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An attempt to solve the taxonomical conflicts in *Basella alba* L.

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Basella alba is a perennial climber with great medicinal potential. The plant is widely used as a green leafy vegetable. There are two forms which are distributed throughout the Southern Asian sub-continent. These forms differ in stem colour, one is green and the other has reddish purple coloured stem. Different workers have different opinions in related with the taxonomy of the *Basella alba*. Some mentioned these two as single species, some as separate species while some have mentioned as the different varieties or cultivars. Henceforth morphological, anatomical, palynological, stomatographic and seed surface studies were undertaken to overcome the taxonomical conflicts. The results supports the opinion as per the IPNI, that both these belongs to the same species, i.e., *alba*. Further molecular studies will elucidate the taxonomical conflicts within the *Basella*.

Keywords: *Basella*, Forms, Taxonomy
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Basella alba (Basellaceae) is a green leafy vegetable which has likely to be originated from Southeast Africa[1].Commonly it is known as Chinese spinach, Ceylon spinach, East Indian spinach, Malabar spinach or cyclone spinach[2,3,4]. Globally it is distributed in tropical Asia, tropical Africa, Brazil, West Indies and Fiji[5]. *Basella alba* is having great biological activities and possess important properties like androgenic, antidiabetic, anti-inflammatory, antimicrobial, antioxidant, antiulcer, antiviral, CNS depressant, hepatoprotective and wound healing[6].

Linnaeus first gave the name *Basella* and also explained distinct two species of *Basella* as *alba* and *rubra* based on the leaf character and stem colour and later in *Hortus Malabaricum* mentioned synonymy for *B. rubra* as *B. alba. Basella* is a green leafy vegetable probably originated from Asia and is with great medicinal potential and also cultivated as a pot herb or as an ornamental[6]. The genus *Basella* comprises five species among which one is pantropical and other are found in Africa and Madagascar[7]. Roxburgh (1832) considered both names i.e., *B. alba* and *B. rubra* as synonymy and considered *Basella alba* as correct name[8] this view was followed by Smitinand[9]. Such conflicting reports have created curiosity regarding the taxonomical studies in *Basella* among the taxonomists[10]. To overcome the taxonomical conflicts the morphological, anatomical, stomatal, palynological and seed cover studies were performed, the results of which will help to put forth the supportive view regarding the status of these two species or forms.

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Material and Methods

The plants were collected and identified as per Cooke[11], Yadav and Sardesai[12], Almeida[13]. The seeds were collected and sown to raise the seedlings. Common conditions were maintained to raise the seedlings for both the forms and morphological, anatomical, stomatal, palynological and seed cover was performed as per standard protocols mentioned below.

i. Morphological studies

The plants were collected and illustrated to find out the similarities and differences and the photo plates were prepared.

ii. Anatomical studies

Free hand sections of root, stem and petiole of both the forms were taken. The sections were photographed under Lawrence Mayo camera attached microscope.

iii. Stomatal studies

Stomatal studies were performed by peel technique method. Microphotography of leaf epidermal peels to

know the structure of stomata was taken by Lawrence Mayo camera attached microscope.

v. Palynological studies

Acetolysis method[14] was employed to perform the palynological studies. The pollen grains structure was studied under the scanning electron microscope JEOL-JSM-6330.

iv. Seed surface studies

Prior to analysis, the seeds were thoroughly washed with acetone and the surface sculpturing studies were performed using scanning electron microscope JEOL-JSM-6330.

Results and discussion

Morphological studies

Basella alba **L. (Red Form) (synonym: *Basellarubra* Roxb.): Fig. 1A, B & C**

It is a fleshy, much branched, perennial climber found commonly around waste places. The plant has tap root system; reddish pink coloured, succulent, glabrous, much branched stem. Leaves are petiolate, alternate, glossy above, entire, thick, broadly ovate, acuminate with cordate base. Flowers are produced in lax spikes. Each flower is small, white with red tinge at apex, bracteate, bracteolate with half divided perianth. Androecium with five inserted stamens which are with short filaments and versatile anthers. Gynoecium with globose ovary, ovules are subsessile, three styles and linear stigmas Fruit of a size of gram having fleshy perianth and thin pericarp. Seed is with scanty albumen, plano spiral embryo and large cotyledons.

