels, Genotypes, and their interaction holds significant importance. The data presented in Figure 2.C demonstrated substantial variations in LFW among the different Humic Acid (HA) treatments (g). The recorded values ranged from 49.16 to 7.09 grams. The highest LFW of 49.16 grams was observed in the HAT4, followed by values of 42.49, 41.33, 39.12, and 36.14 grams for the HAT2, HAT4 , HAT3, and HAT5, respectively. Conversely, the HAT1 exhibited the lowest results in terms of LFW, with a value of 7.09 grams. Significantly, each of these treatments exhibited notable statistical dissimilarities from one another. Vitally, all of these treatments manifested significant statistical distinctions from one another. The reaction of LFW in response to escalating HA levels revealed an intriguing pattern. Observations revealed an augmentation in LFW up to a particular HA level of 60 kg/ha. However, the LFW started to decline beyond that point, resulting in a negative impact. Furthermore, Figure 2.C displayed significant variations in LFW among different genotypes. The data showed maximum (49.16) and minimum (7.09) values for LFW, respectively. The (V4) exhibited the greatest LFW of 49.16 g, while V3 (42.49), V2 (41.33), and V1 (23.80). These discoveries maintain congruence with the investigations undertaken by Dongarwar et al. (2017) who ascertained that the Pusa Reshmi cultivar exhibited significantly superior maximum LFW in comparison to other cultivars (Mani and Anburani 2018). Figure 2.C elucidated substantial divergences in the LFW behavior when analyzing the interplay between HA concentrations and turnip genotypes. The observed data spanned a range from 49.16 g to 7.09 g. The HAT4 V4 treatment demonstrated the highest LFW of 49.16 grams, signifying a substantial interaction between the factors. Comparatively, HAT5 V1, HAT2 V2, and HAT6 V3 exhibited values of 23.80, 23.45, and 22.54 grams, respectively, which were noticeably similar. On the other hand, the lowest response in terms of LFW (7.09 g) was reported in the HAT1 V1 treatment, and these interactions displayed distinctly different behaviors. It is worth noting that all the other treatments yielded inconsistent results in terms of LFW.

Leaf Dry Weight (LDW)

Figure 2.D signifies critical data regarding the implications of varying HA concentrations, genotypes, and their influences on the LDW. The data provided in Figure 2.D clearly demonstrates significant variations in LDW among all the HA treatments. The maxium and minimum values of 13.79 and 1.28 g were LDW. The HAT4 treatment exhibited the highest LDW (13.79 g), followed by the HAT2, HAT3, HAT7, HAT6, and HAT5 treatments with 9.50, 9.0, 6.46, 6.10, and 5.55 grams, respectively. Conversely, the HAT1 treatment had the lowest data for LDW (1.28 g). Importantly, all of these treatments displayed noticeable differences from one another. The response of LDW to increasing HA levels revealed an interesting pattern. It was observed that HA enhanced LDW up to a specific HA level (60 kg/ha). However, there was a decline in LDW beyond that threshold, resulting in a negative impact. This observation is consistent with a previous study conducted by Esringü et al. (2016) demonstrating HA's positive effect on LDW in Walleriana and its overall beneficial impact (Ibrahim et al. 2016). Furthermore, as indicated in Figure 2.D, the LDW displayed significant variations among different genotypes. The range of data observed was from 13.79 to 1.28 g. Notably, (V4) exhibited the highest LDW (13.79 g), followed by V2 (13.35), V3 (9.50) and V1 (4.16 g). These findings are consistent with the investigations carried out by Abdel (2016), who reported analogous outcomes accentuating that the "Cheongdae" and "Chunha" radish cultivars demonstrated elevated LDW and greater percentages of dry matter in comparison to other cultivars. Figure 2.D unveiled substantial discrepancies in the LDW behavior when analyzing the interaction between turnip genotypes and humic acid concentrations. The data ranged from 13.79 to 1.28 g. Notably, the maximum LDW of 13.79 grams was significantly observed in the HAT4 V4 treatment, indicating a significant interaction among the factors. Comparatively, HAT4 V3, HAT3 V3, and HAT5 V2 exhibited LDW results of 5.60, 5.40, and 5.23 grams, respectively. On the other hand, the lowest response in terms of LDW (1.28 grams) was reported in the HAT1 V1 treatment, and these interactions displayed noticeably distinct behaviors. It is important to note that all the other treatments yielded inconsistent results regarding LDW.

Root Fresh Weight (RFW)

Figure 3.A provides crucial insights into the impact of different humic acid levels, genotypes, and their interactions on the RFW of turnip. The data presented in Figure 3.A clearly indicates substantial variations in RFW among the different HA treatments. The obtained results showed a maximum and minimum values of 161.35 and 11.74 g, respectively. The HAT4 treatment exhibited the highest RFW (161.35 g), followed by the HAT3, HAT5, HAT6, HAT2, and HAT7 treatments with 113.07, 86.18, 80.21, 77.13, and 66.42 grams, respectively. Conversely,

the HAT7 treatment recorded the lowest results for RFW (66.42 g). Importantly, all of these treatments displayed statistical differences from one another. Furthermore, the response of RFW to increasing HA levels revealed an interesting pattern. It was observed that up to a specific HA level (60 kg/ha), there was an enhancement in RFW. However, beyond that point, RFW began to decline, resulting in a negative impact. Similar findings have been reported in studies conducted by Heba et al. (2014) on sugar beet, and Shafeek et al. on Japanese. These studies support the observations made in Figure 3.A regarding the significant behavior of RFW across various genotypes. The data in Figure 3.A exhibited a range of 161.35 to 11.74 g for RFW. Notably, (V4) exhibited the highest RFW data (161.35 g), followed by V3 (127.28), V2 (86.18), and V1 (32.43 g). The interaction between HA concentrations and turnip genotypes, as depicted in Figure 3.A, revealed significant variations in the behavior of RFW. The data ranged from 161.35 to 10.48 g. Notably, the maximum RFW of 161.35 g was significantly observed in the HAT4 V4 treatment, indicating a significant interaction among the factors. Comparatively, HAT2 V3, HAT2 V3, and HAT4 V2 exhibited 71.63, 77.13, and 77.27 g, respectively, which were noticeably comparable. On the other hand, the lowest response in terms of RFW (10.48 g) was reported in the HAT2 V1 treatment, and these interactions displayed distinctly different behaviors. It is important to note that all the other treatments yielded inconsistent results in terms of RFW.

Root Dry Weight (RDW)

Figure 3.B provides crucial insights into the impact of different humic acid concentrations, genotypes, and their interactions on the RDW of turnip roots. The data presented in Figure 3.B clearly demonstrates significant variations in RDW among all the Humic Acid (HA) treatments (g). The maximumand minimum values of RDW were recorded from 32.67 to 1.51 g The HAT4 treatment exhibited the highest RDW (32.67 g), followed by the HAT3, HAT2 , HAT7 , HAT6 , and HAT5 treatments with 14.39 , 12.36 , 11.13 , 10.82 , and 10.7 grams, respectively. Conversely, the HAT2 treatment recorded the lowest results for RDW (10.7 g). Importantly, all of these treatments displayed statistical differences from one another. The response of RDW to increasing HA levels revealed an intriguing pattern. It was observed that up to a specific HA level (60 kg/ha), there was an increase in RDW. However, the RDW started to decline beyond that threshold, resulting in a negative impact. These findings align with similar observations made in studies conducted by Heba et al. (2014) on sugar beet. Furthermore, as indicated in Figure 3.B, the RDW displayed significant variations among different genotypes. The data ranged from 32.67 to 1.51 g. Notably, (V4) exhibited the highest RDW data (32.67 g), followed by V3 (14.39), V2 (12.76) and V1 (3.40 g). These findings further support the studies mentioned above and highlight the impact of genotypes on RDW. The interaction between turnip genotypes and HA concentrations, as depicted in Figure 3.B, revealed significant variations in the behavior of RDW. The data ranged from 32.67 to 1.51 g. Notably, the maximum RDW of 32.67 g was substantially observed in the HAT4 V4 treatment, indicating a significant interaction among the factors. Comparatively, HAT7 V2, HAT6 V2, and HAT2 V2 exhibited root dry weight results of 9.36, 9.32, and 8.80 g, respectively. On the other hand, the lowest response regarding RDW (1.19 g) was reported in the HAT2 V1 treatment, and these interactions displayed noticeably distinct behaviors. It is important to note that all the other treatments yielded inconsistent results in terms of RDW.

Total Yield (TY)

Figure 3.C presents invaluable information regarding the influence of different HA levels, genotypes, and their interactions on turnip TY (t/ha). The data presented in Figure 3.C clearly demonstrate the significant variations in average yields (t/ha) among the various HA treatments. The recorded maximum and minimum values for TY were ranged from 18.91 to 1.71 (t/ha). Notably, the HAT4 crop exhibited the highest TY of 18.91 t/ha, followed by the HAT3, HAT2 , HAT5, HAT6, and HAT7 crops with yields of 14.76, 11.56, 9.75, 9.36, and 8.55 t/ha, respectively. These results highlight the substantial differences in turnip TY among the different treatments. The obtained data indicated that HAT7 exhibited the lowest TY (8.55 t/ha), and it was evident that all of these treatments significantly differed. The response of TY to increasing HA levels showed a positive trend up to a specific HA level (60 kg/ha).

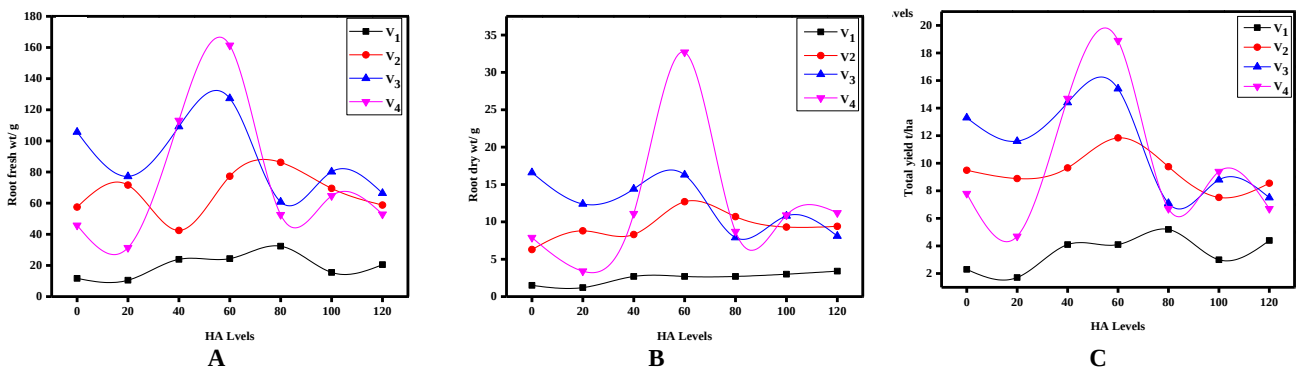

Figure 3. Effect of HA doses on (A) RFE, (B) RDW, and (C) TY

However, beyond that threshold, the total yield began to decline, negatively impacting the TY. Interestingly, the results demonstrated that adding organic manure significantly enhanced the total root output and the physical quality of the roots compared to the untreated control. This highlights the advantage of incorporating organic compost manure alongside fertilizers, surpassing the benefits of using fresh or dry manure alone. The total yield (t/ha) exhibited significant variations among several genotypes, as highlighted in Figure 3.C. The recorded data ranged from 18.91 to 1.71 tonnes per hectare (t/ha). Notably, (V4) achieved the highest TY (18.91 t/ha), followed by V3 (15.37), V2 (11.84) and V1 (5.22 t/ha). Regarding the interaction between HA concentrations and turnip genotypes, Figure 3.C revealed significant behavior about the TY. The data ranged from 18.91 to 1.71 t/ha. Remarkably, the highest TY of 18.91 t/ha was significantly observed in the HAT4 V4 treatment, indicating a significant interaction among the factors. Moreover, 7.52, 7.48, and 7.11 t/ha were also observed in the HAT6 V2, HAT7 V3, and HAT5 V3 treatments, respectively. Conversely, the HAT2 V1 interaction demonstrated the lowest response regarding TY, with a 1.71 t/ha value. It is important to note that these interactions exhibited statistically distinct behaviors. For the remaining treatments, the TY values fell within the intermediate ranges.

In conclusion, in light of the conducted investigation, many inferences can be derived. Firstly, using humic acid at a precise concentration of 60 Kg/ha greatly impacted turnip growth and yield. This intermediary level led to the utmost values in a myriad of growth parameters, encompassing plant height, leaf quantity per plant, leaf expanse, canopy coverage percentage, leaf chlorophyll content, root mass in both fresh and desiccated states, leaf weight when fresh or desiccated, and total yield per hectare.

Secondly, amidst the scrutinized turnip genotypes, the Golden Bal genotype demonstrated unparalleled performance across all gauged criteria. This particular genotype consistently outperformed its counterparts in terms of both vegetative and reproductive characteristics, unequivocally attesting to its superiority concerning growth and yield potential. Moreover, the interplay between humic acid concentrations and turnip genotypes significantly influenced growth and production outcomes. The amalgamation of the Golden Bal genotype with humic acid administered at 60 Kg/ha concentration consistently yielded the most favorable results across all measured parameters. This synergistic interrelation significantly augmented turnip growth and productivity.

Based upon these discerned outcomes, it is strongly advised to cultivate the Golden Bal turnip genotype while concurrently applying humic acid at the prescribed 60 kg/ha concentration to optimize yield within the distinct agroclimatic milieu of Dera Ismail Khan. This well-established combination has unequivocally showcased its profound effectiveness in fostering desirable growth attributes and maximizing the overall productivity of turnip cultivation.

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Applying home-based experiments on locally isolated *Dictyostelium discoideum* to qualitatively demonstrate taxis of social amoebae

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Abstract. *Claudio-Paragas CY, Balaoro-Banzuela RC, Dagamac NHA, Ocenar-Bautista CE. 2023. Applying home-based experiments on locally isolated* Dictyostelium discoideum *to qualitatively demonstrate taxis of social amoebae. Cell Biol Dev 7: 75-81.* Recent years have seen a growing interest in studies on slime molds based in the Philippines, but the inclusivity of Dictyostelids has been largely overlooked. The country has very few studies investigating this category of microbial predators over the past two decades despite their ecological importance in maintaining balance in the soil ecosystem. Thus, we consolidated a multifaceted assessment that examined the behavioral response of locally isolated dictyostelids to an array of external stimuli, particularly under light- and food-induced conditions. The tail-end movement of the motile cells of the clear-cut species, *Dictyostelium discoideum* Raper, was assessed through a proxy indicator based on the fructification of the species. This was done by setting up two simple home-based experimental setups that investigate the effect of light wavelengths and prey cell viability based on the differentiation rate and one choice experiment that looks into the designation of fructification of *D. discoideum*, whether it preferentially differentiates under light or in darkness. Our setups revealed the following: (i) fruiting bodies develop in any light wavelength, but fructification is fastest in white ambient light; (ii) dead microbial cells constitute a lag in fruiting body development; and (iii) the decision-making of *D. discoideum* does not prefer photo avoidance.

