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Reproductive biology of green-stemmed and red-stemmed *Basella alba*

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Abstract

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Basella alba is an important, underutilised vegetable rich in vitamins and consumed in many parts of the world. However, the reproductive biology of this species is insufficiently known; therefore, this study was performed aiming to fill this knowledge gap. Although green-stemmed and red-stemmed *Basella alba* share a lot of reproductive attributes in common, the obtained results showed differences in their flower bud apex colour, anther colour, spike colour, days to 50% flowering, plant height at flower bud initiation and the mean number of flowers per spike. In both forms, the floral attributes favoured self-pollination. However, the possibility of cross-pollination among them due to the activities of pollinators such as ants, moths, butterflies and bees suggests a mixed mating system. Furthermore, both forms attained sexual maturity at varying periods, thus indicating the existence of a temporal prezygotic barrier between them and limiting the chances of their hybridisation. They could also be responsible for the ability to remain in their distinct forms even when they exist together in the same habitat.

Keywords: germination percentage, isolating mechanism, pollen viability, self-pollination.

INTRODUCTION

The genus *Basella* (Basellaceae) comprises five species: *Basella excavata* Eliot, *Basella leandriana* H. Perrier, *Basella madagascariensis* Boivin ex H.Perrier, *Basella paniculata* Volkens and *Basella alba* L. (Eriksson, 2004). *Basella excavata*, *Basella leandriana* and *Basella madagascariensis* are native to Magadascar, *Basella paniculata* is native to South and East Africa, while *Basella alba* has a pantropical distribution (Eriksson, 2004). Earlier recognised *Basella rubra* is currently considered a red-stemmed form of *Basella alba* (Cook, 2010; Deshmukh & Gaikward, 2014).

Green-stemmed and red-stemmed forms of *Basella alba* are common vegetables among the Yoruba tribe of Southwestern Nigeria and are referred to as 'amunututu'. They are good sources of vitamins A and C (Roy et al., 2010). They contain several phytoconstituents such as proteins, alkaloids, carbohydrates, polysaccharides, phenols, flavonoids, carotenoids, minerals and vitamins (Kumar et al., 2012). They are helpful in phytomedicine in treating gonorrhoea, constipation, leprosy, dysentery, ulcer and burns (Dixit & Goyal, 2011; Shantha et al., 2016).

Although there have been reports on some aspects of the morphology and phytomedicinal potentials of green-stemmed and red-stemmed *Basella alba*, there is a lack of information on their reproductive biology. The specific objectives of this research were, therefore, to investigate their reproductive attributes such as seed germination percentage, days to seedling emergence, floral structure, period of anthesis, pollen viability, days to 50% flowering, plant height at flower bud initiation, pollinators, mode of fruit and seed dispersal and reproductive isolating mechanisms.

MATERIALS AND METHODS

Seeds of green-stemmed and red-stemmed *Basella alba* accessions were collected from various locations within Southwestern Nigeria (Table 1). These were authenticated at the IFE Herbarium located at the Department of Botany, Obafemi Awolowo University, Nigeria. Seeds were sown in polythene bags filled with sterilised topsoil and raised to maturity in the screen house of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The experiment was laid out in an utterly randomised design while regular agronomic practices such as irrigation, weeding and staking for optimal growth and development were carried out regularly. A total number of 38 seedlings of green-stemmed *Basella alba* accessions and 45 seedlings of red-stemmed *Basella alba* accessions were raised and studied. The study was carried out from December 2018 to April 2020.

Data were collected from randomly selected plants on the following parameters: the mean number of days to seeding emergence, mean germination percentage, the mean number of days to 50% flowering, floral attributes, mean plant height at flower bud initiation, period to anthesis, percentage pollen viability, types of pollinators, the mean number of fruits per spike, mean number of seeds per spike, the colour of ripe and unripe fruit, colour and types of seeds and mode of seed dispersal.

The germination study was carried out on 8 cm Petri dishes laid with 7 cm Whatman filter paper at temperatures between 20°C and 30°C. Twenty seeds drawn at random were placed on a wet filter paper inside a Petri dish, and this was done in three replicates. The mean number of days to the radicle emergence was documented, and the percentage of germinated seeds was determined.