Basella alba **(Green Form): Fig. 2A, B & C**

It is a fleshy, much branched, perennial climber with tap root system. Stem is greenish yellow coloured, glabrous and branched. Leaves are petiolate, alternate, entire, glossy above, glaucous beneath, ovate and acuminate with cordate leaf base. Flowers are produced in lax spikes. Each flower is small, white, bracteates and bracteolate with half divided perianth. Androecium with five inserted stamens which are with short filaments and versatile anthers. Gynoecium with globose ovary, ovules are subsessile, three styles and linear stigmas Fruit of a size of gram having fleshy perianth and thin pericarp. Seed is with scanty albumen, plano spiral embryo and large cotyledons.

Anatomical studies in *Basella*

Anatomy has great significance in botanical studies and can be employed for various means, viz., proper identification and authenticity of certain medicinal plants, e.g., *Nigellaspp*[15]., *Amaranthus spinosus*[16], *Passiflora incarnata*[17]; physical standardization of plant drugs, *e.g., Gymnanthemum amygdalinum*[18], *Lepidagathis cristata*[19]; for selection of better rootstocks in horticulture, *e.g., Citrus*[20]; to reveal similarity distance among the taxonomically and ecologically diverse tribes[21]; root internal details can denotes probable productivity and tolerance to soil stresses[22]. Anatomical studies in *Basella alba* and *Basellarubra* have been performed by Busuioc and Ifrim[23] which stated negligible difference in mesophyll and vascular bundles. There are some differences in between *Basellarubra* and *Basella alba* in mesophyll tissue of leaf and stem[24]. The mesophyll in *B. alba* was homogenous while that of in *B. rubra* was differentiated into palisade and spongy parenchyma. In the present study it was observed that the internal details of *B. alba* red form and *B. alba* green form are found to be similar in root, stem and petiole (Fig. 1 & Fig. 2). The details with respect to the plant part can be explained as follows:

a. *Basella alba* (Red form)

i. T. S. of root:Fig. 1D

The section is circular in outline. Epiblema is single layered consisting compactly arranged thin walled parenchyma cells. Cortex is made up of loosely arranged parenchyma cells which are followed by endodermis. Vascular bundles are radial and tetrarch. Pith is absent.

ii. T. S. of stem :Fig. 1E

The section is oval to rectangular in shape with single layered barrel shaped parenchymatous cells forming epidermis which is covered with thin transparent waxy layer of cuticle. Next to epidermis is a hypodermis consisting compactly arranged collenchymatous cells. Endodermis is single layered and pericycle is two to three layered. Vascular bundles are in ring manner, they are conjoint, collateral and closed type. Pith is large with loosely arranged cells.

iii. T. S. of petiole :Fig. 1E

The section is heart shaped in outline and shows single layered compactly arranged epidermis which is covered with thin cuticle. Hypodermis consist thin walled parenchymatous cells. Vascular bundles are centroxylic and are arranged in crescent manner.

b. *Basella alba* (Green form) Fig. 2

i. T. S. of root :Fig. 2D

The section is circular in outline. Epiblema is single layered comprising compactly arranged thin

