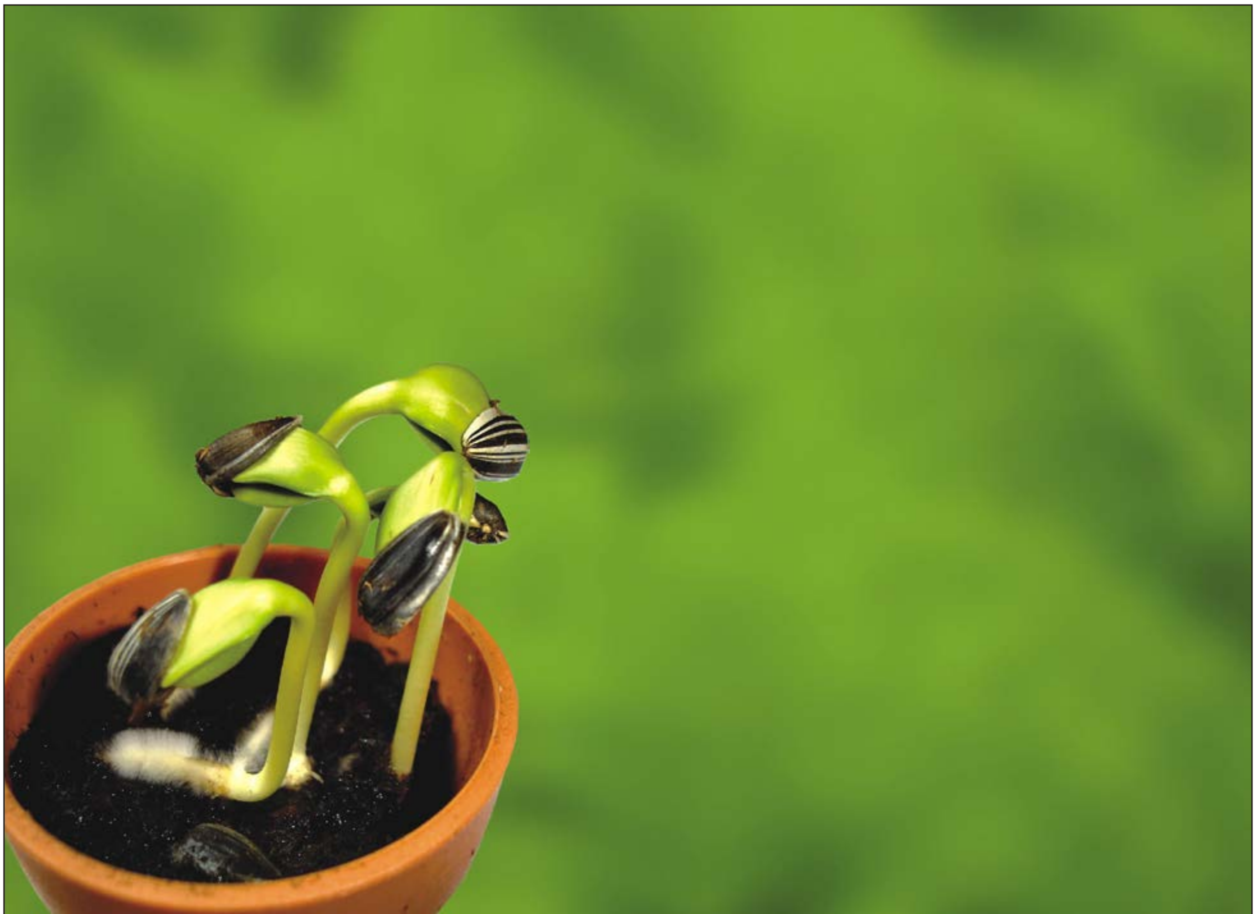

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Study of Intraspecies Variation in Seed Coat Micro-Morphology of *Amaranthus hybridus* by Scanning Electron Microscope

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ABSTRACT

Seed character is an important part of any crop plant. The seeds of *Amaranthus hybridus* (Family-Amaranthaceae) are small in size and not possible to differentiate easily with naked eyes. A little work has been focused on seed morphology of *Amaranthus hybridus* but no specific work on seed micromorphology has yet been documented. Keeping all these views in mind, we have undertaken the venture for observing possible microfeatures under Scanning Electron Microscope (SEM). The micro-morphological typology of seed surfaces was investigated in eight accessions of *Amaranthus hybridus* using scanning electron microscopy. Morphological studies showed that seeds were either pyriform or sub-pyriform or sub-spherical in shape. Pleurogram was found on the seed surface in some accessions. The ornamentations of exo-testa were found to be either polygonal or reticular or crosslinked type or spindle shaped or scalariform or undulated lump with tertiary depositions or slits. The aim of this present study was to explore the seed characters which are useful as a tool for identifying crop species and variations among those accessions too. The micromorphological characteristics of the seed coats may provide valuable information for identification of seeds.