Table 1. Sources of green-stemmed and red-stemmed *Basella alba* plants used in the study. Numbers in brackets represent the number of studied plants within the accession

Accession	Form	Source	Location	Comment
BAIWO (10)	Green-stemmed	Iwo	7.629444 °N 4.191111 °E	Cultivated in home gardens
BAIFE (8)	Green-stemmed	Ile-Ife	7.523056 °N 4.515833 °E	Cultivated in school gardens
BAONDO (5)	Green-stemmed	Ondo	7.236111 °N 5.239722 °E	Cultivated in home gardens
BAEKITI (5)	Green-stemmed	Ekiti	7.616389 °N 5.218333 °E	Cultivated in gardens
BAOYO (5)	Green-stemmed	Oyo	7.419167 °N 3.964722 °E	Cultivated in church gardens
BALAG (5)	Green-stemmed	Ijede	6.942778 °N 4.191111 °E	Cultivated beside home
BRIWO (9)	Red-stemmed	Iwo	7.629444 °N 4.191111 °E	Cultivated in home gardens
BRIFE (8)	Red-stemmed	Ile-Ife	7.523056 °N 4.515833 °E	Cultivated in school gardens
BRONDO (10)	Red-stemmed	Ondo	7.236111 °N 5.239722 °E	Cultivated in home gardens
BREKITI (7)	Red-stemmed	Ekiti	7.616389 °N 5.218333 °E	Cultivated in home gardens
BROYO (5)	Red-stemmed	Ogbomoso	8.146111 °N 4.259167 °E	Cultivated in school gardens
BRLAG (6)	Red-stemmed	Ijede	6.942728 °N 3.098056 °E	Cultivated in home gardens

The pollen viability study was carried out by collecting pollen grains from freshly dehisced anthers onto microscope slides. These were stained with cotton-blue-in-lactophenol for 30 minutes. A total of 100 pollen grains were examined using the light microscope for percentage stainability following the methods of Bolaji & Nwokeocha (2013). The well-formed and deeply stained pollens were considered viable, while those with a collapsed outline, partially stained or not stained, were considered non-viable.

The quantitative reproductive data obtained were subjected to the General Linear Model (GLM) analysis of variance (ANOVA). Results of data analysis were presented as the mean and standard error (mean ± SE). In addition, differences between means were evaluated by applying Duncan Multiple Range Test (DMRT) at $p < 0.05$. Statistical analyses were performed employing System Analysis Software (*SAS*, version *9.0*).

RESULTS

Analysis revealed that germination of green-stemmed *Basella alba* seeds was 72.40 ± 0.97%, while that of red-stemmed *Basella alba* was 75.5 ± 0.48%, and no significant differences between them were found (Table 2). The mean number of days to seedling emergence was 10.12 ± 3.15 for green-stemmed and 13.02 ± 4.11 for red-stemmed plants. The mean plant height at flower bud initiation was 87.70 ± 9.05 cm for green-stemmed *Basella alba*, while 119.49 ± 6.80 cm for red-stemmed plants. Significant differences were found between plant height at flower bud initiation ($p = 0.005$). The mean number of days to 50% flowering was 100.10 ± 3.60 for green-stemmed, whereas red-stemmed plants spent significantly longer ($p < 0.001$) time to flowering, i.e. 200.20 ± 4.07 days (Table 2). The study revealed that the mean number of flowers per spike of green-stemmed plants was significantly higher ($p < 0.001$) than of red-stemmed plants (22.16 ± 3.13 and 16.45 ± 1.83 flowers, respectively).

Anthesis of both forms of *Basella alba* occurred between 8:00 a.m. and 12:00 noon. The pollens of green-stemmed plants were powdery, light yellow. The pollens of red-stemmed *Basella alba* differed by deep yellow colour. No significant differences between the mean pollen viability were found (93.70% and 94.50 %, respectively).