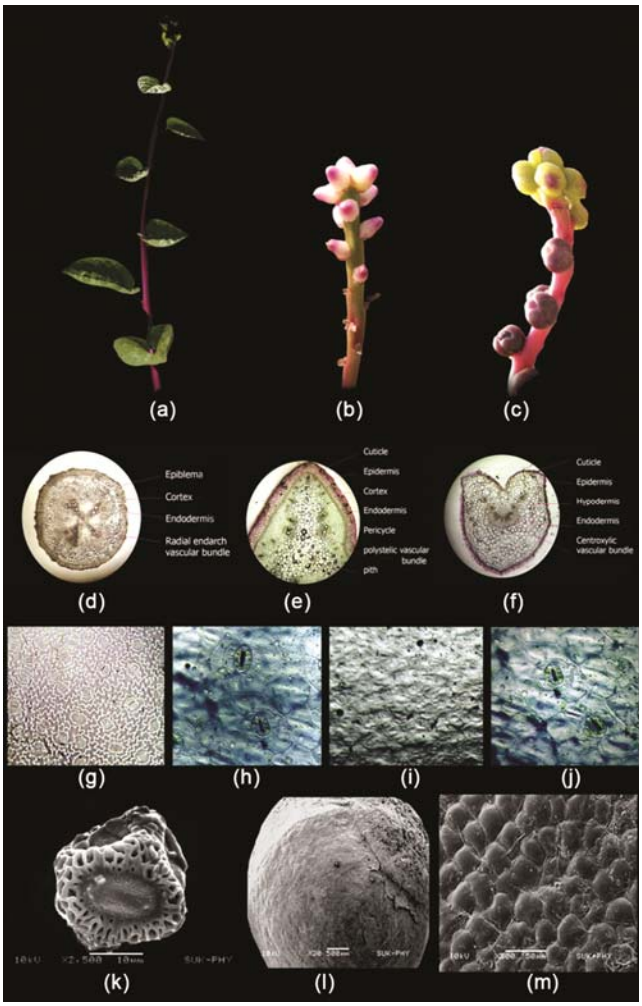

Fig. 1 — *Basella alba* L. (synonym *Basellarubra* Roxb): A. vegetative twig; B. Inflorescence; C. Fruiting twig; D. T. S. of root; E. T. S. of stem; F. T. S. of petiole; G. stomata on adaxial leaf surface (10x); H. stomata on adaxial leaf surface (40x); I. stomata on abaxial leaf surface (10x); J. stomata on abaxial leaf surface (40x); K. pollen grain under SEM; L&M. seed surface under SEM.

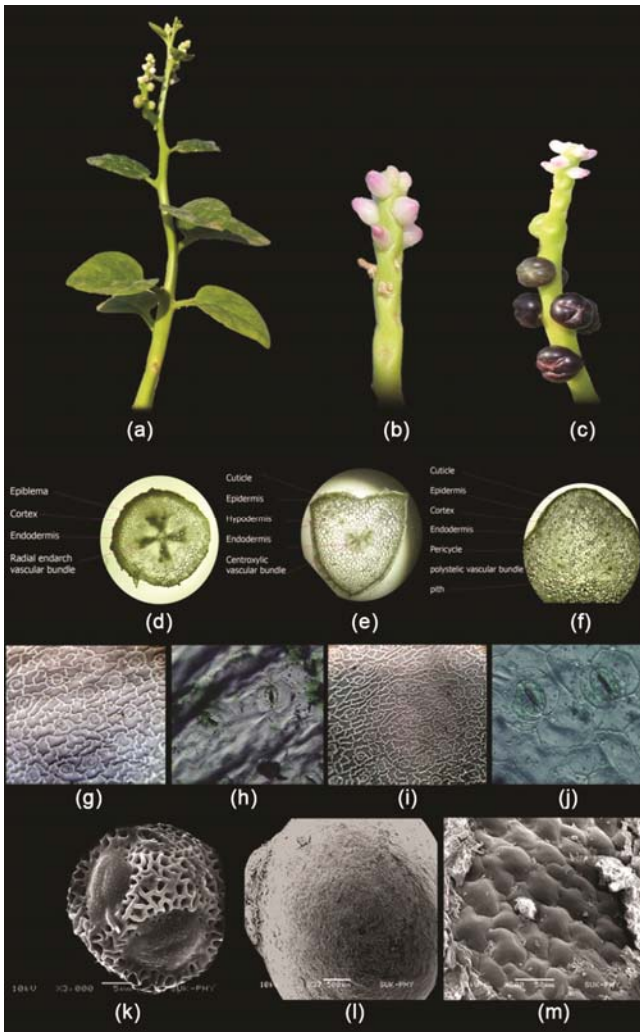

Fig. 2 — *Basella alba* L.:A. vegetative twig; B. Inflorescence; C. Fruiting twig; D. T. S. of root; E. T. S. of stem; F. T. S. of petiole; G. stomata on adaxial leaf surface (10x); H. stomata on adaxial leaf surface (40x); I. stomata on abaxial leaf surface (10x); J. stomata on abaxial leaf surface (40x); K. pollen grain under SEM; L&M. seed surface under SEM.

walled parenchyma cells. Cortex consist loosely arranged parenchymatous cells which is followed by endodermis. Vascular bundles are radial and tetrarch. Pith is absent.

ii. T. S. of stem :Fig. 2E

The section is oval to rectangular in outline and possess thin layered cuticle followed by single layered thick walled epidermis. Hypodermis is made up of few layered compactly arranged collenchymatous cells. Stele consist pericycle, endodermis and vascular bundles. Pericycle is two to three layered made up of sclerenchymatous cells. Endodermis is single layered and the vascular bundles are conjoint, collateral and closed arranged in the ring manner. Pith is broad consisting loosely arranged parenchymatous cells.

iii. T. S. of petiole :Fig. 2F

The section is heart shaped in outline and shows single layered compactly arranged epidermis which is covered with thin cuticle. Hypodermis consist thin walled parenchymatous cells. Vascular bundles are centroxylic and are arranged in crescent manner.