Key words: *Amaranthus hybridus*, micromorphology, pleurogram, exo-testa, SEM

INTRODUCTION

The seed coat (referred to as testa) is the outer covering of every mature seed. It interacts with the internal structures of the seed and the external environment. Seed coats exhibit complex and highly diverse morphology and anatomy, providing valuable taxonomic characters (Barthlott, 1981, 1990). Seeds and leaves of *Amaranthus hybridus* are highly nutritious and utilized as human and for animal food (Tucker, 1986). Morphology and anatomy of seed research contribute to knowledge of taxonomy, evolution and ecology of angiosperm species (Cortez and Carmello-Guerreiro, 2008). It is difficult to study such groups because most species are indistinguishable vegetatively and they are useless for taxonomical identification. The ultrastructural importance of seed surface, as a reliable approach for resolving taxonomic problems is well accepted (Heywood, 1971; Buth and Ara, 1983; Brochmann, 1992; Koul *et al*., 2000). The importance of seed morphology for classification has long been accepted (Hooker and Thomson, 1861). Recent works of successful applications of seed morphology include studies on *Veronica* (Martinez-Ortega and Rico, 2001), Massonieae (Pfosser *et al*., 2003), *Aeschynanthus* (Mendum *et al*., 2001), *Phyllocladus* (Bobrov *et al*., 1999), Caryophyllaceae (Yildiz, 2002), Brassicaceae (Khalik and van der Maesen, 2002) and Balsaminaceae (Shimizu, 1979).

Current research focuses on micromorphology studies of seed coat to identify the different ecotypes of *Amaranthus hybridus*. The seed coats of some Amaranth genotypes grown in the tropical plains of West Bengal was examined by Scanning Electron Microscopy (SEM). Eight exomorphic parameters, including shape, length, width, colour, mass and surface texture were detected from 50 seeds for each accession.

It has been revealed that the differences in seed characteristics were not sufficient to use for sectional delimitation of *Amaranthus hybridus* but helps to separate the different accessions of *Amaranthus hybridus*.

MATERIALS AND METHODS

Plant materials: Eight genotypes viz. IC 95609, IC 35482, IC 120617, IC 95597, IC 35626, IC 95595, IC 32190 and IC 95589 of *Amaranthus hybridus* were procured from National Bureau of Plant Genetic Resources (NBPGR), New-Delhi. These seeds were sown in the research field of the Crop Research Farm Burdwan University (CRF) (23°53'N latitude and 83°25'E longitude) in West Bengal. The seeds used in this study were taken from the ripened fruits of *Amaranthus hybridus*. Fifty seeds of every accession were taken at random; in each of these, mass, length, width and the relation Length-Width (L/W) calculated and the presence or absence of pleurogram in the seeds was recorded.

Preparation of seed material for scanning electron microscopic analysis: Mature dry seeds (without fixation) were mounted on aluminum stubs with double-sided adhesive carbon type and sputter-coated with gold in IB-2 ion coater. For each genotype, at least 20 seeds were randomly selected and studied. The coated materials were examined and photographed with a (S-530) scanning electron microscope at an accelerating voltage of 20 kV. In the micromorphological description of seed surface texture, the following concepts were used: arrangement of epidermal cells, primary sculpture and secondary sculpture (Barthlott, 1981).

The presence or absence of different seed morphological characters were treated as binary characters in a data matrix i.e., coded 1 and 0 respectively. The relationship between the taxa studied and expressed by average taxonomic distance (dissimilarity), termed demonstrated as phenogram was analysed on the basis of recorded characters using Statistica 6.0.

