IMPACT OF ARID CLIMATE ON SEX DISTRIBUTION, MORPHOLOGICAL AND ANATOMICAL ORGANIZATION OF A MEDICINAL HERB AERVA JAVANICA (BURM. F.) JUSS. EX SCHULT (AMARANTHACEAE) IN SOUTHERN ALGERIAN SAHARA

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Abstract

“Ahaggar”, is the most arid land of North African regions which faces serious challenges due to the lack of sufficient water and the increase of drought state (prolonged dry-spells or delayed). These arid conditions have substantial negative impact on the harvest yield of agriculture and affect the morphology, anatomy and biodiversity development of many plant species.

This study conducted on Aerva javanica in situ (in its natural setting) should help to analyze how increased temperatures might affect some morphological, anatomical and physiological characters of this Saharan medicinal herb. The effects of climate changes appear to be impacting this plant in its male and female specimen’s distribution, morphology (vegetative and reproductive systems), and anatomical organization in cross-sections taken from the root, stem and leaf.

The anatomical results have also provided the evidence of the photosynthetic mechanism in C4 of this plant. For the first time, we have shown the difference of resistance of this plant by sex distribution, which is really different of other countries of Africa and the presence of two types of assimilatory parenchyma in both leaves and stems. This anatomical adaptation known “kranz anatomy” confers to the plant a high performance in water deficit conditions, salt stress and high exposure to the temperature and to the intense light.

Key words: Aerva javanica (Bur. F) Juss ex Schult, Amaranthaceae, Morphological study, Anatomical study, Dioecious, Glandular and eglandular hairs, Schizogeny, Kranz anatomy, C4 pathway.

Introduction

In recent decades, climate changes in seasonal patterns, weather events and temperature ranges is becoming the most preoccupied point of scientific researchers in the world. Arid regions are expected to undergo significant changes, high temperature, less precipitation and vegetation disruption which could have a high feedback on effects of CO2 and photosynthesis on plants (Shinwari & Quiser, 2011; Shinwari et al., 2012). Indeed, all these factors seem to have a big impact on the structure of many plant species in North African Sahara, while a wide range of plant species are of medicinal importance (Ilyas et al., 2012).

The environmental conditions have an effect not only on plant growth but also on the competition between plants, animal grazing, light exposure, soil moisture, etc. Pippa et al. (2011) suggested that research would be needed to establish the relevance of climate changes factors to the growth and survival of many plant species.

Aerva javanica (Bur. F) Juss. ex Schult, is a perennial herb belonging to Amaranthaceae (Cronquist, 1988, Bremer et al., 2009) called by the Touareg people “Timkerkezt”, in Arabic “Aroua” (Saiki & Sahki, 2004, Maiza, 2008) in French “cotton of the desert” and in English “snow bush” (Khar, 2007), extensively used in traditional medicine mostly against snakes and scorpions bites (Hammiche & Maiza, 2006). It is one of the most used medicinal plants in the arid land of Tamanrasset (the capital of Ahaggar, highland area in southern Algeria). This plant grows in tropical and subtropical Africa, Indian Ocean (Madagascar), tropical and subtropical Asia and naturalised in Western Australia (Miller & Cope 1996, Spooner, 2007, Palmer & Lally, 2011). Earlier investigations have shown the precise data on the habitat of Aerva javanica related mainly to Saharan populations and were collected in Northern Africa (central Sahara) such: Mauydir, Tafedest, Hoggar and Tassili, where it grew between 0 and 1500 m of altitude (Saiki & Sahki, 2004). On the other hand, Mossa et al. (1987) have reported that A. javanica could be used in dune stabilization in Saudi Arabia by assisting the revegetation of degraded range lands.

The present study has been carried out to evaluate all characters enabling plant to acquire its adaptation to arid conditions, sex distribution in its natural situ (general aspect and detail of different parts of the plant) and anatomical studies including cross sections of the leaves, stems and roots. Also to review different plant strategies to cope with drought, and report a kind of leaf photosynthesis, whole process takes place in response to slowly imposed water deficits under arid conditions.

A. javanica grows in clumps with much intertwined shrubs called “Desert cotton” covered by thin hairs on the whole aerial parts to face to sand storm along with other adaptation’s characters as the shrinkage in the size of leaves and photosynthetic mechanism in C4 by the presence of two types of assimilatory parenchyma. This C4 photosynthesis process constitutes a solution to cope to unfavorable conditions including high temperatures and dryness. Ehleringer et al. (1991, 1997) reported that the efficiency of photosynthesis could be decreased by 40% under harsh conditions. Thus, the C4 photosynthetic carbon cycle could evolve as an adaptation to high light intensities and drought conditions (Gowik & Westhoff, 2011).