Stomatalstudies: Fig. 1 & Fig. 2 G,H,I & J

Stomatogenesis has long been studied by morphologists, physiologists and taxonomist[25]. Chachad and Vaidya[26] mentioned the importance of stomatographic studies in plant systematics. The type

and arrangement of stomata is one of the key factors to solve the taxonomical conflicts in closely aligned species[27,28]. Stomatal studies in *Basella alba* and *B. rubra* have been performed by Adenegan-Alakinde and Mabel[24]. They reported paracytic and anisocytic stomata in *B. alba* abaxial and adaxial surfaces with frequency 4/100 m^2 while in *B. rubra* the abaxial surface showed paracytic, anisocytic and abnormal stomata with frequency 2/100 m^2 and the adaxial surface was with paracytic and cyclocytic stomata with frequency 3/100 m^2. During the present study it was found that the both the forms of *Basella* possess only amphistomatous stomata on both abaxial as well as adaxial surfaces while the frequency of both the forms on abaxial surface was about 35-40/mm^2 and that of adaxial surface was 28-31/mm^2.

Palynological studies in *Basella alba* :Fig. 1&2: K

Palynology play important role to trace an account of closely related plant groups[29]. To reveal the plant's systematics several workers have studied the palynological aspects in various taxalike *Polygonum*[30], *Bauhinia*[31], *Ceratonia*[32], *Arabidopsis*[33], *Ocimum*[29], *Rumex*[34]. Likewise different workers have studied different plant families such as Apocynaceae[35], Malvaceae[36], Sapotaceae[37], Leguminosae- the Caesalpinioideae[38], Naucleeae[39], Leguminosae[40,41], Asteraceae[42]. Nowicke[43] reported that the pollen grains in *Basella* are cuboidal or prismatic in shape. Recently Roy *et al.,*[2] reported that the pollen grains in *Basella* are rugate, disc shaped and exine with reticulate ornamentation. In the present study it was found that the both the *Basella* forms pollen grains are closely related with each other in their structure and sculpture which supports that these two are not the separate species instead might be considered as forms.

Seed coat studies with SEM: Fig. 1& Fig. 2: L&M

Due to stable morphological characters seed coat structure has great importance in the plant systematics and it works as an additional tool in the field of ethnobotany for the proper identification of the plant taxa[44].The structure and sculpture of the seed coat can be examined with the help of SEM, the result of which has great importance in separation of closely related taxa at generic and intra generic level[46]. Kasem *et al.,*[47] mentioned that the angiosperm taxa can be segregated on the macro and micro structures exhibited by the seeds. To develop informative structural details of the seed coat in *Lycopersicon esculentum* Mill. SEM studies have been performed[48]. For the delimitation of the taxa in families Apocynaceae and Asclepiadaceae seed morphological studies has cited significant importance[49]. In present study it was recorded that the seeds of both the forms of *Basella* are circular to oval in shape with protruded structure at the hilum region. Seed coat ornamentation showed reticulate, hexagonal pattern. These results supports that the two forms of *Basella* might be longs to same species.

Summary and Conclusions

In present study it was found that the both the forms revealed the morpho taxonomic similarities. The anatomical studies with reference to root, stem and petiole also exhibited negligible differences. It was revealed that both the forms of *Basella* possess similar type of stomata and near about same stomatal frequency which supports the view that these two forms are not separate taxa but are the forms of single species. Palynological studies with the help of SEM also showed similarities in pollen structure and sculpture. The study of seed surface with the help of SEM also exhibited more similarities in between the two forms. On the basis of all these studies it can be concluded that the *Basella* two forms belongs to same species. As per the IPNI, the red and green forms are not the separate species but belongs to single species, i.e., *alba*. In present study for the sake of our convenience we have mentioned the forms as red form and green form of *Basella alba.* In addition to these, the molecular studies will elucidate the taxonomical conflicts in between these two plants.

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