RESULTS

The seeds were examined under Scanning Electron Microscope (SEM) following standard protocol. Table 1 shows various morphological characters of seed of different accessions of *Amaranthus hybridus.*

Table 1: Overall seed morphology of different cultivars

Accession	Colour	Seed length (mm)	Seed width (mm)	Seed length: Width (ratio)	1000 seed weight (g)	Surface	Outline
IC 95609	Blackish red	1.116	0.992	1.125	0.562	Glossy	Sub-pyriform
IC 35482	White	0.970	0.875	1.108	0.467	Not glossy	Pyriform
IC 120617	Dark brown	1.178	1.054	1.117	0.580	Glossy	Sub-spherical
IC 95597	Black	1.255	1.116	1.124	0.610	Glossy	Sub-spherical
IC 35626	White	0.992	0.914	1.085	0.558	Not glossy	Pyriform
IC 95595	White	1.001	0.899	1.113	0.544	Not glossy	Pyriform
IC 32190	White	0.998	0.930	1.073	0.519	Not glossy	Pyriform
IC 95589	Black	1.209	1.069	1.130	0.570	Glossy	Sub-Spherical

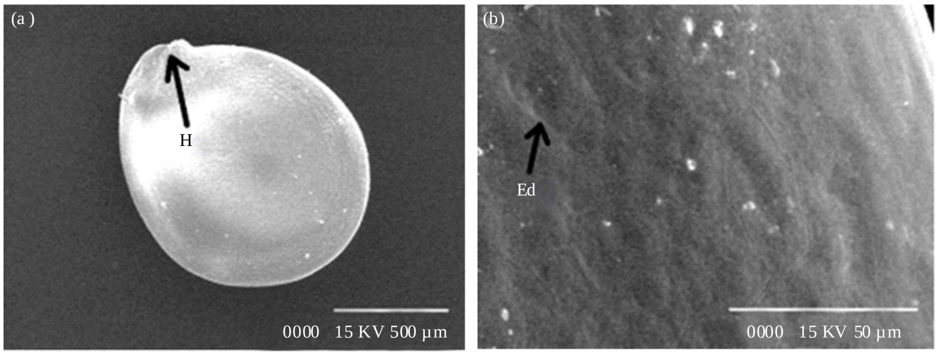

Fig. 1(a-b): Scanning electron microscopic micrographs of (a) Seed morphology (IC 95609) and (b) Seed surface (IC 95609), H: Hilum, Ed: Epidermal cell

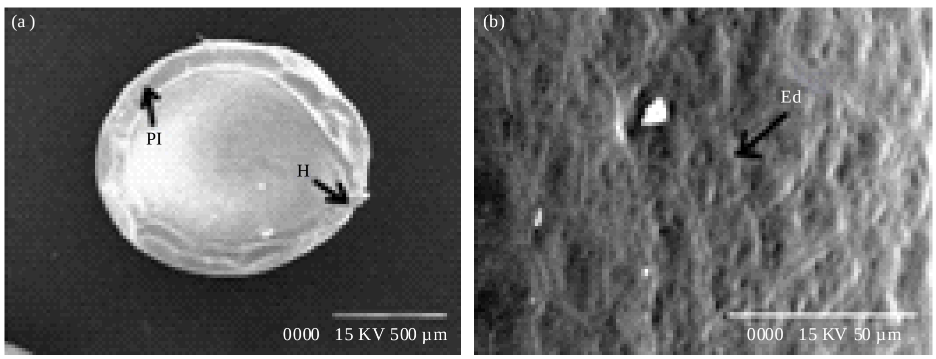

Fig. 2(a-b): Scanning electron microscopic micrographs of (a) Seed morphology (IC 35482) and (b) Seed surface (IC 35482), H: Hilum, Ed: Epidermal cells

Accession IC 95609: Seed black in colour, sub-pyriform, elliptic, hilar elevation present, Epidermal cells ornamentation uniform, totally obscuring its reticular pattern, Pleurogram absent (Fig. 1a-b).

Accession IC 35482: Seed white in colour, pyriform with pleurogram, epidermal cells ornamentation obscurely reticulate with slits of different size and shape. A few irregular shaped tertiary depositions present (Fig. 2a-b).

Accession IC 120617: Seeds dark brown in colour, seeds sub spherical lacking pleurogram. Thread like epidermal cells ornamentation, cross linked forming polygonal meshes; meshes with closely placed undulations and slits in between (Fig. 3a-b).

Accession IC 95597: Seeds black in colour, tertiary depositions of variable size frequent. Epidermal cells ornamentation forming reticulation with quadrangular meshes, tertiary depositions present (Fig. 4a-b).