Keywords: Eumycetozoans, morphogenesis, protist, soil ecology, spores

INTRODUCTION

One of the most intriguing aspects in the study of slime molds is reflected in the unique life cycle of Dictyostelids, which conforms to a picture-perfect manifestation of social cooperation among microbial communities. This underlying behavioral trait is often linked with multicellularity, microbial sociality, and faunal societies. The cellular slime molds, dictyostelids, are diverse groups of single-celled eukaryotic organisms ubiquitously found in most soils (Liu et al. 2020). Having phagotrophic nutrition that engulfs microbial communities, these microbicidal predators are considered great bioindicators of soil microbial activity. They are vital to maintaining balance in the soil microhabitat (Coleman and Wall 2015). Dictyostelids are naturally used as model organisms in studies to answer concerns regarding multicellularity, social evolution, and cell biology (Müller-Taubenberger 2013). According to a study by Ostrowski et al. (2008), it has been found that affiliated genes among samples are connected to the organisms' behaviors, such as kin discrimination. In contrast, dictyostelids are observed to have a different interaction among aggregating with isolates genetically similar to them compared to ones being more geographically distant.

Arguably, the most well-studied dictyostelid species, *Dictyostelium discoideum* Raper, has a unique life cycle consisting of vegetative, social, and sexual phases (Li and Purugganan 2011). Its vegetative cycle is characterized by food and nutrition, where free-living haploid cells prey upon bacteria in their surroundings and divide mitotically at set intervals. Upon starvation when food becomes scarce, *D. discoideum* cells stop dividing and may enter its social or sexual cycle (Kin and Schaap 2021). On the one hand, the sexual phase of *D. discoideum* is initiated through the fusion of two haploid cells of different mating types, which is followed by the cannibalization of surrounding dictyostelids cells, giving rise to a specialized structure known as the macrocyst, where recombination and meiosis take place (Schaap 2011). On the other hand, the social cycle of *D. discoideum* commences with the aggregation of starving haploid cells, resulting in the formation of a multicellular motile slug and ultimately culminates in the production of spore-bearing fruiting bodies where the cycle can be reinitiated (Marée and Hogeweg 2001). One of the defining features of the social cycle of *D. discoideum* is marked early into the cycle, where the aggregation of individual amoebae is orchestrated by cascading cAMP-signaling pathways, which are generated by an interconnected network of adenylyl cyclases (Kawabe and Schaap 2023). The movement of haploid *D. discoideum* cells is owed largely in part to its section of cyclic AMP (cAMP), which induced a chemotactic gradient for the signal relay, kinesis, and the expression of genes

responsible for development and differentiation (Eidi et al. 2021). The multicellular slug contains differentiated cells with distinct localizations-prestalk cells predominate the anterior region, while prespore cells are otherwise localized in the posterior region (Inouye 1992).

Taxis in *D. discoideum* play an underlying role in their development and differentiation, particularly evident during the migratory slug phase, where its kinesis is mediated by light (phototaxis), temperature (thermotaxis), pH (acidotaxis), and wind (rheotaxis). The interplay among these environmental gradients orients the slug towards the soil surface, simulating an optimal environment for better spore dispersal following fructification (Fisher 1997; Marée and Hogeweg 1999). Behaviors of these cellular slime molds have received greater attention in temperate countries and have exhibited a shortfall in tropical regions since they were discovered by Brefeld in 1869. Recent experiments done in the Philippines are mostly with myxomycetes with their heavy metal biosorption and enzyme production (Macabago and dela Cruz 2014; Rea-Maminta et al. 2015), whereas the studies regarding dictyostelids have done isolation to discover their food preferences (Yulo and dela Cruz 2012b). Moreover, the recently published studies also concentrated on the slug phase of dictyostelids instead of studying the part of their fructification process (Kosugi and Inouye 1989; Marée and Hogeweg 1999). These bioassays conducted for the cognitive nature of social amoebae also utilized mostly expensive and not readily accessible equipment in the laboratory, as opposed to areas such as developing countries with a lack of funds and laboratory spaces.

Hence, this study aims to develop simple experimental assays that investigate the dynamics of food based on cell viability and phototaxis that also bear decision-making capabilities in lower eukaryotes, as exemplified by *D. discoideum.*

MATERIALS AND METHODS

Isolation of the *D. discoideum* employed in this study follows a modification of the protocol established by Cavender and Raper (1965). In this technique, 10 g of each collected soil sample from a montane habitat in Northwestern Philippines was diluted in 90 mL of distilled water to yield a 1:10 dilution. Then, 5 mL of the soil suspension was diluted in 7.5 mL of distilled water to yield a 1:25 dilution. Lastly, 5 mL from this suspension was transferred to Hay Infusion Agar (HIA, boil 10 g hay in 1 L distilled water for 20 minutes), and 15 g of agar was added to the mixture to reach a final dilution of 1:50. Subsequently, 0.4 mL 24-hour old suspension of *Escherichia coli* was added to the suspension to act as a food source which rendered the culture as two-membered (Yulo and dela Cruz 2012a; Guyer et al. 2017). Typical morphology of *D. discoideum* that includes stalk, spore characteristics, and branching pattern was observed to confirm identification for the specific species alongside the bases of various journals for further verification of their traits. They are then purified via isolation onto fresh new HIA plates with *E. coli* using a combination of agar blocking and spore touch technique.

Setup I: Fructification of *D. discoideum* in varying light wavelengths

The progression of the life cycle in *D. discoideum* cultures was investigated under different wavelengths of light based on 5 setups (Figure 1): (i) white light (380-780 nm); (ii) red light (620-780 nm); (iii) yellow light (570-585 nm); (iv) blue light (440-490 nm); and (v) dark setup. Commercially available lightbulbs were placed on top of the prepared light chambers in an undisturbed area. They ensured proper measurements for all agar plates and easily opened lids for observation. Moreover, the wavelengths of each are based on the standard measurement of each type of light. All these setups are repeated in 6 replicates per setup. The dark setup utilized aluminum foil, covering the whole plate; therefore, no light could seep through the samples. The *D. discoideum* colonies were isolated in HIA plates using agar block transfer by cutting a block of agar with the organism and transferring it to another blank agar plate. They followed by the spore touch technique, which is done by using a modified small glass pipette to become needle-like and rupturing the spores using it to introduce them onto the surface of the agar. Therefore, the spore touch technique ensured accurate and unbiased results by utilizing three spores for each plate. This was followed by introducing 0.4 mL 24-hour-old *E. coli* suspension as a food source. Cultures were incubated at room temperature for 24 hours under the above light. Life cycle structures (slug, aggregate, early fruiting body, mature fruiting body) were checked and counted every 6 hours. The data was then translated into binary information to see if fruiting bodies were present (1) or absent (0) at the time interval when it was checked. A logistic regression was then performed using the software JASP to account for their numerical equivalent regarding their fructification statistically.

Setup II: Fructification of *D. discoideum* in varying cell viabilities

Growth of dictyostelids using different viabilities of *E. coli* cultures (Figure 3) was assessed by first purifying *D. discoideum* colonies through agar block and spore touch technique onto fresh HIA plates. Upon introduction of spores, 0.4 mL of 24-hour old suspension of *E. coli* was inoculated in three different setups based on the viability of the *E. coli* cultures that were used as a food source: (i) live culture; (ii) dead culture (autoclaved); and (iii) mixture of dead (heat-killed) and live culture. Plates were then incubated under diffused white light or in the dark for 24 hours at room temperature. Observations were recorded at 6 hr., 12 hr., and 24 hr. time points based on the presence of mature fruiting bodies. Similar to the statistical analysis in Setup I, the data was initially transformed into binary information to see if the fruiting bodies were present (1) or absent (0) at the time interval when it was checked. A logistic regression was then performed using the software JASP to statistically account for which of those life stages dominates on the food source (cell viability) setup.

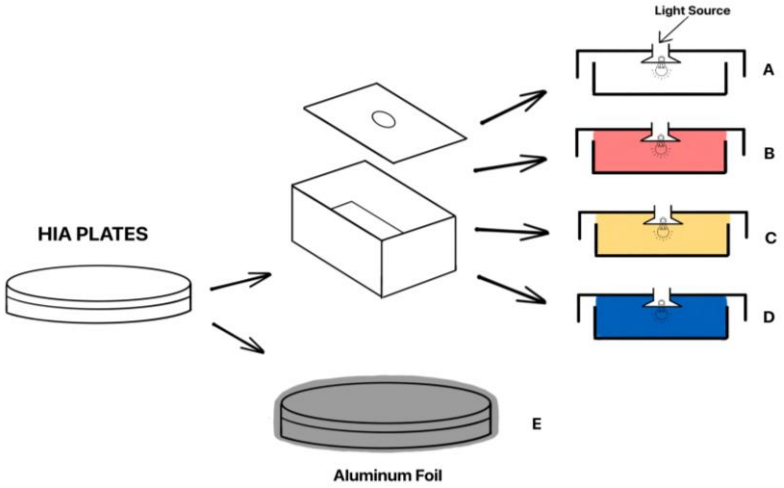

Figure 1. Experimental setup for *D. discoideum* fructification under different light wavelengths: A. White light, B. Red light, C. Yellow light, D. Blue light, and E. Dark setup

Figure 2. Sample experimental setup for observation of fructification under different light wavelengths

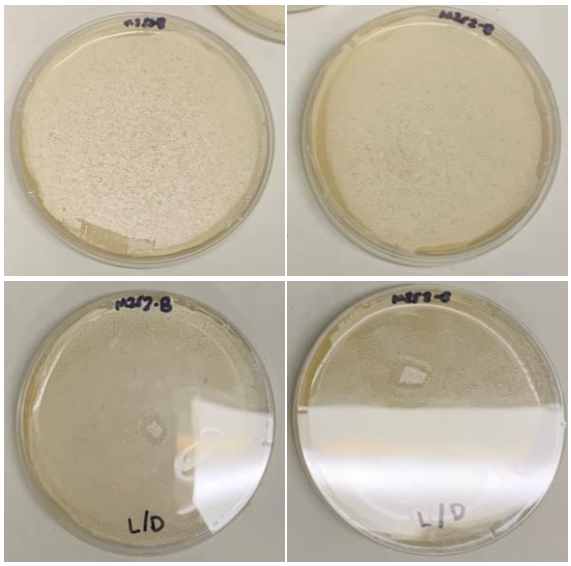

Figure 3. Sample experimental setup of agar plates in fructifying *D. discoideum* in varying cell viabilities

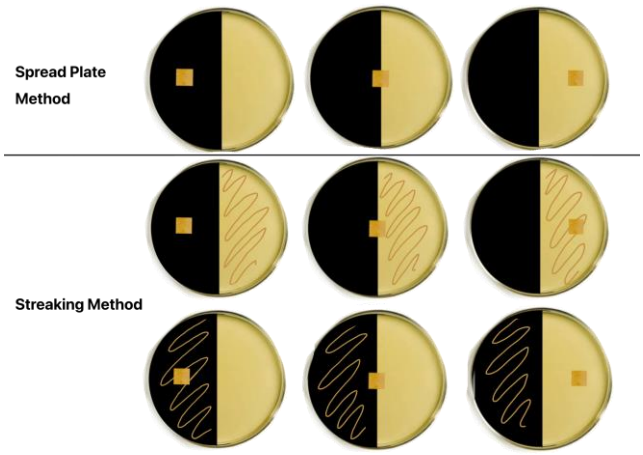

Figure 4. Experimental setup used for the phototaxis bioassay in which the plates differentiate in agar blocks and food source placement

Setup III: Light-based decision-making capabilities of *D. discoideum*

Given the wavelength of light preferred by *D. discoideum* in setup I and the preference for either live or heat-killed *E. coli* as a nutrition source in setup II. The directionality of *D. discoideum* toward or away from the light in the presence of food sources is assessed by producing HIA plates that are half-coated with black paint on both their top and bottom surfaces. This is then followed by the described isolation of *D. discoideum* from the purified plates employing the combination of agar blocking and spore-touch technique. The setup shown in Figure 2 consists of 9 plates with varying placements of the food source and the agar block relative to the light and dark halves of the plate to elucidate the preference of *D. discoideum* for either nutrition or light. Incubation is done under diffuse light which ran for 72 hours with observation points set at 24-hour intervals. The directionality is recorded in binary based on which side of the plate is

dominantly occupied by fruiting bodies-light side (1) or dark (0). This is then applied to the JASP software for logistic regression and data analysis.

RESULTS AND DISCUSSIONS

Response of *D. discoideum* morphogenesis to varying light wavelengths

In the response of *D. discoideum* isolates to different wavelengths of light, logistic regression was utilized to reveal the corresponding fructification patterns based on a 24-hour progression scale (Figure 5). Here, it can be observed that among the light wavelengths, the white light setup exhibited the earliest appearance of mature fruiting bodies (Figure 6). This can be seen as early as the 10th-hour mark. This is followed by the red light, which is evident between the 18th to 19th hour, and the yellow and blue light on the 21st hour. The dark light setup is the last setup to reveal any form of mature fruiting bodies, which can be seen between the 23rd and 24th hr mark.

Response of *D. discoideum* fructification to varying cell viabilities

Binary scores (based on presence/absence) of *D. discoideum* fruiting bodies designated in 6-hour intervals differed substantially among cell viabilities under diffused light, resulting in the earliest fructification of said species in live *E. coli* culture, followed by the mixed *E. coli* culture, and lastly, dead *E. coli* culture. The results of this experimental setup coincide with the previously discussed assay (see Setup I), which denotes a relatively delayed fructification of *D. discoideum* in the dark setup. Notwithstanding, this assay established that this phenomenon is regardless of the cell viability on which the species feeds as evaluated by how similar the fructification had appeared temporally.