Table 2. Comparison of reproductive characteristics between green-stemmed and red-stemmed forms of *Basella alba*. Different letters in superscripts across a row indicate significant differences between the means ($p < 0.05$)

Attributes	Green-stemmed (n = 38)	Red-stemmed (n = 45)
Percentage of seed germination (%)	72.40 ± 0.97^a	75.50 ± 0.48^a
Number of days to seedling emergence	10.12 ± 3.15^a	13.02 ± 4.11^a
Plant height at flower bud initiation (cm)	87.70 ± 9.05^a	119.49 ± 6.80^b
Days to 50% flowering	100.10 ± 3.60^a	200.20 ± 4.07^b
Mean number of flowers per spike	22.16 ± 3.13^a	16.45 ± 1.83^b
Percentage pollen viability (%)	93.70^a	94.50^a
Mean fruit length (cm)	1.07 ± 0.03^a	1.15 ± 0.02^a
Mean fruit diameter (cm)	2.20 ± 0.02^a	2.25 ± 0.03^a
Mean number of fruits per spike	12.21 ± 4.59^a	10.24 ± 3.20^a
Mean seed length (cm)	0.65 ± 0.01^a	0.64 ± 0.04^a
Mean seed diameter (cm)	1.25 ± 0.04^a	1.20 ± 0.02^a

The fruits of *Basella alba* are spherical juicy drupes. The unripe fruits were green and deep purple when they ripened for both forms of this species. The mean fruit length was 1.07 ± 0.03 cm and mean diameter was 2.20 ± 0.02 cm for green-stemmed *Basella alba*, while the mean fruit length was 1.15 ± 0.02 cm and mean diameter was 2.25 ± 0.03 cm for red-stemmed *Basella alba*. No significant differences between the two forms of *Basella alba* by the mean fruit length ($p = 0.638$) and fruit diameter ($p = 0.223$) were revealed.

The mean number of fruits, and thus seeds, per spike was 12.21 ± 4.59 for green-stemmed *Basella alba*, while red-stemmed plants had 10.24 ± 3.20 (Table 2). According to the mean number of fruits per spike, no significant differences were found between the two forms ($p = 0.407$).

The mean seed length was 0.65 ± 0.01 cm, while the mean diameter was 1.25 ± 0.04 cm for green-stemmed *Basella alba*. The mean seed length of red-stemmed plants was 0.64 ± 0.04 cm, and their mean diameter was 1.20 ± 0.02 cm (Table 2). No significant differences were found between the green-stemmed and red-stemmed plants' mean seed length (p = 0.352) and seed diameter (p = 0.374). The two forms flowered and fruited sequentially throughout the remaining period of their life cycles.

DISCUSSION

Reproductive biology and systematics are interrelated (Anderson et al., 2002). Its importance in the taxonomic description of species has been harnessed by many researchers (Folorunsho & Olorode, 2008; Ratha & Paramathma, 2009; Bolaji et al., 2020). This study revealed that green-stemmed and red-stemmed *Basella alba* plants were similar in their reproductive characteristics. There were no significant differences between the two *Basella* forms regarding seed germination percentage and the number of days to seedling emergence, pollen viability, mean fruit length, mean fruit diameter, the mean number of fruits per spike, mean seed length and mean seed diameter (Table 2). The lack of significant differences in these traits supports the opinion that the two *Basella* forms belong to the same species. This point of view has been expressed by Roy et al. (2010).

Although the inflorescence, fruits and seeds were similar in many respects, notable differences were observed in their flower bud apex and anther colours. While the flower bud was pink and the anther light yellow in green-stemmed *Basella alba*, the flower bud apex was deep purple, and the anthers were deep yellow of red-stemmed *Basella alba.* This could be why some researchers (Henry et al., 1987; Roy et al., 2010) consider them as varieties. Understanding species' reproductive biology helps clarify characters' potential use and values in systematic treatments (Anderson et al., 2002).

The percentage germination of the seeds was notably high (72.40 ± 0.97 for green-stemmed and 75.50 ± 0.48 for red-stemmed *Basella alba*). According to Labhane et al. (2014), the traditional method of studying the viability of seeds is by considering the percentage of their germination. The number of days to seedling emergence for both forms was similar (10.12 ± 3.15 for green-stemmed and 13 ± 4.11 for red-stemmed *Basella alba*). The high viability of the seeds and the ability of the seedlings to emerge within a few days could have significantly contributed to their reproductive success and ability to thrive easily under varying environmental conditions. According to Norman et al. (2021), seed germination and seedling emergence are the most critical and vulnerable phases of a crop cycle. Therefore, they can predict the extent of a species' success. Palada & Crossman (1999) have also noted that *Basella alba* adapts easily to various soils and climates and is considered one of the best tropical leaf-vegetable throughout the tropical world.