Accession IC 35626: Seeds white in colour, pyriform in outline with pleurogram, converging hilum. Epidermal cells ornamentation with infrequent cross walls. A few tertiary granular depositions are present (Fig. 5a-b).

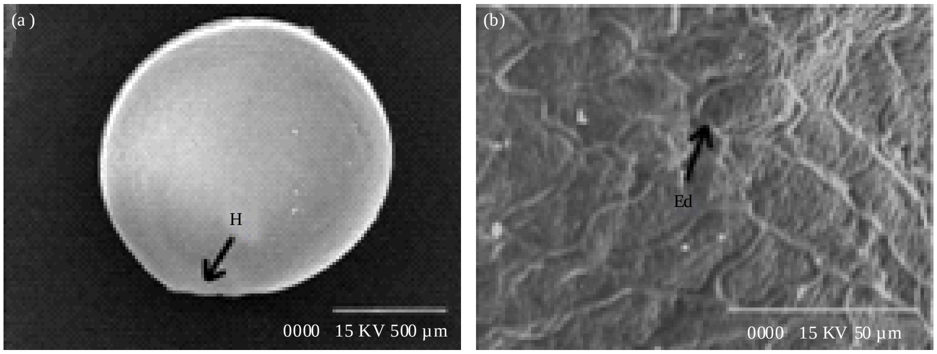

Fig. 3(a-b): Scanning electron microscopic micrographs of (a) Seed morphology (IC 120617) and (b) Seed surface (IC 120617), H: Hilum, Ed: Epicuticular deposition

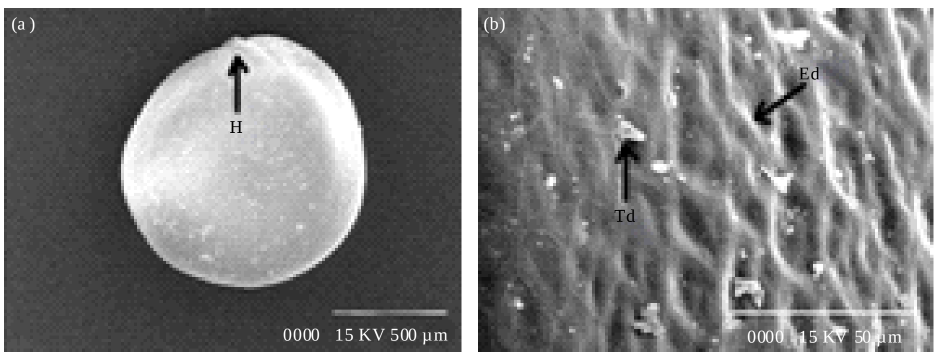

Fig. 4(a-b): Scanning electron microscopic micrographs of (a) Seed morphology (IC 95597) and (b) Seed surface (IC 95597) H: Hilum, Ed: Epidermal cell, Td: Tertiary deposition

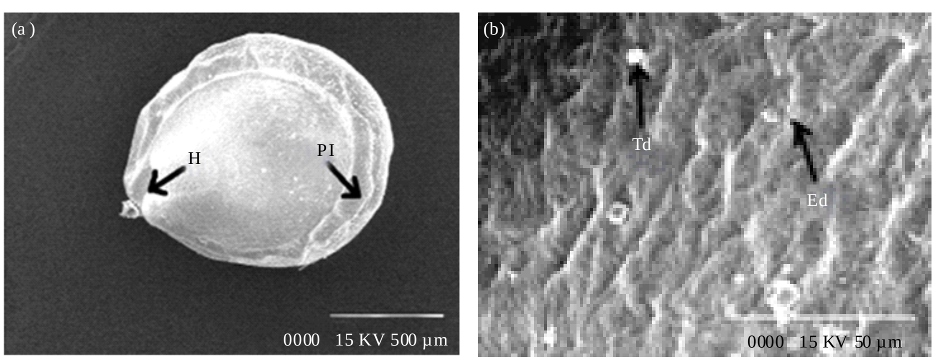

Fig. 5(a-b): Scanning electron microscopic micrographs of (a) Seed morphology (IC 35626) and (b) Seed surface (IC 35626) H: Hilum, Ed: Epidermal cell, Td: Tertiary deposition, Pl: Pleurogram

Accession IC 95595: Seeds white in colour with pleurogram. Epidermal cells ornamentation having irregular shaped slitted undulating lumps (Fig. 6a-b).