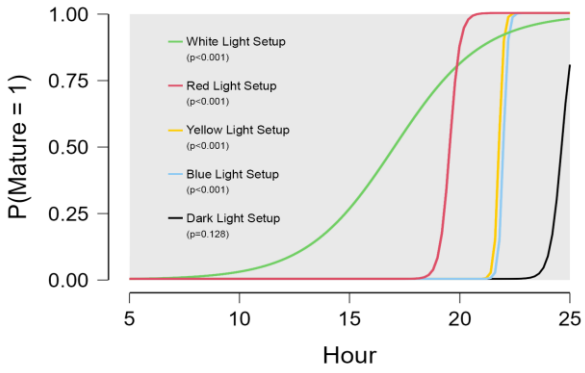

Figure 5. Fructification of dictyostelids in multiple wavelengths of light. Logistic regression curves show the probability of mature fructification bodies of *D. discoideum* based on 24-hour time progression and wavelengths of light

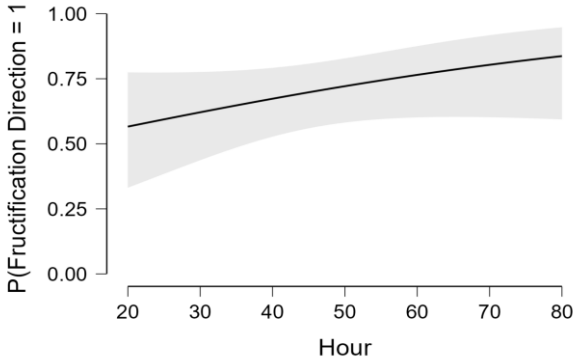

Figure 7. Fructification of *D. discoideum* under diffuse light using varying setups with plates that are half covered to portray darkness. Logistic regression plots illustrate the presence of the dictyostelids' mature fruiting bodies, contingent upon the presence of light

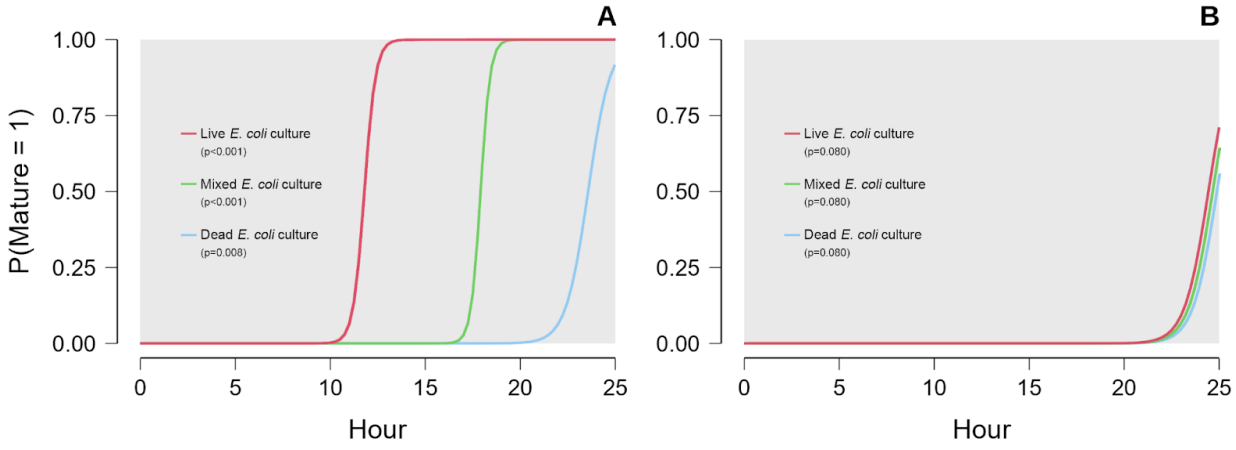

Figure 6. Fruiting body development of dictyostelids in different *E. coli* viabilities. Logistic regression curves show the rate of fructification of dictyostelids on 24-hour progression based on light setup: A. White, B. Dark

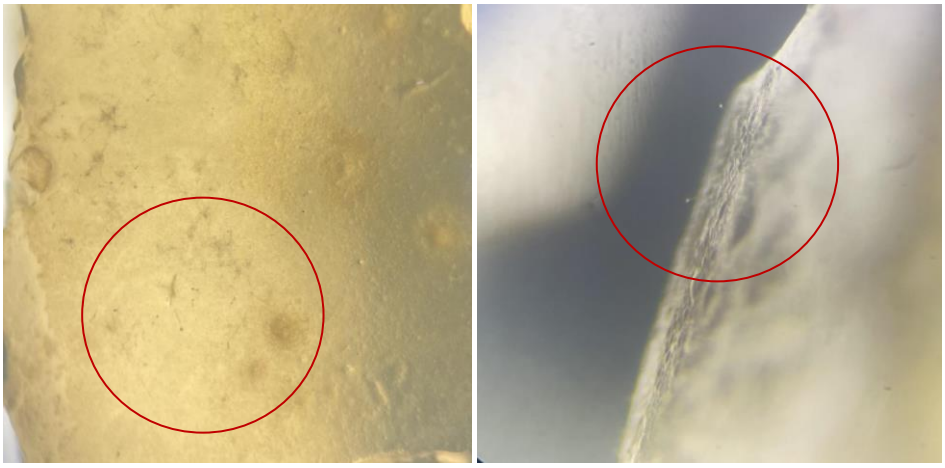

Figure 8. Two samples in different agar plates were observed to have positive phototaxis based on their placement

Light-based decision-making capabilities of *D. discoideum*

Upon subjecting the data to logistic regression analysis, binary scores were used (light=1, dark=0) based on the directionality of *D. discoideum* on the plate (Figure 7). Therefore, it demonstrates that the initial fructification of the dictyostelids, which were already expressing their preferred path, occurred at the 12th hour, exhibiting a progressive increase in abundance of positive plates until the 72nd hour. These fruiting bodies displayed that despite the method employed for food (streaking or spread plate) (Figure 4), a higher likelihood of occurrence will initially be on the side of the agar plate exposed to light, a trend that persisted consistently across the majority (70%) of the samples.

Discussion

This study showcases three simple home experiments that demonstrate the effects of certain conditions in the development of *D. discoideum.* Efforts to understand indigenous protists in the Philippines focus mainly on the taxonomy and diversity of isolates, and related studies on fruiting body development or food preferences are still limited in the tropics. Therefore, to address the basic cellular development among social amoebae using *D. discoideum* as a model, varying light intensity for growth and cell viability as a food source was tested with a simple setup that demonstrates cellular decision-making using phototaxis.

Effect of various light wavelengths on the morphogenesis of D. discoideum

Chemotactic aggregation in *D. discoideum* induces directed cell movement incorporating different forms, such as growing solitary cells and the more developed multicellular organism profile (Loomis 2015). As such, the spatial distribution of the selected cellular slime molds was subjected to different wavelengths of light and was observed for 24 hours. There has been reported evidence that suggests a photosensory transduction complex is present in *D. discoideum* involving at least five proteins: RasD, ErkB, filamin, PKB, and ErkB (Bandala-Sanchez et al. 2006). Therefore, the phototaxis behavior of the species can be attributed to the complex mentioned above, as evidenced by the morphogenic response observed in the bioassay. Throughout the lifecycle of *D. discoideum*, the motile stage, when they are slugs, becomes light-sensitive, allowing them to move towards locations with optimal light exposure to become mature fruiting bodies (Miura and Siegert 2000). With this, it was observed that the white light setup (380 to 780 nm) initiated the earliest fructification from *D. discoideum.* It contains every electromagnetic (EM) radiation in the visible light spectrum, thus cannot be limited to a specific wavelength. White light was also used in a study that displayed results for positive phototaxis using fluence-response (Hong et al. 1981). Red light (600-700 nm) consecutively showed species growth, followed by yellow light (570 nm) and blue light (450-495 nm). Hence, variation in light wavelength has successfully exhibited a significant difference in fructification.

Depletion of nutrients of *D. discoideum* follows different alternative pathways for survival – aggregation, which involves fructification, developing solitarily, or fusing and attracting cells, eventually forming a macrocyst (Schaap 2011). Such dictyostelids exhibit chemotaxis involving signaling complexes, attractant-induced cAMP synthesis, and signal relays. Present complexes in the species can be expected to make essential contributions in phototaxis, such as the protein RasD; a study involving gene disruption for RasD results in a near-total loss of phototaxis in mutant aggregates of Dictyostelium (Wilkins et al. 2000). The light deemed to influence the growth rate of the cultures, having a statistically significant difference between the multiple wavelengths (Chang et al. 1983). When exposed to dark conditions, dictyostelids form macrocysts, using ethylene as a certain trigger (Chang et al. 1983; Amagai 1984). This survival strategy, however, displays slow and inefficient germination, supporting the results of the morphogenesis assay under the dark setup. The mass movement of the cells in *D. discoideum* can suggest an oriented movement such as phototaxis

supported by different proteins but also triggers chemotactic aggregation primarily led by cAMP that completes the signaling pathways for morphogenesis.

Effect of various cell viabilities to the fructification of D. discoideum

The phagocytic nature of cellular slime molds has been extensively investigated in past literature, which designates a biomedical value to these organisms due to their generalist feeding behavior that predates diverse microbial species that include but not limited to gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*), Gram-positive bacteria (*Micrococcus luteus*, *Bacillus subtilis*, and *Staphylococcus aureus*) and yeasts (*Saccharomyces cerevisiae*, *Candida famata*, and *Rhodotorula* sp.) (Yulo and dela Cruz 2012b). In this experimental setup, *D. discoideum* isolates were inoculated in HIA plates with *E. coli* in varying viabilities (live culture, mixed culture, and dead culture) to assess their rate of fructification conforming to food supply pressure under light and dark setups. The assay's earliest recorded presence of *D. discoideum* was inducted 12 hours post-inoculation in the two-membered culture of live *E. coli* under diffused light. The time it took for the purified cellular slime molds to reach fructification in this setup surpasses that of related studies, which, under optimal conditions, took 24 hours on average to operate the completion of the said species' life cycle (Fisher 2001). Conversely, sporocarp formation in cultures with dead *E. coli* occurred most slowly. The underlying phenomena regarding the progression of cell aggregation in the isolates can be linked with the expression of an autocrine factor (pre-starvation factor, or PSF) by *D. discoideum* cells (relative to cell density), which triggers a series of gene expression pathways responsible for the cell differentiation and ultimately, fructification (Rathi and Clarke 1992) The availability of a bacterial food source poses an inhibitory effect on the onset of the pre-starvation response such that this response is induced following the progressive accumulation of PSF and consumption of bacteria, leading to a high PSF/bacteria ratio. Based on this conjecture, the lagged response in the setup with dead *E. coli* cells can be attributed to the increased ability of heat-killed bacteria to bind to the surface of *D. discoideum* cells more tightly than living *E. coli cells*, thus contributing to a much higher inhibitory activity thereby delaying the fructification of *D. discoideum* cells even further. This finding was denoted in a similar study conducted by Burdine and Clarke (1995) that reports greater fructification inhibitory activity of dead *K. aerogenes* than that of living bacterial cells. However, regardless of the delayed response shown by the dead *E. coli* and growth progression in all other setups, no significant difference is established in the fructification rate. This goes to show that predation of the *D. discoideum* and development of fruiting bodies is not a matter of preference towards cell viability of the food source but of the consequential mechanism that leads to the initiation of pre-starvation response brought about by various factors such as inhibition by the food source and PSF levels.

Light-based decision-making capabilities of D. discoideum

Phototaxis bears significance for multiple species as it assists in setting their course toward more favorable environments for their survivability, facilitated by their sensory and signaling mechanisms, thus enabling them to move towards conducive environments (Brodrick and Jékely 2023). This action is particularly evident for *D. discoideum*, as data suggests their response shows a preference for the lighted area, which may be due to the activated photoresponsive pigment protein and consequent intracellular signal transduction pathways (Poff et al. 1974; Poff and Butler 1974). The directional movement of dictyostelids may be influenced by light and nutrition, whereas in the particular experimental setup, despite the lack of *E. coli* on the illuminated side of the plate, the majority of the isolates still exhibited positive phototaxis eventually resulted in fruiting bodies (Figure 8). In their natural habitat, they commonly find their food beneath the soil and move towards the surface where their fructification takes place, optimizing the releasing of spores where different vectors are present for more proper dispersal (Yulo and dela Cruz 2012b).

Phototactic turning is triggered at the tip, with the slugs perceiving light exclusively within the anterior prestalk zone (Francis 1964; Poff and Loomis 1973; Fisher et al. 1984). Slugs are sensitive to light and subtle temperature gradients, enabling them to navigate toward an ideal site for fruiting (Khaire 2003). Most studies addressing the phototaxis of dictyostelids have predominantly concentrated on elucidating their response during the slug life cycle. However, extending this investigation to their fructification phase based on light stimuli is imperative to comprehend the phototactic mechanisms governing significant implications in their ecological adaptation and reproductive strategies. The presence of light was proved to be a crucial environmental cue used by *Heterostelium pallidum* (formerly known as *Polysphondylium pallidum*) to help with the transition of their slug stage to aggregation and fruiting body formation (Harper 1932). In a study by Fukuzawa (2018), *D. mucoroides* developed fruiting bodies under light conditions than in the dark, producing macrocysts (another response to food deprivation). Light can also influence the direction of the migration of *D. discoideum* by directly altering mitochondrial functions, along with responses to cAMP produced in discrete pulses synchronized in cells found at the anterior tip (Poff and Loomis 1973). Understanding the light-responsive behaviors of *D. discoideum* becomes a prerequisite to support further comprehension of the developmental biology of cellular slime molds and subsequent research regarding soil and microbial ecology.

The multifaceted behaviors of *D. discoideum* concerning light and its wavelength and cell viabilities remain contingent upon nutrient availability, phototaxis, chemotaxis, pre-starvation response, and such. This study has developed novelty by veering away from sophisticated laboratory bioassays that can be challenging among many laboratories in developing countries. As such, simple home experiments were created, which can even be employed in educational settings of the academia to demonstrate taxis as

a basic response among biological organisms. Interestingly, such simple bioassays can shed new light on many other unknown cellular ecology and adaptive significance of enigmatic groups of indigenous social amoebae.

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Effects of light intensity on seed germination and early growth seedlings of *Spondias mombin* in Bangladesh

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Abstract. *Das N. 2023. Effects of light intensity on seed germination and early growth seedlings of* Spondias mombin *in Bangladesh. Cell Biol Dev 7: 82-88.* The experiment was conducted to determine the effects of light intensity on the seed germination and early growth of *Spondias mombin* L. under four light-intensity treatments (40%, 60%, 100%, and under a closed natural forest canopy) in polybags containing a mixture of topsoil and cow dung in a 3:1 ratio. The results showed that the closed natural forest canopy and 100% light intensity treatments resulted in unsatisfactory germination of *S. mombin* seeds. The 40% light intensity treatment (medium shading) significantly increased seed germination, with the highest cumulative seed germination at 40% light intensity (71.61%) and the lowest at 100% light intensity (26.58%). Intense light delayed germination. Light intensity significantly ($P \leq 0.05$) affected most of the morphological and physiological parameters of the seedling early growth. The early growth phase of seedlings did not perform well under heavy shade, indicating that *S. mombin* requires shading during early growth. Seedlings under 100% light intensity died shortly after emergence. The findings demonstrated that the best growth and most stable seedlings were obtained under 60% light intensity. Therefore, adequate exposure to light at the nursery stage is necessary for the optimum growth performance of *S. mombin* seedlings for agroforestry and afforestation purposes in Bangladesh.