This paper reports the variation of sex expression distribution, morphological and anatomical characters in relationship to photosynthetic pathway of A. javanica in North African land which could have a powerful adaptation strategy to arid conditions.
Materials and Methods

Plant collection and identification: Fresh plant was collected from Tamanrasset, at Oued Amsel (AI: 1254 m; Long: 05° 30.816 E; Lat: 22° 40.644 N) on sandy-loam soil in March 2010-2015 (Fig. 1A).

The plant species was identified at the National Institute of Forestry Research of Tamanrasset (INRF), Algeria with the help of Floras of Sahara (Maire, 1933, Ozenda, 2004). The voucher specimen was deposited in the Herbarium of the Laboratory of Research on Arid Zones, University of Sciences and Technology Houmediene (Collection 2012).

Morphological observations: Several observations were made for morphological characterization. The size and appearance of plants and their organs (roots, stems, leaves, flowers and fruits) in their natural environment were studied with naked eyes or binocular, and photographs were taken.

Anatomical studies: Free hand thin transverse sections were made from fresh materials (stem, leaves and roots) using a razor blade according to a perpendicular plane to the axis of the object, the slices are then processed to sodium hypochlorite 12°C1 then acetic acid 1% to remove the cytoplasm of the cells. The sections were stained with Methyl green and Congo Red (double staining) and then stored in distilled water with few drops of glycerin 1% and observed in the optical microscope (Langeron, 1949, Furelaud et al., 2011). Photographs were taken; moreover, further details have been shown on drawing assessment.

Results

Aerva javanica is a common plant in the “Ahaggar”, widely distributed on different soils, loamy sandy, stony mountains to rock. It is a dioecious plant with unisexual flowers male and female, with a wide proportion of male plants compared to the rest of the world results in a high distribution of male plants compared to females in several regions (personal communication).

Morphological description: Fresh vegetative and flower systems of Aerva javanica were studied and identified by their morphological characters as per literature.

Saharan plant densely hairy (Fig. 1B), with thick foliage, woolly white to greyish much branched. Erect shrub with much branched stem at the base, and tap root deep up to the same length as the aerial part of the plant forms tufts of woolly which is named desert Cotton (Fig. 1C), the tufts are higher in wadis beds where the soil is sandy loam, it can exceed 1.20 m height and 1.80 m wide and occupies over 4 m space with a stout taproot (Fig. 1D).

The leaves are simple, alternate, short petiolate, oval, lance-shaped, hairy pinnately veined, length varies between 1 to 2 cm on sandy soil and from 2 to 6 cm on mountainous soils with the greyish appearance having abundant of trichomes on the upper surface (Fig. 1E and 1F).

There are two types of inflorescences, female (whitish with a purplish hue, Fig. 2A) and male (whitish with a golden yellow, Fig. 2B). Both male and female inflorescence form woolly terminal panicles (Bartha, 1970, Palmer & Lally, 2011).

Each inflorescence is composed of 10 small racemes arranged on a twig of 20 cm length of the plant. Each raceme has a main axis (6 cm) and laterally borne having very small flowers (2 mm) with pedicels of equal length.

The female flower is hypogenous, surrounded by three smooth transparent bracts, composed by 5 tepals with a nectariferous disc (10 nectariferous glands) in intrastaminal position, ovary, with a short style and bifid feathery stigma (Figs. 2. A1, A2, A3 and A4).

The male flower consists of 5 tepals surrounded by bracts, stamens (5 fertile and 5 sterile or abortive stamen “staminodes) attached at their basis, the pollen grain is pantoporate, specific to the Amaranthaceae family (Müller & Borsch, 2005), sterile ovary with a short style and small bifid stigma (Figs. 2. B1, B2, B3 and B4). After reproduction, the fruit is a pyxis, ovoid blackish purple, surrounded by a persistent perianth (post-floral development) (Fig. 2C and 2D).
A: Morphological aspects of female inflorescences. 

B: Morphological aspects of male inflorescences. 

C-D: Pyxis fruit generated by the female flower. 
Fig. 2. Morphology of male and female reproductive system of *Aerva javanica* (A: Female reproductive system. B: Male reproductive system. C-D: Fruit).
Fig. 3. Anatomical aspect of the stem of *Aerva javanica*.
A: General anatomical aspect of the cross section of the stem of *Aerva javanica* (x45). B: Anatomical structure of assimilatory parenchyma of the stem (x400). C-D: Morphology of glandular and non glandular trichomes on epidermis (x450). E: Detail of schizogeny cells (x450). F: Diagram of a portion of the stem. G: Portion of a cross section of the stem (x400).