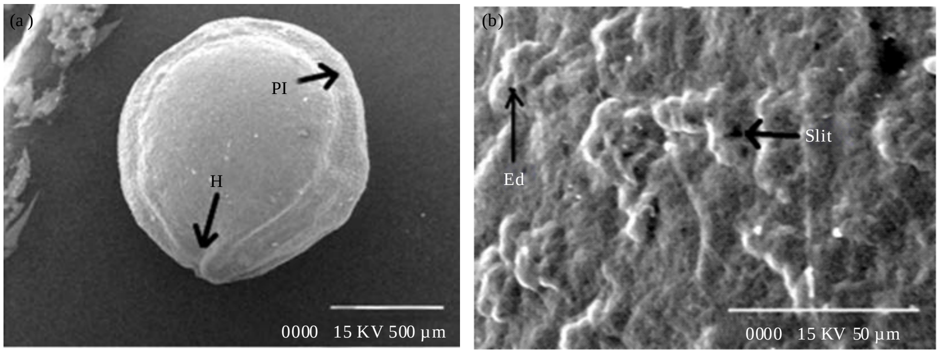

Fig. 6(a-b): Scanning electron microscopic micrographs of (a) Seed morphology (IC 95595) and (b) Seed surface (IC 95595), H: Hilum, Ed: Epidermal cell, Pl: Pleurogram

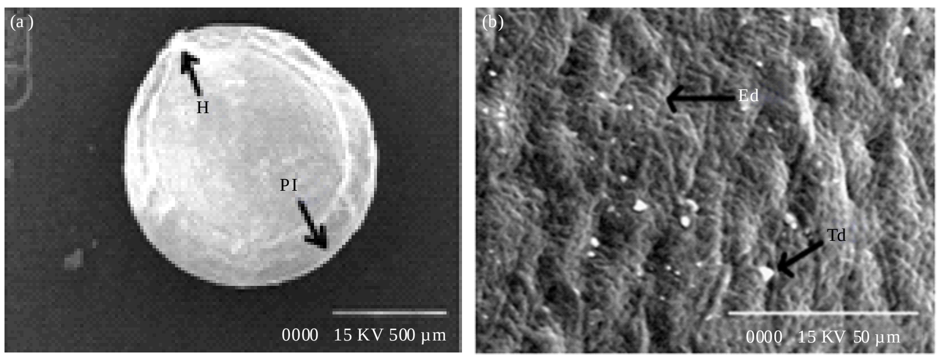

Fig. 7(a-b): Scanning electron microscopic micrographs of (a) Seed morphology (IC 32190) and (b) Seed surface (IC 32190), H: Hilum, Ed: Epidermal cell, Td: Tertiary deposition, Pl: Pleurogram

Accession IC 32190: Seeds white in colour, pleurogram present, epidermal cells ornamentation reticulated with meshes having scalariform slits. Tertiary depositions granular and frequently distributed (Fig. 7a-b).

Accession IC 95589: Seeds black in colour, sub-spherical. Tertiary depositions are of variable shapes and size. Epidermal cells ornamentation distinctly reticulate with spindle shaped meshes (Fig. 8a-b).

All the accessions were somewhat related slightly above 2.4 linkage distance as revealed in Fig. 9. At the linkage distance of 1-8, two clusters are recognizable, viz. Cluster I and Cluster II, each with four units, viz., IC 95609, IC 120617, IC 95589 and IC 95597 in the former and IC 35482, IC 35626, IC 32190 and IC 95595 in the latter. This speaks of their overall similarity to an appreciable extent. However, at the linkage distance of 1.6, as many as 6 clusters are recognizable of which four are solitary and thus unique (viz. IC 95609, IC 95597, IC 35482 and IC 95595). The units IC 120617 and IC 95589 in one hand and IC 35626 and IC 32190 on the other bear closest relationship, being linked at a distance slightly above 1.4.