It is noteworthy that from the number of days to 50% flowering and plant height at flower bud initiation, the red-stemmed *Basella rubra* spent a much longer time (Table 2) in the vegetative phase than the green-stemmed. This implies that the two *Basella alba* forms attain sexual maturity at varying periods.

Conversely, even though the period of anthesis, pollinators, fruit and seed dispersal agents were similar for both *Basella alba* forms, the timing of their sexual maturity is such that green-stemmed *Basella alba* would have flowered and started to produce fruits long before red-stemmed *Basella alba* begins to flower, thereby making hybridisation between the two forms limited. This indicates the temporal prezygotic barrier, which could be responsible for their ability to remain in their distinct forms even when they occur in the same habitat. Prezygotic barriers include spatial, temporal or behavioural differences leading to sexual isolation, and they are the most critical and effective barriers, given that they act early in the life cycle of an organism to impose the strongest impediment to gene flow, thereby preventing hybridisation (Widmer et al., 2009; Henrich & Kalbe, 2016).

The bisexual nature of the flowers and the location of the anthers above and very close to the stigma, favour self-pollination in both forms. However, the bright colouration of the perianths of the two species attracted pollinating insects such as ants, bees, moths and butterflies, thereby encouraging cross-pollination, suggesting that the breeding system in both forms is a mixed mating system. According to Holsinger (1996), the continuum between self-pollination and outcrossing in plants results in a mixed mating system. Understanding the breeding system of these two *Basella alba* forms could enhance their effective genetic improvement through hybridisation programmes by breeders, thereby enhancing their utilisation by consumers. It is also key to their effective conservation. According to Anderson et al. (2001), the knowledge of the reproductive system of plants is a central element in their effective conservation. Vivian-Smith et al. (2007) have also noted that flowers of *Anredera cordifolia* (Basellaceae) attract pollinators such as bees and ants.

Hybridisation between green-stemmed and red-stemmed *Basella alba* could be enhanced by varying their planting periods. Their flowering periods overlap so that outcrossing between them is enhanced. This is worthwhile considering the temporary prezygotic isolation between them because of differences in the time it takes for both species to attain the generative stage. Breeders could explore this information in carrying out hybridisation programmes for the genetic improvement of *Basella alba*. The knowledge of pollination, breeding systems and seed dispersal of plant species could be used to enhance their conservation and restoration (Hamrick et al., 1991; Karron, 1991; Ramirez, 2006). We suppose that humans mainly disperse seeds of both forms of *Basella alba*. Vivian-Smith et al. (2007) have reported that *Anredera cordifolia* (Basellaceae) is also primarily spread by humans and water.

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REFERENCES

Anderson G.J., Bernardello G., Stuessy T.F., Crawford D.J., 2001: Breeding systems and pollination of selected plants endemic to the Juan Fernandez Islands. – American Journal of Botany, 88: 220–233. https://doi.org/10.2307/2657013

Anderson G.J., Johnson S.D., Neal P.R., Bernardello G., 2002: Reproductive biology and plant systematics: The growth of a symbiotic association. – Taxon, 51: 637–653. https://doi.org/10.2307/1555019

Bolaji A.O., Nwokeocha C.C., 2013: Issues concerning reproductive isolation in rice hybrid swarm involving *Oryza sativa* Linn., *O. longistaminata* A. Chev. et Roehr. and *O. glaberrima* Steud. Located in Jebba, Nigeria. – International Journal of Biological and Chemical Sciences, 7(5): 2040–2049. https://doi.org/10.4314/ijbcs.v7i5.21

Bolaji A.O., Idowu-Aiye M., Moronfade H.O., 2020: Reproductive biology of four weedy *Euphorbia* species from Ile-Ife, Nigeria. – Ife Journal Science, 22(1): 1–8. https://doi.org/10.4314/ijs.v22i1.1

Cook A., 2010: Linnaeus and Chinese plants: A test of the linguistic imperialism thesis. – Notes and Records of the Royal Society, 64: 121–138. https://doi.org/10.1098/rsnr.2009.0051

Deshmukh S.A., Gaikward D.K., 2014: A review of the taxonomy, ethnobotany, phytochemistry and pharmacology of *Basella alba* (Basellaceae). – Journal of Applied Pharmaceutical Science, 4(1): 153–165. https://doi.org/10.7324/japs.2014.40125

Dixit U., Goyal V.C., 2011: Traditional knowledge from and for elderly. – Indian Journal of Traditional Knowledge, 10: 429–438.