Keywords: Germination, light intensity, seedling growth, *Spondias mombin*

INTRODUCTION

Seed germination and seedling establishment are fundamental factors in plant growth and development. Light availability is a major ecological factor influencing seed germination, seedling survival, and establishment (Guenni et al. 2008). Germination of many species requires specific light requirements, with species responding to slight variations in the light spectra associated with the season or shaded habitat, triggering or inhibiting germination (Fenner and Thompson 2005). Knowledge of the effect of light on germination is essential in the propagation of plant species for restoration purposes (Khurana and Singh 2001) and for a better understanding of germination ecology (Baskin and Baskin 2014). Although soil moisture content strongly affects light penetration into the soil, it generally appears to be physiologically and ecologically significant amounts of light (Tester and Morris 1987). Thus, the germination response to light may vary between habitats. For example, in shaded environments such as forests, intense light can be associated with a canopy gap that increases the probability of seedling's establishment (Khurana and Singh 2001).

Light is essential for photosynthesis and influences various physiological processes, such as stomatal function, electrolyte absorption, and transportation (Muhammad et al. 2021). There is a direct relationship between light intensities and plant growth rate, as plant growth increases as light intensity gradually increases to a certain extent (Wang et al. 2021). Plants growing at high light intensities allocate more biomass to the underground part for root growth, facilitating water and nutrient absorption and reducing leaf temperature to meet plant growth needs (Balliu et al. 2021). Plants grown in full sunlight have thicker stems, well-developed, shorter internodes, and better development of palisade tissues in leaves (Rina et al. 2019). However, plants grown in a poor-light environment allocate more biomass to the aboveground part for leaf growth to fully absorb limited light energy and meet the photosynthetic needs of plants (Pearcy 1999). Increasing light intensity positively impacts plant growth; the stem grows faster in the dark than in light conditions (Jeong et al. 2013). Light directly affects the vegetative and flowering phases of plants.

Spondias mombin L. belongs to the family Anacardiaceae and is endemic to tropical regions in Asia, America, and Africa (Mitchell and Daly 2015). The *Spondias* spp. (Anacardaceae) also found in the biodiversity-rich primary forest of Bangladesh (Sarker et al. 2015). The *S. mombin* is a hermaphrodite tree with a trunk diameter range of 20-40 cm and a height range of 8-25 m (Mitchell and Daly 2015). The fruit is oval-shaped, 5-10 cm long, with a thin and hard shell, and an average weight of 150-240 g, and its fruit has been used as an antipyretic and diuretic (Mohammed et al. 2011). The shape of the seed looks like a virus due to the various fibers on it, with an oil content of 31.5% (Oyelade et al. 2005). The plant has been traditionally known for its medicinal and food source (Okwu 2001). The fruits, leaves, bark, seeds, and roots treat various diseases. Young leaves are

cooked as a vegetable, but excessive consumption of the fruit can cause dysentery (Ayoka et al. 2008). Urugulaga and Laghton (2000) reported that this plant has broad-spectrum antibacterial and antifungal properties. Furthermore, collecting, processing, and marketing *S. mombin* products help reduce poverty by providing employment and strengthening the rural economy (Leakey et al. 2005; Das 2014d).

The seeds of different tree species are fundamental in silviculture for natural and artificial regeneration, as these are essential, flourishing, high-quality trees. However, dormancy is a significant constraint in working with seeds, even when all other conditions are constant. Previous research on seed germination and seedling establishment has mainly focused on the influence of external environmental factors on storage method and duration (Wawrzyniak et al. 2020), temperature and water (Das 2014a, 2015a; Khaeim et al. 2022), and dormancy-breaking technology (Babaei-Ghaghelestany et al. 2020). Seeds placed across the entire light energy spectrum significantly affected germination and growth, with maximum light effectiveness suitable for promoting seed germination and seedling growth. Seeds from some plant species require heavy shade to keep the soil moist and fresh before germination, while others do not require shade to germinate without shade. The study on light requirements for seed germination and early growth seedlings of *S. mombin* species is still very limited. Therefore, this study aimed to determine the effect of different light intensities in nurseries and closed natural forest canopy on *S. mombin* seed germination and early growth characteristics.

MATERIALS AND METHODS

Study area and seed collection

The experiment was conducted in the nursery of Bangladesh Agricultural University, Bangladesh (24° 44' N and 90° 24' E) in February-July 2021. The air temperature ranged between 24° and 33°C, with a relative humidity of 66-78% during the experiment. The seeds were collected from 9- to 15-year-old and healthy trees. All seeds were dried under the sunlight and stored in airtight polybags until applied to treatments. The collected seeds were sorted to discard the damaged and discolored seeds, and only healthy dried seeds were used for the experiment.

Experimental design and early growth seedling

The germination test was carried out in a medium of topsoil and cow dung in a ratio of 3:1 by sowing the seeds in 4 × 6 cm polybags. During the experiment, the potting media was used for filling the polybags, followed by (i) A mixture on top and floor forest soil (up to 5 cm depth), (ii) Polybags, (iii) Decomposed cow dung, and (iv) Compost. Then, the beds were laid systematically and slightly higher than the surrounding areas so that water did not remain long. The polybags were filled with soil and fertilizer, and the seed was sown in each polybag. One seed was planted in each polybag at a depth of 0.5-1.5 cm. Watering was carried out manually once a day during the experiment. Afterward, the seed germination and seedlings were exposed to four light intensities: direct light (100% light intensity), 60%, and 40% light intensity, and under a closed natural forest canopy. Direct sunlight was obtained by arranging the seedlings under total exposure (100%). 60% light intensity was achieved by placing seedlings under growing media covered with a single layer of synthetic green (1 mm) fine mesh net, and 40% light intensity was achieved by placing seedlings covered with double layers of 1mm mesh net of synthetic green. The amount of Photosynthetic Active Radiation (400 to 700 nm waveband) was measured using LI-COR 190 Quantum Sensor. The close natural forest canopy implies trees that grow densely where the leaves and branches at the top form a canopy or ceiling, which inhibits light penetration to the forest floor (Ita et al. 2022). Weeding in the pot was carried out to eliminate competition with weeds.

Cumulative germination was monitored daily for 60 days until no further germination. The parameters observed were seedling height (cm), collar diameter (mm), number of leaves, total leaf area (cm^2), Root Dry Weight (RDW) (g), and total biomass (g) at six months old. The seedling height (cm) was determined using a calibrated ruler. Collar diameter assessment used Vernier Caliper (Das et al. 2018). Visual count determined the number of leaves, while the leaf area (Sarker et al. 2013; Das 2014b, c) was assessed by the graphical method (Oni and Bada 1992). Biomass increment was estimated monthly (Das 2015b; Das and Sarker 2015). A total of 20 seedlings were randomly selected and carefully uprooted. The seedlings were separated for biomass determination, and then the dry weights of these components were determined after oven drying at 80°C for 24 hours. The dry weight of leaves, stems, and roots were measured. The total biomass of the seedlings was calculated as follows: Total biomass = leaf biomass + stem biomass + root biomass.

Determination of seed germination

The number of seeds germinated in each treatment was recorded. The 1st and last day of seed germination were recorded periodically. At the end of the germination period, the rate of germination and the germination percentage (Maguire 1962) were calculated using the following equations:

$$G_p = \frac{N_g}{N_t} \times 100$$

i.e.,

$$G_r = \sum \frac{N_g}{days\ of\ count}$$

$$G_r = \sum \frac{N_g}{days\ to\ first\ count} + \cdots + \frac{N_g}{days\ to\ final\ count}$$

Where:
G_p: Germination percentage
N_t: Total number of seeds planted
N_g: Number of germinated seeds
G_r: Germination rate

Data analysis

Data on seed germination were statistically analyzed to determine treatment variations using R statistical software version 4.1.2 (R Development Core Team 2021). The Duncan Multiple Range Test (DMRT) (Duncan 1955) and Analysis of Variance (ANOVA) were carried out to analyze the data. One-way ANOVA was used to compare seed germination and early growth characteristics of seedlings in various treatments. DMRT was conducted to compare mean germination percentages, end date of germination, and germination periods. The significance among treatment means was analyzed using DMRT.

RESULTS AND DISCUSSION

Effects of light intensity on the seed germination of *Spondias mombin*

Seed germination of *S. mombin* was started and concluded on the 7th and 43rd Days After Sowing (DAS), with the highest germination rate within the first three weeks. Germination percentages at 21st DAS ranged from 13.1-33.7%. The highest germination was obtained at the treatment of 40% light intensity, which is significantly different ($P \leq 0.05$) from other treatments (Figure 1). The highest cumulative germination was obtained at the treatment of 40% light intensity (71.61%), while the least was at the treatment of 100% light intensity (26.58%) (Table 1).

Light intensity significantly affected the germination of *S. mombin* seeds. The 40% light intensity treatment had a significantly higher germination rate than other treatments. The germination rate for *S. mombin* seeds sown at 40% and 60% light intensity was not significantly different. Three treatments of 40%, 60% light intensity, and under closed natural forest canopy) had higher germination than 100% light intensities (Table 1). There was no significant difference in germination starting dates among the treatments; however, there were significant differences ($P \leq 0.05$) in germination end dates, germination periods, germination percentages, and rates of germination (Table 1).

Table 1. Effects of light intensity on seed germination of *Spondias mombin* at nursery stage

Treatment	Germination Starting Date (d)	Germination End Date (d)	Germination Period (d)	Germination Percentage (%)	Rate of Germination
Under forest canopy	8.35±0.43	42.11±0.32b	33.76±0.36b	46.67±3.43a	1.41±0.11b
40% light intensity	7.16±0.25	40.01±0.23a	32.95±0.27b	71.61±2.38b	1.59±0.06a
60% light intensity	7.58±0.16	41.02±0.24a	33.34±0.22b	54.93±2.64a	1.53±0.07a
100% light intensity	8.93±0.23	43.06±0.46b	34.93±0.34a	26.58±3.51c	1.22±0.10c

Note: The same letter(s) in the column indicates no significant difference. Data are mean ± SD at $P \leq 0.05$

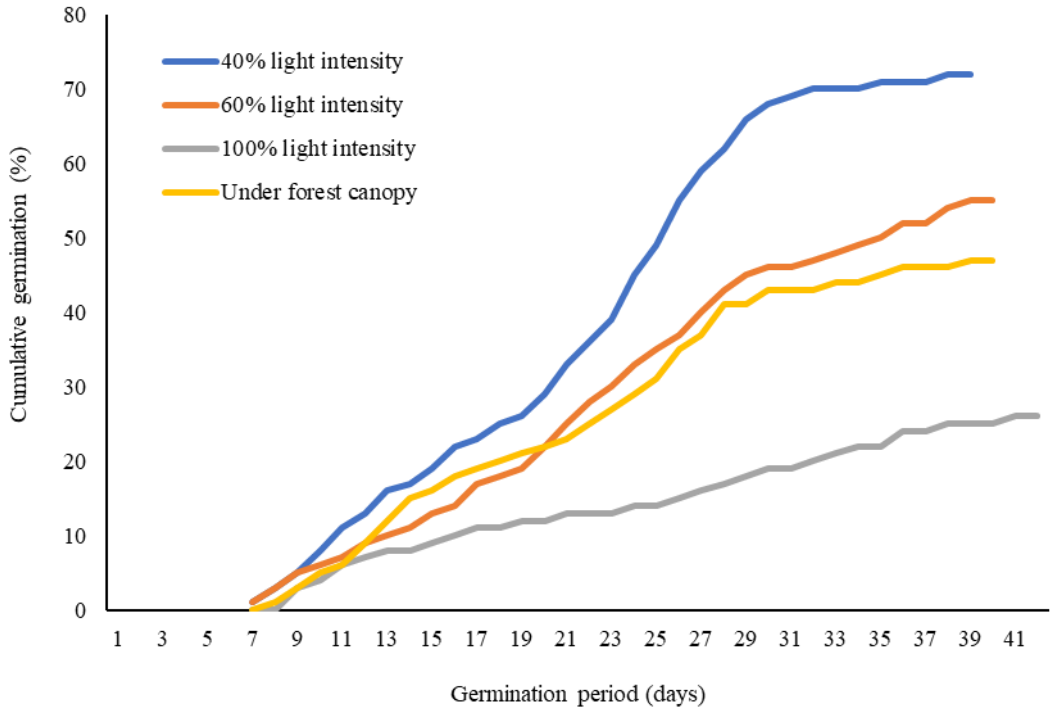

Figure 1. Cumulative germination (%) throughout the germination period of *Spondias mombin* with different light-intensity treatments

Table 2. Growth parameters of *Spondias mombin* seedlings affected by light intensities.