Fig. 4. Anatomical structure of the leaf of *Aerva javanica*.
A: General anatomical aspect of the cross section of the leaf (x40). B: Diagram showing anatomical appearance of the cross section of the leaf. C: Morphology of glandular trichomes on epidermal cells of the leaf (x450). D: Detailed portion of a cross section at the midrib (x400). E-F: Detailed anatomical aspect of the cross section at the blade part of the leaf (x400).


Fig. 5. Anatomical aspect of the root of *Aerva javanica*.
A: General anatomical aspect of the cross section of the root (x45). B: Detail of schizogeny cells (x450), C: Diagram showing anatomical appearance of a portion of the cross section of the root (x400).

Anatomical analysis: All cross sections taken from leaves, stems and roots have revealed the classical primary and secondary anatomical structure.

The surfaces of plant organs are often covered by various glandular and non-glandular trichomes developed from epidermal cells. The glandular trichomes are exclusively uniseriate clusters, interrupted by secretory glands unicellular star-shaped cells surrounded by a thick cellulose wall. Non glandular trichomes are also uniseriate clusters and long (Fig. 3C and 3D).

In the leaves and stems, the epidermis was single layered and the surface covered by numerous glandular and non glandular branched trichomes with typical stars shape at the ending (tips) of glandular ones, the number and length of trichomes increase facing to drought conditions. In addition, large number of submersed (pressed) stomata on two faces of epidermis were also observed.

Anatomy of the stem: The observation of the cross sections of the stem (Fig. 3A) showed from the outside towards the inside the following tissues:

The transverse section of the stem showed single layered called epidermis formed by rectangular cells jointed together topped with an impervious covered with thick cuticle (Fig. 3A, B).

Beneath the epidermis, there were 3 to 4 layers of an angular collenchymas which were very thick isodiametric cells arranged in small angles distributed on the whole range of the cut. Between the corners, there were layers of parenchyma cells with two types of cells, 2 to 3 layers of cells with more or less elongated thin wall wrinkled followed by 1 layer of very thick cubical parenchymatic cells (Fig. 3B).

There were parenchyma cells with intercellular spaces. In addition to the secretory structures mentioned, there were a significant number of “schizogeny”. These ducts are complex having a distinct outer epithelium without intercellular spaces and an inner epithelium in which schizogenous spaces containing products of secretion yellowish, brown or greenish color (Fig. 3E). The number of these cell structures is important in the vicinity of the assimilator parenchyma and within the pith’s parenchyma rich in Calcium oxalate stones. The number and the diameter of the “schizogeny” was increased with aging of the stem and the color of the secretor’s products darkens with time.

Parenchymatic cortex cells were 5 to 7 layered with sclerenchyma cells sheathed numerous small vascular bundles constituted by xylem and phloem. It was observed that the primary structure of the stem had been transformed by the growth of the vascular cambium. A sinusous to a ring shape of vascular bundles of varying size, embedded in interfascicular parenchyma and enclosing parenchymatous pith was formed by the cambial activity.

The cambium was located between the xylem and the phloem. Beneath it, there were xylem tissue composed regular trachea and tracheid very thick lignified cells between the secondary xylem cells (Fig. 3F and 3G).

Anatomy of leaves: The cross-sectional view of the leaf exhibited a bilateral symmetry (Fig. 4A and 4B). At the level of the midrib, the upper and lower epidermis of the leaf was single rectangular thin cellulosic layered covered by the thick cuticle, many epidermis cells developed outer side both glandular and non glandular trichomes (Fig. 4C), followed by an angular collenchymas constituted of 3 to 4 layers thick cellulosic cells distributed on both sides of the central rib alternating with “schizogeny”.

In the center, there was a big crescent-shaped vascular bundle surrounded by perivascular cells which were small thick cubic form charged by chlorophylls, sheathed by many layers of mesophyll cells (Fig. 4D).

In the blade parts, there was an epidermis single layered covered by the thick cuticle. In the cross-section, cuticle was present on both abaxial and adaxial epidermis, larger rectangular cells with sunken stomata particularly on adaxial epidermis, a lot of glandular and non-glandular trichomes were observed.

Beneath the adaxial epidermis, there was 3 to 4 layers of elongated mesophyll cells with intercellular spaces very charged of chlorophylls. This tissue was found also in the abaxial epidermis with less number of layers cells. Many vascular bundles with xylem and phloem structures surrounded by bundle sheath cells are alterned by “schizogeny” along the blade (Fig. 4E and 4F).

