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Comparative anatomical studies on some species of the genus *Amaranthus* (Family: Amaranthaceae) for the development of an identification guide ☆

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ABSTRACT

A study of anatomical features of mature leaves and stems (at fruiting stage) of 12 *Amaranthus* taxa (Family: Amaranthaceae) shows high variation between them and supplied new characters. The internal structures were evaluated to clarify their effectiveness in solving taxonomic complexity and identification difficulty in this genus. Observation of the transections of blades showed that the epidermis is uniseriate, ground tissue consists of angular collenchyma and thin parenchyma. The vascular bundles shape has three patterns crescent, ring, ovate. Also they may be united or separated while the midrib shape in cross section has two patterns in which U-shaped, cordate or crescent bundle occurs. All leaves are petiolate. The examination of the petioles exhibits new and varied characters such as petiole shape (cross section), vascular bundles (shape, number, arrangement). While the resulted characters from the observation of the stem structure showed less variation. Nineteen qualitative characters with 38 character states resulted from leaf anatomy. Only (8) characters were sufficient to generate an identification anatomical key. DELTA program was used in key-generation. Also different measurements were carried out by a photo analysis program (Image J), such as lamina thickness, mesophyll thickness, area of upper and lower epidermal cells and thickness of upper and lower epidermal cells to exhibit most possible dissimilarities between the studied species.

Introduction

The number of *Amaranthus* species varies in literature. The genus *Amaranthus* L. (Amaranthaceae Juss.) consists about 65–80 (Bojian et al., 2003; Mosyakin and Robertson, 1996; Mujica and Jacobsen, 2003; Iamonico, 2014a, 2014b.). 455 species names for the genus *Amaranthus* are known with the following statistics: 105 (23.1%) accepted, 262 (57.6%) synonyms and 88 (19.3%) unassessed (The plant list, 2013). However due to the few studies on *Amaranthus* systematics the number still tentative. Also the nomenclature of *Amaranthus* is critical since hundred names were published during the centuries and the nomenclatural disorder/misapplication of names create intricate problems in its taxonomy (Costea et al., 2001; Iamonico, 2014a, 2014b, 2016a, 2016b; Nestor, 2015).

Furthermore, some *Amaranthus* species are aggressive annual weeds in the world, causing impacts on the natural floras and vegetation and on cultivated field *Amaranthus* presence can lower grain yield and quality as well as hinder mechanical harvest (Wax, 1995). Some of this species also have been shown to possess allelopathic chemicals that reduce seedling vigor of several crops and weed species (Menges, 1987, 1988), while others may induce toxicosis and death in dairy cattle (Kerr and Keleh, 1998). The grain *Amaranths* are ancient crops with increasing prospects as potential food and feed resources because of their high grain protein and starch quality and high-nitrogen, highly digestible vegetative tissues (Cai et al., 1998a,b). Some *Amaranthus* species have medicinal importance as *A. spinosus* (Pal et al., 2013).

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Among the morphological studies on different *Amaranthus* species; Abdul Rahaman and Oladele (2003) and Gaafar et al. (2015) studied the stomata; Alege and Daudu (2014), Pal et al. (2013), Munir et al. (2011), Hong et al. (2005) studied the leaves. Kraehmer (2013), Carlquist (2003) and Metcalfe & Chalk (1950, 1979)

As a consequence, some issues of the taxonomy of *Amaranthus* still remain not completely solved, although some approaches was carried out (Hauptli and Jain, 1978; Joshi and Rana, 1991; Bojian et al., 2003, Mosyakin and Robertson, 2003; Hussein, 2005; Pinto and Velasquez, 2010; Iamonico, 2012; Iamonico and Das, 2014).

Because of the poor published data about the leaf and stem anatomies, especially concerning the taxonomical implications, we here present a study aims to evaluate the taxonomic value of leaf and stem structures of some *Amaranthus* species, with the final aim to facilitate identification *Amaranthus* and improve the taxonomy of the genus.