Treatment	Total Height (cm)	Collar Diameter (mm)	Height/ Diameter Ratio (%)	Number of Leaves	Total Leaf Area (cm^2)	Number of Branches	RDW (g)	Total Biomass (g)
Under forest canopy	9.5±0.28b	0.45±0.03b	59.8±2.47b	11.7±0.58b	13.6±0.75c	0.9±0.01	1.4±0.02	4.1±0.11c
40% light intensity	11.3±0.31b	0.53±0.06b	56.2±1.86b	12.6±0.51b	15.4±0.89c	0.9±0.02	1.5±0.02	5.2±0.18b
60% light intensity	13.9±0.29a	0.68±0.04a	41.3±2.01a	14.8±0.32a	19.6±0.81a	1.0±0.01	1.5±0.02	6.9±0.21a
100% light intensity	10.8±0.36b	0.69±0.07a	43.7±2.94a	12.1±0.67b	17.5±0.62b	0.9±0.01	1.6±0.03	5.8±0.19b

Note: The same letter(s) in the column indicates no significant difference. Data are mean ± SD at $P \leq 0.05$

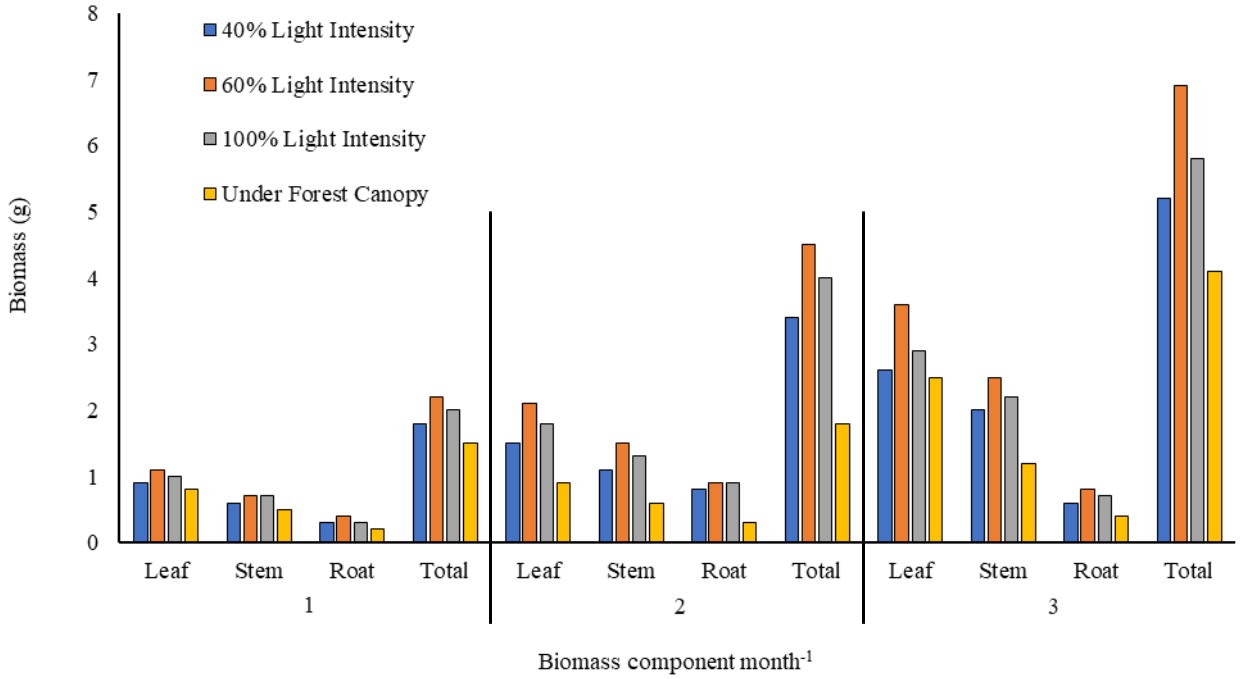

Figure 2. Effect of light intensity on biomass accumulation of *Spondias mombin* seedlings

Effects of light intensity on the growth of the seedlings of *Spondias mombin*

At the end of 12 weeks, the mean height of *S. mombin* seedlings ranged from 9.5-13.9 cm. The highest was obtained at 60% light intensity, while the lowest was at the closed natural forest canopy treatment. There was no significant difference in the heights of seedlings at the treatments of 40% and 100% light intensities (Table 2). At 12 and 21 weeks of growth, the collar diameter of seedlings ranged from 0.45-0.68 mm. The mean collar diameter at 60% light intensity was significantly higher than those at 40% and forest canopy. The effect of light intensity on collar diameter differed significantly ($P \leq 0.05$) (Table 2). The Height/Diameter (H/D) ratio of the seedlings was initially increased at the 8th and 14th weeks and then gradually decreased with the increase of age. At the end of the 15th week, the H/D ratio under closed natural forest canopy and 40% light intensity did not differ. The H/D ratio of closed natural forest canopy and 40% light intensity were significantly higher than those at 60% and 100% (Table 2).

The mean number of leaves of *S. mombin* seedlings varied from 11.7 to 14.8. The number of leaves produced at the treatments of 40%, 100% light intensities, and closed natural forest canopy were not significantly different. The 60% light intensity treatment had significantly higher numbers of leaves than other treatments (Table 2). Light intensities had a significant ($P \leq 0.05$) effect on the seedling's total leaf area. Seedlings exposed to 60% light intensity had the largest leaf area (19.6 cm^2). The smallest leaf area was obtained at a closed forest canopy, with a mean value of 13.6 cm^2. However, the treatments did not affect the number of branches (Table 2). Light-intensity treatments did not affect Root Dry Weight (RDW) (Table 2).

After three months of growth, the mean accumulation of total biomass of *S. mombin* seedlings ranged from 4.1-6.9 g. In the first month of growth, biomass accumulation did not differ in all treatments. In the second month, biomass accumulation was higher at 60% light intensity than at 40%, 100% light intensity, and forest canopy (Figure 2). In the third month, biomass accumulation was higher at 60% and 100% light intensities than at 40% light intensity and closed natural forest canopy.

Discussion

The best treatment for germination of *S. mombin* was at 40% light intensity, considering the germination period, percentage, and germination rate. The successful germination at 40% light intensity due to *S. mombin* seeds require a low light to germinate. The S. *mombin* seeds had some form of dormancy since their ungerminated seeds were viable at the end of the experiments. A higher germination rate of *Chrysophyllum albidum* G.Don seeds was also obtained at 40% light intensity than that of 60% and 100% light intensities (Onyekwelu et al. 2012). The result of *S. mombin* seed germination may help cultivate large quantities of *S. mombin* outside their habitats, promoting ex-situ conservation.

The results also showed that light intensity significantly ($P \leq 0.05$) affected most of the morphological and physiological parameters of the seedlings. The results showed that *S. mombin* seedlings had a moderate annual growth rate of 25-41 cm and a yearly diameter growth rate of 0.56-1.08 cm. However, this annual growth rate depends on the light intensity. Ekeke et al. (2006) reported a mean height growth of between 24.7 and 32.9 cm for *Dacryodes edulis* after 12 weeks, which is higher than the height of *S. mombin* in this study. In silviculture, the H/D ratio is used as an indicator of stability, growth vigor, and the ability of trees to resist wind damage (Wang et al. 1998). A low H/D ratio indicates stable and robust growth, while a high ratio indicates the opposite growth. The *S. mombin* seedlings treated with 60% light intensity were more stable than those treated with under-forest canopy and 40% light intensity. *S. mombin* seedlings in the closed natural forest canopy treatment and treatment of 40% light intensity had a higher H/D ratio, so they may not be suitable as planting stock.

The *S. mombin* seedlings treated under a closed natural forest canopy produced fewer leaves and a lower total leaf area than the other treatments. The low number of leaves and total leaf area might have led to low photosynthetic activity and low biomass production (Egharevba and Osunde 2001). In the present study, biomass accumulation was higher in the seedlings under 60% than for those under 40%, 100% light intensity, and closed natural forest canopy. Chen et al. (2023) showed that the growth characteristics of *Liquidambar formosana* Hance seedlings were significantly better under sunlight than under shade, and the root, leaf, stem, and total biomasses were significantly higher under sunlight compared to heavy shade conditions. Furthermore, more biomass was allocated to the leaves to ensure photosynthesis in an adequate light environment; consequently, more matter and energy accumulated. The stress tolerance of *S. mombin* may affect its biomass distribution pattern in a low-light environment. It suggests that shade-tolerant plants invest more biomass in the stem and propel the stems and roots to store more material to improve their low-light environment tolerance. The lack of light inhibits photosynthesis under heavy shade conditions, affecting the transport of photosynthetic products to the root system and inhibiting root growth (Li et al. 2017). In this study, the decrease in light intensity significantly decreased the seedling height, total biomass, and stem diameter of *S. mombin* seedlings. The seedling growth in a closed natural forest canopy was poor, indicating that it may not perform well under heavy shade.

Light directly affects the developmental processes and growth of the above-ground plant parts and indirectly affects the underground roots (Li et al. 2017). Intense light benefited the basal stem diameter and the root system growth of the seedlings, but they had minimum height. Therefore, plants usually develop and elongate to improve light interception in a closed forest environment, resulting in increased stem diameter, seedling height, and decreased root length (Mediavilla and Escudero 2010). The death of young *S. mombin* seedlings at intense light, especially at 100% light intensity, suggested that *S. mombin* may not tolerate full sunlight. The seedling growth under 100% light intensity died shortly after emergence, implying the species needs shading during early growth. Therefore, *S. mombin* seedlings need shade for the establishment and early growth, evidenced by their survival and growth under moderate shade environments (i.e., 60% light intensities). Therefore, the best growth and most stable seedlings were obtained under 60% light intensity. A study by Wardiana and Herman (2011) showed a better collar diameter, a higher number of leaves, and biomass production for *Reutealis trisperma* (Blanco) Airy Shaw seedlings under a reduced light environment (65% light intensity) than full light (100% light intensity). To improve light interception in a closed forest environment, plants usually invest more resources in the growth and elongation of seedlings and thickening of the stem diameter, resulting in increased seedling height, stem diameter, number of leaves, total leaf area, and biomass production. Veenendaal et al. (1996) obtained similar results for *Terminalia superba* Engl. & Diels and *Entandrophragma utile* (Dawe & Sprague) Sprague, while Osunkoya et al. (1994) reported on twelve forest tree species that showed reduced growth with decreasing light intensity.

Previous studies revealed that enhanced light within a specific range promotes seedling growth (Xia et al. 2020; Chen et al. 2020). The study results were similar to the morphological characteristics observed in the seedlings of *Pinus massoniana* Lamb. (Pinaceae) and *Quercus mongolica* Fisch. Ex Ledeb. (Fagaceae) under different light intensities (Li et al. 2017). Under heavy shade conditions, the lack of light inhibits photosynthesis in the aboveground parts, which affects the transport of photosynthetic products to the root system, and, therefore, root growth is inhibited (Li et al. 2017). Insufficient energy is invested in the root length, seedling height, and stem diameter, inhibiting plant growth.

In conclusion, light is one of the factors affecting *S. mombin* germination and seedling early growth, as demonstrated by 40% and 60% light intensities. Seedlings under closed natural forest canopy had poor performance by decreasing survival percentage on heavy shading, corresponding to a low H/D ratio essential for growth and development. To enhance the natural regeneration of *S. mombin*, silvicultural measures such as thinning or gap openings are suggested for increasing light irradiance in the forest understory. Therefore, the *S. mombin* tree species

distributed throughout the country constitute potential stocks for agroforestry, afforestation, reforestation, and breeding programs.

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Seed phenotypic variations in cowpea, *Vigna unguiculata*, from selected open markets in Edo State, Nigeria

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Abstract. *Ikhajiagbe B, Ogwu MC, Omage ZE. 2023. Seed phenotypic variations in cowpea,* Vigna unguiculata, *from selected open markets in Edo State, Nigeria. Biodiversitas 24: 89-101.* Understanding the phenotypic variation of *Vigna unguiculata* (cowpea) can facilitate sustainable utilization and support protein security goals. This study aimed to investigate the existence and level of seed phenotypic variations within and among three local cultivars of cowpea, namely Ife Brown, Ekpoma Local, and Sokoto White in Edo State, Southern Nigeria. This information will assist utilization, conservation planning, and breeding efforts. Key qualitative and quantitative characters were collected and analyzed using parametric and non-parametric tests. Results showed that there were no variations in the qualitative parameters among the seeds of cvs. Ekpoma Local and Sokoto White. However, cv. Ife Brown varied significantly, particularly in seed color. Significant variations (P>0.05) existed in the seed quantitative parameters. The seed volume was the most diverse, with a coefficient of variation of 13.15-14.14. Further, the seed volume of cv. Sokoto White was the most diverse. In terms of overall variation, the group mean sum of squares for cv. Ife Brown was 146.95, compared to 26.18 and 31.23 for cvs. Ekpoma Local and Sokoto White respectively, indicating that cv. Ife Brown was the most likely variable cultivar. There is a need for molecular characterization to ascertain the diversity observed in the cowpea seeds.

Keywords: Cowpea characterization, legume security, local cultivars, plant genetic resources, seeds diversity, *Vigna unguiculata*

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp.) is a major legume crop of the global South hemisphere. The crop originated and was first domesticated in southern Africa, but is now cultivated in almost all parts of sub-Saharan Africa and parts of Asia and the Americas (Lazaridi and Bebeli 2023). It is a good source of dietary protein as well as a consistent source of income for both local and commercial farmers. As an abiotic stress tolerant and adaptable crop, cowpea is one of the oldest known human food sources with protein security roles (Herniter et al. 2020; Ifie et al. 2020; Jayawardhane et al. 2022). The seeds contain around 25% protein and 64% carbohydrate, while the young leaves, pods, and peas are high in vitamins and minerals (Simion 2018; Magashi et al. 2019; Ifie et al. 2019, 2020).

West and Central Africa is the world's largest producer of cowpea. Africa accounts for 95.9% of the total 7.4 million tons of cowpea seeds produced (Ifie et al. 2019; Ikhajiagbe et al. 2019; International Institute of Tropical Agriculture 2020). Nigeria is the world's largest producer and consumer of cowpeas. Cowpea is a staple food in Nigeria and Africa due to its hardiness, versatility and popularity (Omoigui et al. 2020).

Cowpea is the most important arable food legume in Sub-Saharan Africa (Adewale et al. 2011), and there are numerous varieties of cowpea both in and outside of Nigeria. The seed shapes, sizes, colors, texture, pigmentation, and growth patterns of cowpea varieties in Nigeria differ greatly (Iseghohi et al. 2019). Unfortunately, the ever-changing environment, volatile global economy, and intensification of low-input agricultural production have resulted in a dramatic increase in soil depletion and nutrient depletion in many Sub-Saharan regions (Magashi et al. 2019). This challenges food production and food stability, and while cowpea serves as a crop that meets global nutrient needs, this will only last so long before variation is lost due to cowpea succumbing to the consequences of climate change. Fortunately, genetic variation and abundance in cowpea may be used to create varieties that are more resistant to production constraints. As a result, in order to extend the collection, adequate awareness of genetic variation within current germplasm is needed. This allows breeding programs to pick and evolve more improved varieties quicker, not only in terms of yield but also of nutritional benefit (Magashi et al. 2019). Overall, germplasm with a greater genetic base acts as a buffer, providing resistance to climatic and other environmental changes and maintai6ning long-term food stability.

This challenges food production and food stability, especially for an essential crop like cowpea that contributes towards global nutrient needs like protein security. To this end, cowpea germplasms with a greater genetic base can act as a buffer, providing resistance to climatic and other environmental changes and maintaining long-term food stability (Nkhoma et al. 2020; Mekonnen et al. 2022).

Given this, when higher-precision diversity analysis approaches are not accessible to scientists, phenotypic assessment of genotypes for morphological classification remains a viable mechanism for identifying genetic heterogeneity within a population.