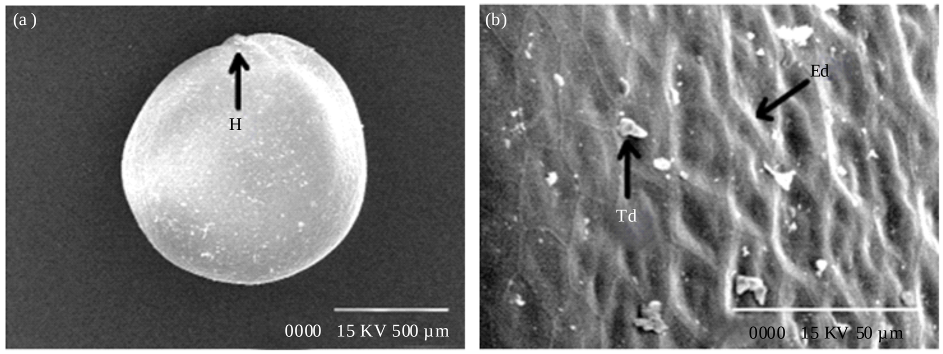

Fig. 8(a-b): Scanning electron microscopic micrographs of (a) Seed morphology (IC 95589) and (b) Seed surface (IC 95589), H: Hilum, Ed: Epidermal cell, Td: Tertiary deposition

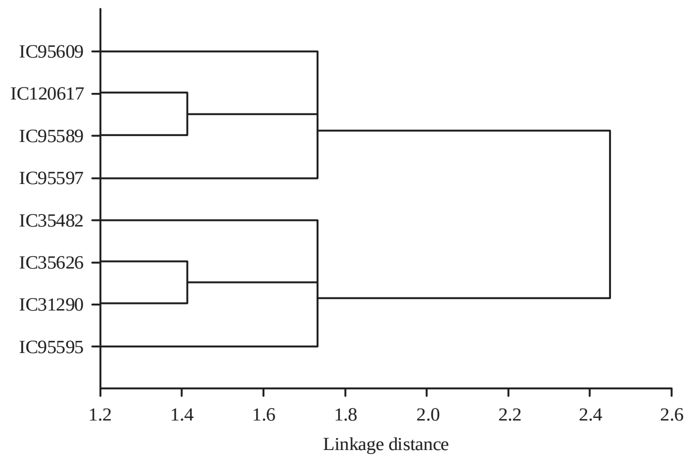

Fig. 9: Phenogram showing form relationship among 8 cultivars

DISCUSSION

Seed morphology and its orientation patterns has been taxonomically important to identify the species and genera. Present study revealed that the genotypes possess considerable diversity in seed size, shape, colour and seed coat micromorphology. In this study, seed coat colour was different in different accessions viz. four accessions were white, two accessions were black, one dark brown and one was blackish red. The colour of the seeds is very important in distinction of the taxonomic status of *Draba* and *Bolboschoenus maritimus* (Browning *et al*., 1997). Each and every accession was examined under scanning electron microscope which have been briefly elaborated in this context. Similarly, the surface was glossy and shiny in some cases and the remaining are not (Table 1). However, a lot of morphological differentiation and variation were observed by Raynal (1974) and Grisebach (1839) on different species of *Nymphoides*. A similar observation was also noted by Koul *et al*. (2000) and Hedge (1965).

The important features of the seed testa topographies of 8 taxa were examined. Taxa, representing different surface pattern groups is given in Table 1. At higher magnification (SEM X 60 and 1000), the seed surface ornamentations could be divided into a total of five different patterns: reticulate, polygonal, scalariform, undulating lumps and spindle shaped. The detail SEM study has also been elaborated showing its hilum, seed coat ornamentation pattern, tertiary deposition and pleurogram. It is noteworthy that remarkable variations were also exhibited in each SEM micrograph. Pleurogram is an important character for identification of Chamaecrista observed by De-Paula and Oliveira (2012). Pleurogram was observed in IC 35482, IC 35626, IC 95595 and IC 32190.

A large variation in seed size, shape and weight was observed among the accessions. A simple correlation was also exercised having bivariate correlation model with different characters like seed length vs seed width and seed length vs seed weight following Panse and Sukhatme (1955). The seed length correlated with seed width positively (r = 0.138). Similarly, the seed length was correlated with seed weight positively (r = 0.431).

On the basis of different micromorphological features of *A. hybridus*, considerable diversity in seed size, colour and seed coat micromorphology, a single linkage for eight variable were drawn which have been cited in (Fig. 9). The phenogram exhibits the relationship among the 8 accessions on the basis of overall similarities of seed characters. From this phenogram, whatever form relationships revealed, may prove useful in better taxonomic resolution of *A. hybridus*.

CONCLUSION

This study was only a preliminary investigation on the available accessions of *Amaranthus hybridus* and this only in throws some light on the micromorphology and relationship among the accessions of *Amaranthus hybridus*, although it is a complex genus. It is such a crop material which possesses maximum variation found through seed study. There might be many clues for identifying the accessions by seed characters.

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