Anatomy of the root: The cross section of the root contains from outside towards inside following tissues (Fig. 5A): Periderm, which made up the outermost layer of the root, outer side the cork tissue which is formed by 10 layers of thin suberized cells in parallel disposition, it has found in much areas residual epidermis with unicellular root hair.

Inside of the cork cambium, a layer with 1 to 2 slightly thick rectangular cells called living secondary tissue (phelloderm) very rich on schizogeny full of secretion products (Fig. 5B).

Beneath the periderm, there were multi-layered circular and ovaidal cells with triangular intercellular spaces, many boundaries of sclerenchyma were appeared just outer phloem. This sclerenchyme will be a well sheat around vascular bundles with age.

The vascular cambium, composed of 2 to 3 layers, located between the xylem and the phloem, gives rise to a secondary xylem inside and to a secondary phloem to the outside. At early stage it functioned between xylem and phloem then many parenchymatic cells lose the properties that they had, changing size, shape and reverting as cambial dedifferentiated cells, will join to vascular cambium in sinuous form then in circular shape. Several concentric circles of continued vascular cambium with xylem and phloem secondaries in rays were observed by aging with many “schizogeny” in phloem’s tissues (Fig. 5C).

The xylem tissue was composed of regular trachea, tracheid and vessels surrounded by parenchyma cells very thick lignified cells. Xylem cells were also presented in the center, therefore the pith is not seen in the center.

Discussion

The field study provided data on the distribution of Aerva javanica’s male and female plants, as well as the observation of many male populations compared to the rest of the world. Contrary to the previous investigations which were reported 92, 3% of female populations against 7, 7% of males worldwide (Khan et al., 1970), it was
extremely difficult to find specimens with male flowers according to Miller & Cope (1996), Spooner (2007) and Movaliya & Zaveri (2014). Due to the comparative observations, in Ahaggar, the wide distribution of males than females, suggested that probably males might be more resistant to arid conditions and climate changes than females. This theory is confirmed by the data collected by Hultine et al. (2016) who suggest that males would generally be less sensitive to increased aridity than co-occurring females, and therefore, male-biased extreme sex ratios are possible in a significant number of populations. We think that this plant sex ratio could be a result of climate changes in this arid land and the males have developed a high capacity of adaptation to females. 

*A. javanica* has been studied in different aspects in Algeria. Indeed, it has been described by Quezel & Santa (1962) as a perennial Saharan plant, dioecious with unisexual flowers, very hairy, with thick white woolly foliage color.

Most of *A. javanica* is generally found in Tamanrasset (North of Africa) on rocky plateaus and stony plains with the wadi bed loamy sandy soil, flowers in the period of December-June when temperature exceeds 45°C, sunny hot and dry climate. 

In comparison, the growth and development (flowering period) of this plant species are available in July- September in India and Pakistan (Qasim et al., 2010). Movaliya & Zaveri (2014) have reported that *Aerva* blooms and be very wooly in rainy season during this period at Bhavnagar district of Gujarat. However, what distinguishes Indian species from Algerian ones. Is it the nature of soil or the climate conditions?

The information provided on the Bhavnagar region district of Gujarat state in India where *A. javanica* was plentiful has grew in a hot semi-arid climate and slight sodicity and salinity in soils and a water deficit throughout the year (Oza, 2003). The comparison between Asiatic and Mediterranean *Aerva* indicated that geographical position of this plant species (the nature of soil or the climate conditions) could have an effect on its distribution, plant sex-ratio and the morphology of leaves and flowers. 

The comparative study of *Aerva javanica* in different biotopes highlights its complex morphological and anatomical variations setting up in response to climate change and harsh environmental conditions (Sahki & Sahki, 2004). As a result, its morphology reminiscent of a ball of wool is intended to face the Saharan sand wind (Qureshi & Bhatti, 2009).

In plants developed in sandy areas, the leaves are longer, exceeding 6cm long compared to those of mountainous regions where the leaves are short (1 to 2 cm). Soliman (2006) noted that the plants of Saudi Arabia their leaves have a size between 2 to 4 cm and came in many forms (lanceolate, oblong, ovate, sub-oriicular) whereas only one form of leaves was observed in Ahaggar. This variation could be due to factors of environment. *A. javanica* seems to have an effect on the dune stabilization, very much used to assist the vegetation of degraded areas (Soliman, 2006). The size and shape (physiognomy) of leaves have been widely used as proxies for temperature and moisture variables (Bailey & Sinnott, 1915, Dilcher, 1973, Greenwood & Wing, 1995, Parrish & Spicer, 1988, Qureshi & Bhatti, 2009, Soliman, 2006, Wilf et al., 2003, Wing et al., 2000, Wolfe & Upchurch, 1987, Wolfe, 1995).