According to Oyenuga (1968), cowpea is an indigenous grain legume in Nigeria, but despite the popularity of the crop, not much is known about the morpho-genotypic variation of the crop. Nigeria is a secondary center of diversity of cowpea and the largest producer of the crop. Therefore, there is an urgent need to document and understand the morpho-genotypic variation to boost production, breeding, utilization, and conservation of the crop within key cultivation and distribution regions of the country like Edo State (Ortiz 1998; Edet and Ishii 2022). This study aimed to assess the level of variability in key seed phenotypic characteristics among three prominent *V. unguiculata* cultivars sold in Benin City, Edo State, Nigeria.

MATERIALS AND METHODS

Study area

Benin City was selected as the study area and is the state capital of Edo, which is situated in southern Nigeria. Benin City has a total area of 1,204 km^2 and is located approximately 40 km (25 miles) north of the Benin River and 320 km (200 miles) by road east of Lagos (465 miles^2). Benin City is the main hub of activity in the state, with a population of 1,782,000 as of 2021. It is also the epicentre of the Nigerian rubber industry (Osawaru et al. 2012, 2013, 2014).

Samples collection

Three cowpea (*V. unguiculata*) cultivars were purchased from three random locations within ten local markets from four local government areas in Benin City, Edo State (Figure 1; Table 1). The samples were 90 in total, 30 samples for each cultivar. The four local government areas and their respective markets include Ikpoba Okha (Santana, Ekiosa, and Oregbeni Markets), Oredo (Ugbighoko, Oba Market, and New Benin Markets), Egor (Egor and Uselu Markets) and Ovia North-East (Ekosodin and Oluku Markets). The total distance covered was 48.11 km (29.89 miles) and the GPS locations were retrieved using Garmin eTrex ® 10 handheld systems (Garmin Limited) (Figure 1; Table 1).

Morphological assessment procedure

The morphological assessments of the seeds were examined based on two categories, namely quantitative and qualitative characteristics. A total of 10 qualitative and 5 quantitative characters were scored on each of the cowpea varieties. Ten seeds of each variety from each local market were measured. Variegated testa color and moderate size describe *V. unguiculata* cv. Ekpoma Local, while cv. Sokoto White is distinguished by the pale grey testa and medium size and *V. unguiculata* cv. Ife Brown has a distinct uniform brown color except for the eyes (Figure 2).

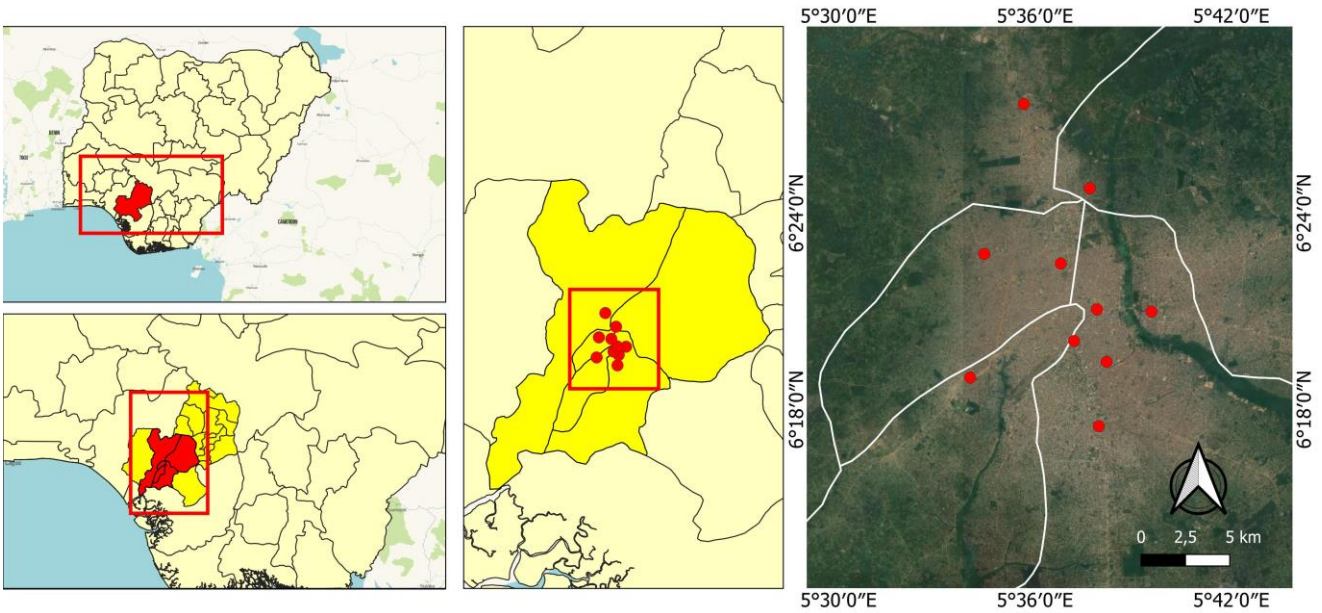

Figure 1. Map of the study area showing the source of cowpea (*Vigna unguiculata*) used in the study and distances apart

Table 1. Source of *Vigna unguiculata* seeds used in the study

Place of purchase	Local government area	Cultivar	Seed code	GPS location
Santana market	Ikpoba Okha	Ife Brown	IKCbMsL1 - IKCbMsL3	6°17'28.6" - 6°17'30.9N
		Ekpoma Local	IKCeMsL1 - IKCeMsL3	5°37'56.7" - 5°37'58.1"E
		Sokoto White	IKCsMsL1 – IKCsMsL3	
Ekiosa market	Ikpoba Okha	Ife Brown	IKCbMeL1 – IKCbMeL3	6°19'20.0" - 6°19'27.3"N
		Ekpoma Local	IKCeMeL1 – IKCeMeL3	5°38'13.0" - 5°38'11.5"E
		Sokoto White	IKCsMeL1 - IKCsMeL3	
Oregbeni market	Ikpoba Okha	Ife Brown	IKCbMoL1 – IKCbMoL3	6°20'58.7" - 6°20'59.5"N
		Ekpoma Local	IKCeMoL1 – IKCeMoL3	5°39'37.0" - 5°39'33.8"E
		Sokoto White	IKCsMoL1 - IKCsMoL3	
Ugbighoko market	Oredo	Ife Brown	ORCbMuL1 - ORCbMuL3	6°18'58.8"- 6°18'58.5"N
		Ekpoma Local	ORCeMuL1 – ORCeMuL3	5°34'03.0" - 5°34'01.4"E
		Sokoto White	ORCsMuL1 – ORCsMuL3	
Oba market	Oredo	Ife Brown	ORCbMoL1 - ORCbMoL3	6°20'03.9" - 6°20'05.3"N
		Ekpoma Local	ORCeMoL1 – ORCeMoL3	5°37'10.8" - 5°37'10.2"E
		Sokoto White	ORCsMoL1 - ORCsMoL1	
New Benin market	Oredo	Ife Brown	ORCbMnL1 - ORCbMnL3	6°21'03.0" - 6°21'04.3"N
		Ekpoma Local	ORCeMnL1 – ORCeMnL3	5°37'51.7" - 5°37'52.5"E
		Sokoto White	ORCsMnL1 – ORCsMnL3	
Ekosodin market	Ovia North-East	Ife Brown	OVCbMeL1 - OVCbMeL3	6°24'45.6" - 6°24'45.5"N
		Ekpoma Local	OVCeMeL1 – OVCeMeL3	5°37'40.8" - 5°37'40.8"E
		Sokoto White	OVCsMeL1 – OVCsMeL3	
Oluku market	Ovia North-East	Ife Brown	OVCbMoL1 - OVCbMoL3	6°27'21.1" - 6°27'20.0"N
		Ekpoma Local	OVCeMoL1 – OVCeMoL3	5°35'40.9" - 5°35'38.3"E
		Sokoto White	OVCsMoL1 – OVCsMoL3	
Uselu market	Egor	Ife Brown	EGCbMuL1 - EGCbMuL3	6°22'28.9" - 6°22'27.6"N
		Ekpoma Local	EGCeMuL1 – EGCeMuL3	5°36'50.4" - 5°36'47.6"E
		Sokoto White	EGCsMuL1 – EGCsMuL3	
Egor market	Egor	Ife Brown	EGCbMeL1 - EGCbMeL3	6°22'44.3" - 6°22'45.2"N
		Ekpoma Local	EGCeMeL1 – EGCeMeL3	5°34'28.7" - 5°34'27.1"E
		Sokoto White	EGCsMeL1 – EGCsMeL3	

Note: Local government Area: Ikpoba Okha [IK], Oredo [OR], Egor [EG], Ovia North-East [OV]; **Varieties** [C]: Ife brown [Cb], Ekpoma local [Ce], Sokoto white [Cs]; **Cultivars** [C]: Ife brown [Cb], Ekpoma local [Ce], Sokoto white [Cs]; **Markets** [M]: Santana market [Ms], Ekiosa market [Me], Oregbeni market [Mo], Ugbighoko market [Mu], Oba market [Mo], New Benin market [Mn], Ekosodin market [Me], Oluku market [Mo], Uselu market [Mu], Egor market [Me]; **Location** [L]: Location [L1], Location [L2], Location [L3]

Quantitative characteristics

Key quantitative characteristics parameters measured included seed length, seed width, seed thickness, 10-seed weight, and 20-seed volume. This was carried out using methods previously described by Osawaru et al. (2012, 2013, 2014), Chime et al. (2017), Ogwu et al. (2018), Aiwansoba et al. (2019), and Obongodot et al. (2022) by using the measurement from 10 common-sized seeds with the aid of a Uline digital venire caliper (H-7352). The linear dimensions measured were in cm and their average values were calculated and recorded. 10-seed weight was determined by weighing ten seeds of common sizes with a high-precision A & D EK-6000i- Class NTEP-approved weighing scale. The 20-seed volume was achieved by the water displacement method. Twenty seeds were dropped into a 5 mL - 2 l cylinder containing 95% ethanol and 5% water. The volume displaced was recorded as the volume of the seed.

Qualitative characteristics

The modal phenotypic and qualitative parameters assessed include eye pattern, eye color, seed shape, brilliance of seeds, splitting of testa, testa texture, color variegation, basal color, pattern of variegation, and basal color of variegated seeds as clarified in the works of Ohanmu et al. (2019a) and Ikhajiagbe et al. (2020). The testa basal color was determined using the application, Color Namer®. The qualitative characters which were determined visually were scored by nominal codes from a descriptor for cowpea by The International Board for Plant Genetic Resources (1983) (Table 2).

Data analysis

Collected data were assessed to reveal their sums of squares and least significant differences (LSD) to ascertain the source of variability among seed parameters as well as two-way analysis of variance to reveal their level of significant difference. The results were presented as a mean of 10 random determinations where necessary. The SPSS® version 21 Statistical package was used for statistical analyses.

Table 2. Qualitative characters assessed in the study and their descriptive keys

Qualitative characteristics	Descriptive key
Seed shape	1 kidney, 2 ovoid, 3 crowder, 4 globose, 5 rhomboid
Splitting of testa	0 absent, 1 present
Testa texture	1 smooth, 3 smooth to rough, 5 rough (fine reticulation); 7 rough to wrinkled
Testa color variegation	0 absent, 1 present
Testa basal color	Using the application, Color Namer® and ranged from light peach, brown, sand, light brown, tan, pale peach, beige, peach, camel, pale brown, cocoa, dull orange, butterscotch, sand brown, pinkish tan, pinkish grey, to apricot
Pattern of testa variegation	1 dense black uneven spot/dot on brown background basal color with clean eye, 2 sparse black dots on creamy brown background with a concentration around the hilum, 3 patchy light brown dots on dark brown background
Basal color of variegated seeds	0 non variegated seeds, 1 cream, 2 brown, 3 black
Eye color	0 eye absent (white, cream), 1 brown splash or grey, 2 tan brown, 3 red
Eye pattern	0 absent, 1 very small, 2 kabba group (the eye fills the narrow groove all around the hilum and the body has some form of speckling and a blue hallow is found around the hilum), 3 narrow eyes (hilum ring. Eye fills the narrow groove around the hilum and spills out of this grove in front of the hilum but for a short distance but has an indistinct front margin), 4 small eye, 5 Holsten group (i.e., the eye circles the back of the hilum in a narrow ring, widens at the sides and then extends the margin of the eye is very distinct), 6 Watson group (eye encircles the back of the hilum as a narrow ring, widens at the sides and spills over the non-micropylar end of the seed with an indistinct margin. The extra width at the sides of the hilum distinguishes this group from 3, narrow eyes).
Brilliance of the seed	1 shiny, 2 medium, 3 matt

RESULTS AND DISCUSSION

Seed phenotypic quantitative characterisation

Results of the assessments of the quantitative characteristics of *V. unguiculata* cv. Ife Brown is presented in Table 3. It was observed that no significant differences in seed length, seed width, or seed thickness were observed. Seed length ranged therefore from 1.10 to 1.39 cm respectively. The seed width, on the other hand, varied from 0.79 to 0.97 cm, while seed thickness from 0.52 to 0.64 cm respectively. However, significant differences (P>0.05) were observed in the 20-seed volume as well as the dry seed weight shown in this study. Whereas the seed sample with the lowest 20-seed volume (OVCbMeL1) was sourced at Ovia North-East local government area from Ekosodin market with a volume of 4.10 mL; compared with the 20-seed volume of 8.00 mL obtained from (EGCbMuL2) Uselu market at Egor local government area. Similarly, the lowest seed weight obtained for *V. unguiculata* cv. Ife Brown was 2.72 g from Santana market at Ikpoba Okha local government area (IKCbMsL1), and highest 4.17 g from Uselu market at Egor local government area (EGCbMuL2).

The quantitative characteristics of *V. unguiculata* cv. Ekpoma Local is presented in Table 4. The results show no significant differences in all the morphological parameters measured (P>0.05). The seed length ranged from 0.78 to 0.87 cm and 0.60 to 0.71 cm for the seed width. Seed thickness varied from 0.44 to 0.47 cm, while the 20-seed volume ranged from 2.10 to 3.20 mL. No significant changes in seed weight occurred as seed weight ranged from 1.48 to 1.74 g respectively.

The results of quantitative characteristics assessment of *V. unguiculata* cv. Sokoto White is presented in Table 5. The seed length of *V. unguiculata* cv. Sokoto White ranged from 0.74 to 0.92 cm (P>0.05). No significant changes in the seeds collected from the various sampling sites were recorded. 20-seed volume was the least (2.12 mL) at the location ORCsMoL3 compared to 3.89 ml of the 10-seed volume collected at EGCsMeL3.