Eriksson R., 2004: Basellaceae. – In: Smith N. (ed.), Flowering plants of Neotropics: 44–45. Princeton.

Folorunsho A.E., Olorode O., 2008: Biosystematic studies in Annonaceae II. Vegetative and floral morphological studies of some genera of Annonaceae in Nigeria. – Research Journal of Botany, 3(1): 1–8. https://doi.org/10.3923/rjb.2008.1.8

Hamrick J.L., Godt M.J.N., Murrawski D.A., Loveless M.D., 1991: Correlations between species traits and allozyme diversity: Implications for conservation biology. – In: Falk D.A., Holsinger K. (eds), Genetics and Conservation of Rare Plants: 75–86. New York.

Henrich T., Kalbe M., 2016: The role of prezygotic isolation mechanisms in the divergence of two parasite species. – BMC Evolutionary Biology, 16: 245. https://doi.org/10.1186/s12862-016-0799-5

Henry A.N., Kumari G.R., Chithra V., 1987: Flora of Tamilnade, India, Ser. 1, 11. Coimbatore.

Holsinger K.E., 1996: Pollination biology and the evolution of mating systems in flowering plants. – Evolution Biology, 29: 107–149.

Karron J.D., 1991: Patterns of genetic variation and breeding systems in rare plant species. – In: Falk D.A., Holsinger K.E. (eds), Genetics and Conservation of Rare Plants. New York.

Kumar V., Bhat Z.A., Kumar D., Khan N.A., Chashoo I.A., Ara I., 2012: Gastroprotective effect of leaf extracts of *Basella alba* var. r*ubra* against experimental gastric ulcers in rats. – Brazillian Journal of Pharmacognosy, 22: 657–662. https://doi.org/10.1590/s0102-695x2012005000032

Labhane N.M., Dongarwar N.M., Borkar S.J., 2014: In vivo study of morphology of embryo in the medicinal plant, *Basella alba* Linn. (Basellaceae). – International Journal Life Sciences, 2(4): 329–333.

Norman P.E., Danquah A., Asfaw A., Tongoona P.B., Danquah E.Y., Asiedu R., 2021: Seed viability, seedling growth and yield in White Guinea Yam. – Agronomy, 11(2): 1–10. https://doi.org//10.3390/agronomy11010002

Palada M.C., Crossman M.A., 1999: Evaluation of tropical leaf vegetables in the Virgin Islands. – In: Perspectives on New Crops and New Uses: 388–393. Alexandria.

Ramirez N., 2006: Reproductive biology and plant species selection for habitat restoration in the Vezuelan Gran Sabana Plateau. – INCI, 31(5): 114–124.

Ratha P.K., Paramatha M., 2009: Potentials and *Jatropha* species wealth in India. – Current Science*,* 97: 1000–1004.

Roy S.K., Gangopadhyay G., Mukherjee K.K., 2010: Is stem twinning form of *Basella alba* L. a naturally occurring variant? – Current Science, 98(10): 1370–1375.

Shantha T.R., Patchaimal P., Prathapa M., Kumar R., Tewari D., Bharti V., Venkateshwarlu G., Mangal A.K., Padhi M.M., Dhiman K.S., 2016: Pharmacognostical standardization of upodika, *Basella alba* Linn, an important ayurvedic antidiabetic plant. – Ancient Science of Life, 36(1): 35–41. https://doi.org/10.4103/0257-7941.195411

Vivian-Smith G., Lawanson B.E., Turnbull I., Downey P.O., 2007: The biology of Australian weeds. 46. *Anredera cordifolia* (Ten). Steenis. – Plant Protection Quarterly, 22(11): 2–10.

Widmer A., Lexer C., Cozzolino S., 2009: Evolution of reproductive isolation in plants. – Heredity, 102: 31–38. https://doi.org/10.1038/hdy.2008.69

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