This study offers a powerful knowledge of the anatomical structures of the vegetative systems (leaves, stem and root) of this plant. The observation analysis of cross sections has presented a good tool to use in botanical identification of this medicinal species and could highlight the main mechanisms of its adaptation. The results obtained show that the plant developed considerable variations in anatomical structures for inducing a reduction of water losses to cope with the conditions of aridity and salinity of the desert.

The presence of a thick cuticle covering epidermal cells having long and branched glandular trichomes like the other species of warm regions (Judd et al., 2002; Werker, 2000) and reduce the water loss by evaporation (Smail-Saadoun, 2005; Yves et al., 2003), interrupted by a significant number of stomata (Camefort, 1982), as well as a high number of “schizogeny” filled with products of secretion very used in traditional medicine.

The presence of thick cuticle relatively with many hidden stomata on external surfaces of vegetative parts help the plant to reduce evaporation and the solar heating and temperature rise. Also, long branched trichomes inhibit water loss by creating a high humidity environment at the surface of aerial part. The characters described above, and particularly branched trichomes may likely offer protection to stomata (Werker, 2000, Judd et al., 2002).

In the stem and leaf sections, there were two types of assimilator parenchyma cells. In the stem, elongated and cubic cells were present but in the leaf, the vascular bundles called “the bundle sheath” contained a special mesophyll cells formed by cubic cells surrounded by an elongated cells, this anatomical organisation called “Kranz Anatomy” (Berry, 2001). This special anatomy of leaves can also be thought to serve as a source of characters for C4 plant which have virtually no photorespiratory activity (Houari et al., 2012). These observations that emphasized the anatomic aspects of mesophyll and their significance for interpreting the type of photosynthetic pathway, with those of C3 (composed of a layer of cells all lying) and that of C4 (organized in two different seats elongated cell and cubic cell) (Houari et al., 2013).

Sage et al. (2007) demonstrated that genus *Aerva* was polyphyletic with 75% of C3 face to 25% of C4 species, and suggested that these C3 species should be close to the ancestor of the C4 species and thus might reveal insights into the evolution of C4 photosynthesis. This photosynthesis evolution could therefore be a form of adaptation to arid conditions.

This C4 pathway is a good adaptation to the hot and arid climates with a very high rate of salinity, because they may as well fix more CO2 by using less water than plants in C3, or a significant production of biomass, a decrease of the photospiration, an optimization of photosynthetic performance and a better use of strong light intensities or even important syntheses of ATP and NADPH (Rawsthorne, 1992, Gong et al., 2006).

Indeed in this track, the CO2 formed an acid to 4 carbons (and not 3 carbons) involving two morphologically and functionally distinct types of photosynthetic cells. This ability to avoid photospiration makes *A. javanica* harder in arid conditions with closed stomata and low carbon dioxide levels. This concentric distribution of
photosynthetic tissue, recalling the process of photosynthesis to double carboxylation (Rawsthorne, 1992, Sage, 2001, Colin et al., 2012).

Notwithstanding, the results obtained from the observation of the cross section of this plant confirm the works of Maire (1933) compared to its sclerified aspect and the abundance of “schizogeny” filled with products of secretion in all the parts of the plant within the pith’s parenchyma rich in calcium oxalate crystals (Deyssson, 1964).

Conclusion

This study has deepened to show the importance of environment conditions on the sex plant distribution, morphological and the anatomical aspects of Aerva javanica in Ahaggar (Tamanrasset).

Contrary to earlier reports of the use and general characteristics, it is the first report of the detailed plant distribution, morphological and anatomical development facing to arid climate. Also, it included, the observation of the whole range of tissue structures and cytological responsible of secretion and retention of therapeutic substances as well as its type of C4 photosynthesis.

In situ, the male plants are more abundant than females; this suggests that male plants are more resistant than females.

The morphological and anatomical structures suggest that leaf, stem, root and flower have evolved a specialized external morphology and special anatomical features to reduce water loss.

These features include the presence of thick cuticle with many hidden stomata covering its external parts supported by a long non-glandular and glandular branched trichomes with many star-shaped cells.

These structural features that prevent water loss confirm the importance of this species as it is adapted to drought conditions. In addition, A. javanica is characterized by C4 pathway of photosynthesis which could be an important challenge to increase its adaptation feed back to arid conditions. Abundance of schizogeny containing different colors of products of secretion in all organs of the plant has also been observed; thus the entire plant can be used in traditional medicine.

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