The measurable mean and coefficient of variation (CV) of *V. unguiculata* seeds collected from the various markets are presented in Table 6. The results showed a mean of 1.27 cm for seed length, amounting to a CV of 4.97 for cv. Ife Brown. Compared to cv. Ekpoma Local, the mean seed length was 0.81, with a CV of 3.28; whereas, for cv. Sokoto White, the seed length was similar to cv. Ekpoma Local (0.81 cm) with a CV of 5.53. The implication of this is that the variability was more in regards to seed length in cv. Sokoto White, than cv. Ife Brown before cv. Ekpoma Local. In terms of seed dry weight, the mean value of cv. Ife Brown was 3.47 g, which eventually was the highest when compared with the seed weight of cv. Ekpoma Local (1.61 g) and cv. Sokoto White (1.64 g). In terms of CV, the results showed that the seed volume of cv. Sokoto White presented the highest amount of variation. The lowest CV was recorded in the seed thickness of the cv. Ekpoma Local (2.62) (Table 6).

Figure 2. *Vigna unguiculata* seed morphology. A. cv. Ife Brown. B. cv. Ekpoma Local. C. cv. Sokoto White. Purchased from: 1 Santana market, 2. Ekiosa market, 3. Oregbeni market, 4. Ugbighoko market, 5. Oba market, 6. New Benin market, 7. Ekosodin market, 8. Oluku market, 9. Uselu market, 10. Egor market

Table 3. Quantitative parameters of *V. unguiculata* cv. Ife Brown seeds collected at sampling sites

Seed codes	Seed length (cm)	Seed width (cm)	20-Seed volume (mL)	Seed thickness (cm)	10-Seed weight (g)
IKCbMsL1	1.25	0.82	6.00	0.52	2.72
IKCbMsL2	1.24	0.81	6.00	0.53	4.10
IKCbMsL3	1.30	0.84	6.80	0.58	3.39
IKCbMeL1	1.23	0.93	6.00	0.57	3.71
IKCbMeL2	1.24	0.84	5.80	0.57	3.12
IKCbMeL3	1.22	0.87	6.00	0.55	3.41
IKCbMoL1	1.29	0.82	6.10	0.61	3.21
IKCbMoL2	1.10	0.80	5.70	0.58	3.13
IKCbMoL3	1.31	0.79	4.90	0.62	2.90
ORCbMuL1	1.28	0.82	5.00	0.61	3.17
ORCbMuL2	1.23	0.88	5.00	0.58	3.57
ORCbMuL3	1.29	0.84	5.20	0.58	3.46
ORCbMoL1	1.21	0.86	6.00	0.61	3.25
ORCbMoL2	1.26	0.87	6.00	0.60	3.38
ORCbMoL3	1.15	0.86	5.60	0.61	3.36
ORCbMnL1	1.21	0.82	6.00	0.56	3.78
ORCbMnL2	1.16	0.84	6.00	0.58	3.75
ORCbMnL3	1.34	0.86	6.30	0.57	3.52
OVCbMeL1	1.33	0.86	4.10	0.61	3.36
OVCbMeL2	1.25	0.87	6.80	0.62	3.62
OVCbMeL3	1.37	0.97	7.00	0.61	3.49
OVCbMoL1	1.31	0.84	5.70	0.55	3.85
OVCbMoL2	1.30	0.86	5.20	0.57	3.21
OVCbMoL3	1.32	0.84	5.30	0.60	3.65
EGCbMuL1	1.29	0.83	5.00	0.54	3.83
EGCbMuL2	1.39	0.84	8.00	0.59	4.17
EGCbMuL3	1.27	0.85	5.40	0.58	3.91
EGCbMeL1	1.30	0.90	5.40	0.64	3.69
EGCbMeL2	1.31	0.91	6.30	0.54	3.44
EGCbMeL3	1.26	0.88	5.00	0.55	2.91
SD	0.06	0.04	0.75	0.03	0.34
LSD (0.05)	0.69	0.31	1.04	0.16	1.26
P-value	0.172	0.581	0.043	0.077	0.016

Note: SD: Standard Deviation, LSD: Least significant difference

Table 4. Quantitative parameters of *V. unguiculata* cv. Ekpoma Local seeds collected at sampling sites

Seed codes	Seed length (cm)	Seed width (cm)	20-Seed volume (ml)	Seed thickness (cm)	10-Seed weight (g)
IKCeMsL1	0.85	0.63	2.90	0.46	1.59
IKCeMsL2	0.87	0.64	2.40	0.46	1.59
IKCeMsL3	0.84	0.59	3.00	0.47	1.49
IKCeMeL1	0.83	0.60	3.00	0.45	1.58
IKCeMeL2	0.82	0.71	2.00	0.46	1.61
IKCeMeL3	0.85	0.61	3.00	0.46	1.51
IKCeMoL1	0.86	0.65	2.70	0.45	1.55
IKCeMoL2	0.83	0.63	2.80	0.44	1.59
IKCeMoL3	0.81	0.65	3.00	0.45	1.63
ORCeMuL1	0.79	0.62	3.15	0.45	1.69
ORCeMuL2	0.79	0.62	3.00	0.45	1.67
ORCeMuL3	0.83	0.66	3.00	0.49	1.65
ORCeMoL1	0.78	0.62	3.20	0.46	1.65
ORCeMoL2	0.78	0.70	2.50	0.44	1.62
ORCeMoL3	0.79	0.61	2.80	0.44	1.71
ORCeMnL1	0.78	0.63	3.07	0.47	1.61
ORCeMnL2	0.81	0.61	2.90	0.45	1.63
ORCeMnL3	0.78	0.58	2.50	0.44	1.74
OVCeMeL1	0.79	0.63	2.00	0.45	1.63
OVCeMeL2	0.79	0.68	2.60	0.45	1.56
OVCeMeL3	0.78	0.61	2.10	0.44	1.65
OVCeMoL1	0.80	0.61	2.80	0.44	1.60
OVCeMoL2	0.81	0.63	2.20	0.43	1.48
OVCeMoL3	0.85	0.66	3.10	0.45	1.53
EGCeMuL1	0.81	0.62	2.50	0.44	1.65
EGCeMuL2	0.84	0.64	3.00	0.46	1.74
EGCeMuL3	0.81	0.57	3.11	0.45	1.68
EGCeMeL1	0.80	0.62	2.20	0.45	1.52
EGCeMeL2	0.82	0.63	2.20	0.45	1.65
EGCeMeL3	0.83	0.63	3.00	0.46	1.59
SD	0.03	0.03	0.37	0.01	0.07
LSD (0.05)	0.21	0.21	1.09	0.11	0.61
P-value	0.305	0.749	0.665	0.532	0.129

Note: SD: Standard Deviation, LSD: Least significant difference

Table 5. Quantitative parameters of *V. unguiculata* cv. Sokoto White seeds collected at sampling sites

Seed codes	Seed length (cm)	Seed width (cm)	10 Seed volume (ml)	Seed thickness (cm)	Seed weight (g)
IKCsMsL1	0.80	0.61	3.40	0.48	1.64
IKCsMsL2	0.81	0.65	2.40	0.49	1.58
IKCsMsL3	0.89	0.70	2.43	0.46	1.42
IKCsMeL1	0.89	0.63	3.00	0.48	1.67
IKCsMeL2	0.81	0.60	2.80	0.44	1.65
IKCkMeL3	0.92	0.63	3.80	0.50	1.74
IKCsMoL1	0.79	0.63	3.10	0.47	1.57
IKCsMoL2	0.77	0.61	3.00	0.44	1.66
IKCsMoL3	0.76	0.64	2.50	0.52	1.60
ORCsMuL1	0.81	0.63	3.00	0.46	1.49
ORCsMuL2	0.85	0.62	3.00	0.46	1.57
ORCsMuL3	0.78	0.62	2.50	0.45	1.66
ORCsMoL1	0.77	0.61	2.50	0.48	1.76
ORCsMoL2	0.73	0.61	3.00	0.50	1.67
ORCsMoL3	0.78	0.55	2.12	0.40	1.59
ORCsMnL1	0.74	0.61	2.90	0.48	1.64
ORCsMnL2	0.75	0.63	3.11	0.50	1.66
ORCsMnL3	0.77	0.60	3.00	0.44	1.55
OVCsMeL1	0.86	0.65	3.00	0.48	1.63
OVCsMeL2	0.87	0.66	3.60	0.49	1.60
OVCsMeL3	0.82	0.68	3.30	0.50	1.76
OVCsMoL1	0.79	0.63	2.80	0.49	1.65
OVCsMoL2	0.91	0.61	3.00	0.43	1.62
OVCsMoL3	0.76	0.61	2.60	0.46	1.47
EGCsMuL1	0.79	0.63	3.16	0.47	1.91
EGCsMuL2	0.81	0.61	3.00	0.43	1.96
EGCsMuL3	0.77	0.65	3.20	0.48	1.84
EGCsMeL1	0.87	0.70	3.50	0.52	1.48
EGCsMeL2	0.84	0.65	3.30	0.49	1.52
EGCsMeL3	0.80	0.64	3.89	0.49	1.70
SD	0.05	0.03	0.41	0.03	0.12
LSD (0.05)	0.21	0.11	1.23	0.11	0.14
P-value	0.309	0.160	0.746	0.587	0.064

Note: SD: Standard Deviation, LSD: Least Significant Difference

Table 6. Measurable mean and coefficient of variation of *V. unguiculata* seeds collected at sampling sites

Cowpea variety	Plant quantitative parameter	Mean	SD	95% C.I.		CV
				Lower bound	Upper bound	
V. unguiculata cv. Ife Brown	Seed length (cm)	1.27	0.06	1.24	1.29	4.97
	Seed width (cm)	0.85	0.04	0.84	0.87	4.47
	Seed volume (ml)	5.79	0.76	5.5	6.07	13.15
	Seed thickness (cm)	0.58	0.03	0.57	0.59	5.14
	Seed weight (g)	3.47	0.35	3.34	3.6	9.96
V. unguiculata cv. Ekpoma Local	Seed length (cm)	0.81	0.03	0.8	0.82	3.28
	Seed width (cm)	0.62	0.02	0.62	0.63	2.75
	Seed volume (ml)	2.71	0.36	2.58	2.85	13.37
	Seed thickness (cm)	0.45	0.01	0.45	0.46	2.62
	Seed weight (g)	1.61	0.07	1.59	1.64	4.19
V. unguiculata cv. Sokoto White	Seed length (cm)	0.81	0.04	0.79	0.82	5.53
	Seed width (cm)	0.63	0.03	0.62	0.64	4.81
	Seed volume (ml)	2.93	0.41	2.78	3.09	14.14
	Seed thickness (cm)	0.47	0.03	0.46	0.48	5.9
	Seed weight (g)	1.64	0.12	1.6	1.69	7.4

Note: SD: Standard Deviation, CI: Confidence Interval, CV: Coefficient of Variation

The assessment of the sum of squares in an attempt to compare the genetic capabilities and genetic characteristics of the seeds is presented in Table 7. The results indicate that in regard to the mean sum of squares when opposed to cv. Ekpoma Local and cv. Sokoto White, whereas cv. Ife Brown has the greatest variability.

Seed phenotypic qualitative characterisation

The modal phenotypic and qualitative parameters of *V. unguiculata* cv. Ife Brown is presented in Table 8. In terms of seed shape, all seed samples selected throughout the sampling sites were 5 (rhomboid). In terms of splitting of testa, all seeds were predominantly 1 (presence of testa splitting). In terms of testa texture, all the seeds were 7 (rough to wrinkled). Except for testa basal color, which had considerable variation in coloration in the testa of sample seeds, the values for pattern of testa variegation, eye color, and brilliance of seeds amongst others were largely uniform.

The modal phenotypic and qualitative characteristics of *V. unguiculata* cv. Ekpoma Local is presented in Table 9. No changes in seed shape were observed, as the seeds were generally 5 (rhomboid). In terms of testa color variegation, all seeds obtained were generally and unanimously 1 (presence of testa color variegation). The prominent testa basal color for cv. Ekpoma Local was dark brown. All the seeds were obtained from the various markets for cv. Ekpoma Local, has the same brilliance of seeds, 2 (medium). The values for the pattern of testa variegation, eye color, testa texture and eye pattern amongst others were also largely uniform.

The modal phenotypic and qualitative characteristics of *V. unguiculata* cv. Sokoto White is presented in Table 10. The findings revealed that the shape of the seeds, the splitting of the testa, the texture of the testa, and the color variegation of the testa all followed the same pattern throughout the experiment. The seeds of *V. unguiculata* cv. Sokoto White were generally pale grey in terms of testa basal color. There were no variegations in the appearance of testa variegation pattern, basal color of variegated seeds, eye color, eye pattern, and seed brilliance. That is, there were no changes in the above five parameters regardless of the market areas from which they were purchased.

Discussion

Seed quantitative and qualitative phenotypic characterization of three *V. unguiculata* cultivars collected from open markets within Edo State, Nigeria has been completed. Except of seed volume and seed weight, there were no significant differences ($P>0.05$) in the quantitative parameters evaluated for *V. unguiculata* cv. Ife Brown. There were also no significant differences in all the quantitative parameters assessed in cv. Ekpoma Local and cv. Sokoto White. This observation is similar to the reports of Dorvlo et al. (2022) on *V. unguiculata* var. *Videza* from Ghana wherein variations were mainly in seed weight and sizes. According to Fatokun et al. (1992), two unlinked major gene families within cowpea's quantitative trait loci genomic regions account for the majority of variations in seed volume and weight. Due to the yield and commercial value of cowpea seed (dry-grain) size, *V. unguiculata* cv. Ife Brown would likely have more income security value than cv. Ekpoma Local and cv. Sokoto White. Interestingly, cowpea seed weight, length, and weight traits are regulated by one pleiotropic locus (Lo et al. 2019). However, this was not supported by findings in the current study as there were no similar significant differences ($P>0.05$ in seed length and width. In addition to seed weight and volume, the report of other workers suggests the number of days to flowering, number of productive branches, pod length and width, leaf length, and width, number of seeds per pod, and number of pods per plant as key phenotypic quantitative agronomic traits of cowpea (Menssen et al. 2017; Odeseye et al. 2018; Gerrano et al. 2022).