Material and methods

Seeds of 11 *Amaranthus* accessions were supplied by the Royal Botanic Garden at Kew (London, UK) while one *taxon* (*A. tricolor*) was selected from one of the Botanical Gardens in Egypt (National Garden in Nasr City). 12 species of *Amaranthus* were studied in the present study (Table 1). All the plants were grown from seeds.

The leaf structure was examined using five segments (lengths of 1–2 mm) of the blade and petiole (fruiting stage) and then they were immediately fixed in (FAA) according to Johasen (1940). The third node and internode were selected for the stem study. Serial transverse sections (10–20 μm in thickness) were cut with a steel blade on an IHC World KD-1508A rotary microtome and fixed on slides by means of Haupt's adhesive. The sections were stained with a safranin-fastgreen combination, and then mounted in Canada balsam (Sass, 1961).

Observation and photomicrographs were achieved using XSZ-N107 Research Microscope fitted with Premiere MA88-900 digital camera. 14 measurements for leaf and stem internal structures, epidermal cell area were carried out by the ImageJ Ver. 1.49o program (standard software in scientific image analysis) and calibrated by using a standard stage micrometer slide. Each recorded measurement is the average of ten measurements.

Also stomatal density and stomatal index where calculated as described by Wilkinson (1979).

$$\mathbf{SI} = \mathbf{S} \times \mathbf{100}/\mathbf{S} + \mathbf{E}$$

Various data obtained from the description of the leaf anatomy were subjected to automated key generation using version 4.12 of the DELTA suite of program (Dallwitz et al., 2000).

Results

The observation of the internal structure of the leaf and stem of the studied species revealed (14) qualitative characters (Table 3). 28 qualitative characters were resulted and summarized in (Table 2). Also they comprehensively listed in the following text:

Table 1
Species and accessions of *Amaranthus* used in this study.

No.	Species	Accession no.	Origin	Subgenus	Habitat
1	*A. albus* L.	0022453	Germany	*Albersia*	Wild
2	*A. blitum* L.	0006563	France	*Albersia*	Cultivated
3	*A. caudatus* L.	0003919	Unknown	*Amaranthus*	Wild
4	*A. deflexus* L.	0020275	Italy	*Albersia*	Cultivated
5	*A. dubius* Mart.ex Thell.	0006574	Colombia	*Albersia*	Wild
6	*A. graecizans* L.	0020242	Portugal	*Amaranthus*	Wild
7	*A. hybridus* L.	0019657	Bolivia	*Amaranthus*	Wild
8	*A. powellii* S. Watson.	0030999	France	*Amaranthus*	Wild
9	*A. retroflexus* L.	0008394	Italy	*Amaranthus*	Wild
10	*A. spinosus* L.	0017882	Unknown	*Amaranthus*	Wild
11	*A. tricolor* L.	Unknown	Egypt	*Amaranthus*	Cultivated
12	*A.* x *ozanonii* Piszter.	0008383	Unknown	*Amaranthus*	Cultivated

Table 2
Qualitative characters of leaf and stem of the studied species of *Amaranthus*. Character number is preceded by # and states of the same character are assigned serial numbers.

Characters	Species											
	A. albus	*A. blitum*	*A. caudatus*	*A. deflexus*	*A. dubius*	*A. graecizans*	*A. hybridus*	*A. powellii*	*A. retroflexus*	*A. spinosus*	*A. tricolor*	*A. x ozanonii*
#1. Petiole shape in TS/1.crescent/2. rounded/3.cordate/4.V-shaped	1	1	4	1	1	1	1	1	1	2	2	3
#2. Protuberances/1.present/2.absent	1	1	1	1	1	1	1	1	1	1	2	1
#3. Epidermis type/1.uniseriat with radial or pueblos cells	1	1	1	1	1	1	1	1	1	1	1	1
#4. Ground tissue type/ 1.parenchymatous and angular collenchyma	1	1	1	1	1	1	1	1	1	1	1	1
#5. Sand crystals in petiole/1.present/2. absente	1	1	1	1	1	1	1	1	1	1	1	1
#6. Number of vascular bundles/1.8/	1	2	3	4	3	5	4	6	1	7	6	7

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