Vigna unguiculata cv. Ife Brown had diverse testa basal colors, while the other parameters were distributed uniformly. It was the only variety that displayed testa splitting. Due to the obvious large size of cv. Ife Brown, the splitting can be traced to inadequate sorting and handling procedures. Hence, better handling procedures should be adopted. The existence of variegation, set cv. Ekpoma Local apart. The color difference in the testa was predominantly dark brown, while the other parameters were uniformly distributed. Cultivar Sokoto White also had uniformly distributed parameters. The seed volume had the highest coefficient of variation (CV) among the three varieties studied. This implies that the seed volumes for each variety have the greatest degree of heterogeneity. Although cv. Sokoto White had the highest CV for seed volume, cv. Ife Brown had the highest number of squares, while cv. Ekpoma Local had the lowest sum of squares. The difference between cv. Ekpoma Local and cv. Sokoto White was low. *Vigna unguiculata* cv. Ife Brown had the most variation. Though cv. Ekpoma Local and cv. Sokoto White are similar in size, but they differ significantly in testa basal color, variegation presence, eye color, and pattern. The pale grey testa basal color, lack of color variegation, and greyish eye color cv. Sokoto White has all been identified as significant differences. Cultivar Ekpoma Local has a dark brown basal testa color as well as a variegated testa. Phenotypically, the three cultivars assessed in the current are not the same.

There is a high chance of variations arising within legume species, such as *Medicago truncatula, Lotus japonicus, Phaseolus vulgaris, Arachis hypogaea, Cajanus cajan,* and *Cicer arietinum* and these differences can be attributed to environmental, physiological, and genetic influences (Smykal et al. 2022; Salgotra and Stewart 2022). The environmental influences also include the agricultural production preferences of the farmer. As a consequence, our results may be affected by these factors. Cultivar Ife Brown has different testa basal colors, which may be due to the expression of many color factor genes, as seed testa color expression in cowpea is regulated by many genes. Many genes are thought to be involved in the inheritance of seed testa color in cowpea and these are together called *Color Factor* and includes *Watson, Holstein-1,* and *Holstein-2* in a three-locus system (Egbadzor et al. 2014; Zuluaga et al. 2021).

Table 7. Assessment of the sum of squares of measured parameters of *V. unguiculata* collected at sampling sites

Source of variation	Type III sum of squares	Df	Mean square	F	*P*-value
V. unguiculata cv. Ife Brown					
Corrected Model	587.8[a]	4	146.95	1042.6	<0.001
Intercept	857.9	1	857.87	6086.6	<0.001
Group	587.8	4	146.95	1042.6	<0.001
Error	20.4	145	0.14		
Total	1466.1	150			
Corrected Total	608.2	149			
[a.]R Squared = 0.966 (Adjusted R Squared = 0.965)					
V. unguiculata cv. Ekpoma Local					
Corrected Model	104.8[b]	4	26.18	953.8	<0.001
Intercept	231.9	1	231.91	8448.3	<0.001
Group	104.7	4	26.18	953.8	<0.001
Error	3.9	145	0.03		
Total	340.6	150			
Corrected Total	108.7	149			
[b.]R Squared = 0.963 (Adjusted R Squared = 0.962)					
V. unguiculata cv. Sokoto White					
Corrected Model	124.9[c]	4	31.22	819.9	<0.001
Intercept	252.2	1	252.18	6622.2	<0.001
Group	124.9	4	31.23	819.9	<0.001
Error	5.5	145	0.04		
Total	382.6	150			
Corrected Total	130.4	149			
[c.]R Squared = 0.958 (Adjusted R Squared = 0.956)					

Table 8. Modal phenotypic and qualitative parameters of *V. unguiculata* cv. Ife Brown seeds collected at sampling sites

Seed codes	Seed shape	Splitting of testa	Testa texture	Testa color variegation	Testa basal color	Pattern of testa variegation	Basal color of variegated seed	Eye color	Eye pattern	Brilliance of seeds
IKCbMsL1	5	1	7	0	Light peach	0	0	3	3	2
IKCbMsL2	5	1	7	0	Sand	0	0	3	3	2
IKCbMsL3	5	1	7	0	Light brown	0	0	3	3	2
IKCbMeL1	5	1	7	0	Tan	0	0	2	3	2
IKCbMeL2	5	1	7	0	Light brown	0	0	2	3	2
IKCbMeL3	5	1	7	0	Light peach	0	0	2	3	2
IKCbMoL1	5	1	7	0	Pale peach	0	0	2	3	2
IKCbMoL2	5	1	7	0	Beige	0	0	2	3	2
IKCbMoL3	5	1	7	0	Light peach	0	0	2	3	2
ORCbMuL1	5	1	7	0	Tan	0	0	2	3	2
ORCbMuL2	5	1	7	0	Peach	0	0	2	3	2
ORCbMuL3	5	1	7	0	Light peach	0	0	2	3	2
ORCbMoL1	5	1	7	0	Camel	0	0	2	3	2
ORCbMoL2	5	1	7	0	Light brown	0	0	2	3	2
ORCbMoL3	5	1	7	0	Pale brown	0	0	2	3	2
ORCbMnL1	5	1	7	0	Cocoa	0	0	2	3	2
ORCbMnL2	5	1	7	0	Pale brown	0	0	2	3	2
ORCbMnL3	5	1	7	0	Light peach	0	0	2	3	2
OVCbMeL1	5	1	7	0	Dull orange	0	0	3	3	2
OVCbMeL2	5	1	7	0	Butterscotch	0	0	3	3	2
OVCbMeL3	5	1	7	0	Light peach	0	0	3	3	2
OVCbMoL1	5	1	7	0	Sand brown	0	0	3	3	2
OVCbMoL2	5	1	7	0	Sand brown	0	0	3	3	2
OVCbMoL3	5	1	7	0	Tan	0	0	3	3	2
EGCbMuL1	5	1	7	0	Pinkish tan	0	0	1	3	2
EGCbMuL2	5	1	7	0	Pinkish grey	0	0	1	3	2
EGCbMuL3	5	1	5	0	Light brown	0	0	1	3	2
EGCbMeL1	5	1	7	0	Apricot	0	0	2	3	2
EGCbMeL2	5	1	7	0	Light peach	0	0	2	3	2
EGCbMeL3	5	1	7	0	Light peach	0	0	2	3	2

Table 9. Modal phenotypic and qualitative parameters of V. *unguiculata* cv. Ekpoma Local seeds collected at sampling sites

Seed codes	Seed shape	Splitting of testa	Testa texture	Testa color variegation	Testa basal color	Pattern of testa variegation	Basal color of variegated seed	Eye color	Eye pattern	Brilliance of seeds
IKCeMsL1	5	0	3	1	Dark brown	2	1	2	6	2
IKCeMsL2	5	0	3	1	Dark brown	2	1	2	6	2
IKCeMsL3	5	0	3	1	Dark brown	2	1	2	6	2
IKCeMeL1	5	0	3	1	Dark brown	2	1	2	6	2
IKCeMeL2	5	0	3	1	Light brown	2	1	2	6	2
IKCeMeL3	5	0	3	1	Dark brown	2	1	2	6	2
IKCeMoL1	5	0	3	1	Dark brown	2	1	2	6	2
IKCeMoL2	5	0	3	1	Dark brown	2	1	2	6	2
IKCeMoL3	5	0	3	1	Dark brown	2	1	2	6	2
ORCeMuL1	5	0	3	1	Dark brown	2	1	2	6	2
ORCeMuL2	5	0	3	1	Light brown	2	1	2	6	2
ORCeMuL3	5	0	3	1	Light brown	2	1	2	6	2
ORCeMoL1	5	0	3	1	Dark brown	2	1	2	6	2
ORCeMoL2	5	0	3	1	Dark brown	2	1	2	6	2
ORCeMoL3	5	0	3	1	Dark brown	2	1	2	6	2
ORCeMnL1	5	0	3	1	Dark brown	2	1	2	6	2
ORCeMnL2	5	0	3	1	Dark brown	2	1	2	6	2
ORCeMnL3	5	0	3	1	Light brown	2	1	2	6	2
OVCeMeL1	5	0	3	1	Dark brown	2	1	2	6	2
OVCeMeL2	5	0	3	1	Dark brown	2	1	2	6	2
OVCeMeL3	5	0	3	1	Dark brown	2	1	2	6	2
OVCeMoL1	5	0	3	1	Dark brown	2	1	2	6	2
OVCeMoL2	5	0	3	1	Dark brown	2	1	2	6	2
OVCeMoL3	5	0	3	1	Dark brown	2	1	2	6	2
EGCeMuL1	5	0	3	1	Light brown	2	1	2	6	2
EGCeMuL2	5	0	3	1	Dark brown	2	1	2	6	2
EGCeMuL3	5	0	3	1	Dark brown	2	1	2	6	2
EGCeMeL1	5	0	3	1	Dark brown	2	1	2	6	2
EGCeMeL2	5	0	3	1	Dark brown	2	1	2	6	2
EGCeMeL3	5	0	3	1	Dark brown	2	1	2	6	2

According to Tiryaki et al. (2016), the testa color trait is polygenic and influenced by multiple genes in a variety of plant species, including legumes such as cowpea, common bean (*Phaseolus vulgaris*), and soybean (*Glycine max*). This expression of multiple genes results in varying levels of several color pigments in the seed testa, which explains the observed color changes in seed testa (Mavi 2010). Environmental factors like temperature and light intensity may also affect the production of these pigments II (Ohanmu et al. 2019b). According to Bhatt et al. (2016), seed color has also been stated to play a role in seed dormancy and germination in leguminous plants. As a result, further research should be conducted to examine the relationship between seed testa color and dormancy as well as germination. Furthermore, seed size and seed coat color have been used to establish a simple method of improving seed quality for many crop species, including common bean (*P. vulgaris*), cowpea, rapeseed (*Brassica napus*), flax (*Linum usitatissimum*), and *Arabidopsis thaliana* (Tiryaki et al. 2016). In contemporary agricultural systems, where uniformity is preferred, seed differences may cause uncertainty (Mitchell et al. 2016). More so if these differences like seed color, weight, shape, and volume have negative impacts on crop yield like reducing yield numbers and amounts. These seed differences may be unfavourable to both the seller and buyer, particularly when they are undesirable traits to the end users and consumers.

Over 115 common bean germplasm resources were assessed using key morphological characters and it was discovered that the population was highly diverse (Long et al. 2020). A study of the phenotypic diversity of two chickpeas (*Cicer arietinum*) collections was conducted in Ethiopia where data were obtained from three independent places in one region, and the results indicated significant differences in phenotypic and agronomic performance variability between the two collections (Admas et al. 2021). Another research looked at the variance in seed morphologies of 160 *Cucurbita maxima* populations obtained from different parts of Turkey. Sizeable differences in seed shape, color, size, and weight were ascertained (Balkaya et al. 2009). In the study of 56 Japanese native cultivars of common buckwheat (*Fagopyrum esculentum*), a considerable number of variances in seed shape characteristics and husk colors were also detected (Tetsuka and Uchino 2005).

Table 10. Modal phenotypic and qualitative parameters of *V. unguiculata* cv. Sokoto White seeds collected at sampling sites

Seed codes	Seed shape	Splitting of testa	Testa texture	Testa color variegation	Testa basal color	Pattern of testa variegation	Basal color of variegated seed	Eye color	Eye pattern	Brilliance of seeds
IKCsMsL1	5	0	5	0	Pale grey	0	0	1	2	2
IKCsMsL2	5	0	5	0	Pale grey	0	0	1	2	2
IKCsMsL3	5	0	5	0	Pale grey	0	0	1	2	2
IKCsMeL1	5	0	5	0	Pale grey	0	0	1	2	2
IKCsMeL2	5	0	5	0	Pale grey	0	0	1	2	2
IKCkMeL3	5	0	5	0	Pale grey	0	0	1	2	2
IKCsMoL1	5	0	5	0	Pale grey	0	0	1	2	2
IKCsMoL2	5	0	5	0	Pale grey	0	0	1	2	2
IKCsMoL3	5	0	5	0	Pale grey	0	0	1	2	2
ORCsMuL1	5	0	5	0	Pale grey	0	0	1	2	2
ORCsMuL2	5	0	5	0	Pale grey	0	0	1	2	2
ORCsMuL3	5	0	5	0	Pale grey	0	0	1	2	2
ORCsMoL1	5	0	5	0	Pale grey	0	0	1	2	2
ORCsMoL2	5	0	5	0	Pale grey	0	0	1	2	2
ORCsMoL3	5	0	5	0	Pale grey	0	0	1	2	2
ORCsMnL1	5	0	5	0	Pale grey	0	0	1	2	2
ORCsMnL2	5	0	5	0	Pale grey	0	0	1	2	2
ORCsMnL3	5	0	5	0	Pale grey	0	0	1	2	2
OVCsMeL1	5	0	5	0	Pale grey	0	0	1	2	2
OVCsMeL2	5	0	5	0	Pale grey	0	0	1	2	2
OVCsMeL3	5	0	5	0	Pale grey	0	0	1	2	2
OVCsMoL1	5	0	5	0	Pale grey	0	0	1	2	2
OVCsMoL2	5	0	5	0	Pale grey	0	0	1	2	2
OVCsMoL3	5	0	5	0	Pale grey	0	0	1	2	2
EGCsMuL1	5	0	5	0	Pale grey	0	0	1	2	2
EGCsMuL2	5	0	5	0	Pale grey	0	0	1	2	2
EGCsMuL3	5	0	5	0	Pale grey	0	0	5	6	2
EGCsMeL1	5	0	5	0	Pale grey	0	0	1	2	2
EGCsMeL2	5	0	5	0	Pale grey	0	0	1	2	2
EGCsMeL3	5	0	5	0	Pale grey	0	0	1	2	2

Benin City, Edo State (Nigeria) where the majority of the samples used in the study were sourced is a central hub where people travelling from and to diverse parts of the country go through, it is imperative to note that diverse *V. unguiculata* cultivars from different part of the country may have a legacy effect and influence local cultivars like *V. unguiculata* cvs. Ife Brown, and Ekpoma Local. Concerning the current lack of data on cowpea diversity, utilization, breeding, and conservation in Nigeria, a diverse array of criteria is needed to resolve this. Addressing the issue will enable an understanding of the source and extent of variations as well as a correlation of environmental variations within cowpea cultivars and varieties (Ifie et al. 2019; Iseghohi et al. 2019).

In conclusion, *V. unguiculata* cv. Ife Brown sold in markets within Edo state have the greatest phenotypic variations among all the cultivars accessed in this study, even though, it does not originate from and is not native to the state. The observed phenotypic variation in *V. unguiculata* cv. Ife Brown is likely due to a combination of genetic and environmental factors from the western part of Nigeria where it is native. Although, *V. unguiculata* cv. Ekpoma Local is considered native to Edo State, and has many similarities with *V. unguiculata* cv. Sokoto White, it may not originally be from the State. Also, the similarities between both cultivars may be linked to the nearly similar environmental conditions prevalent in Ekpoma and Sokoto. Generally, the findings from this study support the possibility of variations existing within the cowpea seeds available in Edo State, Southern Nigeria. This finding will enable future workers to effectively compare *V.* unguiculata through both morphological and molecular investigations.

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