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STUDY OF THE INTERNAL STRUCTURE OF *Amaranthus retroflexus* LEAF

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Abstract

The Amaranthus retroflexus species studied has a stem up to 100 cm high, branched, light green to reddish in color, with few leaves at the base, and the upper part with leaves covered with hairy hairs. For the microscopic observation of the internal structure of the Amaranthus retroflexus leaf, the seedlings were first fixed in AFE solution (70% ethyl alcohol, glacial acetic acid, 40% formalin) for 48 hours, after which they were washed with distilled water and preserved in alcohol 70%. The internal structure of an Amaranthus retroflexus leaf was observed in cross-section. It consists of two epidermises, one upper and one lower, as well as mesophyll. Stomata open in the morning under the influence of light (photoactive reaction), when the stomatal cells have a higher degree of turgor than the accessory cells. It was also observed that the opening degree of the stomata reaches a maximum in sunny conditions, when the penetration of CO_2, necessary for photosynthesis, is more intense, which determines the transpiration of the leaves at a high intensity.

Key words: *Amaranthus retroflexus, internal structure, stomatal, leaf.*

INTRODUCTION

The study was conducted to examine the internal structure of the leaf of *Amaranthus retroflexus*, a common weed species, using various microscopic techniques. By sectioning the plant organ and creating temporary microscopic preparations, researchers can observe the cellular structures and arrangements within the leaf. This study can provide valuable insights into the anatomy and morphology of the leaf, which can be used to better understand the physiology and ecology of this species. Additionally, this research can contribute to the development of more effective methods for controlling the spread of this weed species in agricultural and natural ecosystems.

The leaf is a lateral vegetative organ of the stem or branches, with a flat shape, which fulfills the fundamental function in the process of photosynthesis, serving also for respiration and transpiration (Athanassova, 1996; Benvenuti & Macchia, 1999; Brainard & Bellinder, 2002). Being the most plastic vegetative organ of the plant, the leaf can metamorphose, adapting to perform other functions: protection, absorption, storage of reserve substances and water, vegetative reproduction (Fischer & Evert, 1982a; Fischer & Evert 1982b; Fischer & Evert 1982c).

The species *Amaranthus retroflexus* has a stem up to 100 cm high, simple or branched, light green to reddish in color, with few leaves at the base, and dense leaves at the top, covered with thin hairs (Dominguez et al. 1994; Ghorbani et al. 1999).

The leaves are ovoid, long-petiolate, bluish-green, alternate, with a pointed tip, usually reddish on the underside (Franssen et al. 2001a; Franssen et al., 2001b). The cotyledons have an elongated shape, a length of approx. 10-12 mm, with the underside usually reddish (Frost, 1971; Hügin, 1986; Hunt et al., 1985).

The flowers are small, clustered in greenish bunches at the extremity of the main stem or branches, with the perianth (the floral sheath differentiated between the calyx (sepals) and the corolla (petals) pointed like a thorn, the inflorescence prickly (Coetzer et al., 2002; Costea, 1998). The flowering period is June - September, and the number of seeds per plant is between 1000-5000 (Gallagher & Cardina, 1998a; Gallagher & Cardina. 1998b).

Figure 1. General aspect of the *Amaranthus retroflexus* species

MATERIALS AND METHODS

For provisional microscopic preparations, the fastest and most used technique for sectioning the plant organs is the manual one, with the anatomical razor/scalpel or blade (Gebauer et al., 1987; Ghorbani et al., 2000). The following types of sections are obtained: transverse when the scalpel passes perpendicular to the axis of the plant organ; longitudinal-radial, when the scalpel passes through the center of the organ, in the direction of its radius; longitudinal-tangential, when the scalpel passes perpendicular to the radius, but does not touch the center of the organ (Lonchamp, 1981; Schonbeck et al., 1980). Manual sectioning is performed as follows: the razor blade is applied to the surface of the object to be sectioned and a horizontal movement is imposed on the razor (Mapes et al., 1997; Marín et al., 2000; Schimpf, 1977). The movement must be uniform, without stops and restarts, so that the sections are thin (microtones) (Costea et al., 2001; Doyon et al., 1986). The thinnest sections are transferred to the port object slide, in a drop of water and covered with the slide (Warwick et al., 1980; Weaver, 1984; Weaver et al., 1986).
For the microscopic observation of the internal structure of the *Amaranthus retroflexus* leaf, the seedlings were first fixed in AFE solution (70% ethyl alcohol, glacial acetic acid, 40% formalin) for 48 hours, after which they were washed with distilled water and preserved in 70% alcohol. For the purpose of constructing the temporary microscopic preparation, a small fragment of the lower epidermis was detached with the help of sharp-tipped forceps, after which it was placed on the microscope slide (degreased beforehand) in a drop of water. The epidermal fragment was carefully stretched to remove creases, then covered with a glass coverslip and gently pressed to remove air bubbles. The obtained preparation was examined under a microscope, successively using 10x, 20x, 40x and 60x objectives.
In order to visualize the shape and arrangement of cell debris under the optical microscope, a temporary microscopic preparation was made by transversally sectioning the lower epidermis of the leaf and placing the respective fragment on the glass slide, in a drop of acetic carmine. The slide was then coverslipped and the slide was studied using progressive 10x, 20x, 40x, and 60x objectives.

RESULTS AND DISCUSSIONS

In the image below you can see the internal structure of an *Amaranthus retroflexus* leaf, in cross-section. It consists of two epidermises, one upper and one lower, as well as mesophyll.

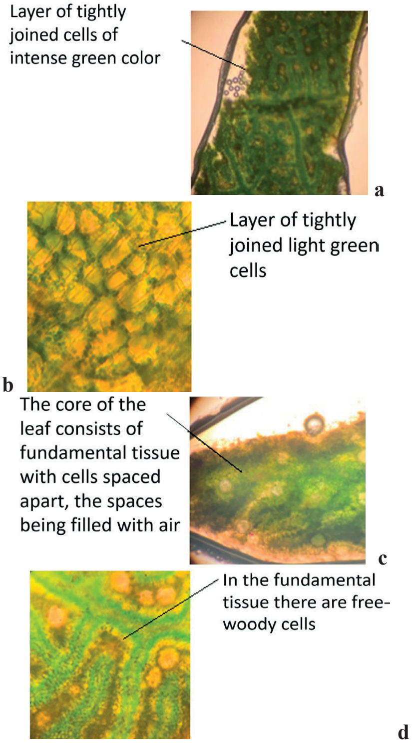

Figure 2. Transverse section through the leaf of *Amaranthus retroflexus*, viewed under the optical microscope, with different objectives (10x, 20x, 40x and 60x): a- upper epidermis; b- lower epidermis; c- mesophyll or core of the leaf; d- fundamental tissue

The upper epidermis, in turn, is made up of a layer of densely green cells closely united to each other. The lower epidermis consists of a layer of tightly joined light green cells. On both epidermises, but especially on the lower one, there are modified cells called stomata. The mesophyll or core of the leaf consists of ground tissue with cells spaced apart, the spaces being filled with air. In the fundamental tissue are found libero-wooden bundles.

On the lower epidermis, pluricellular hairs appear, in the shape of a shield, with a polygonal basal cell, a uniseriate pluricellular pedicel (several oblong-rectangular cells, superimposed on each other), and the gland is unicellular, approximately spherical-pyriform in shape, rich in cytoplasm.

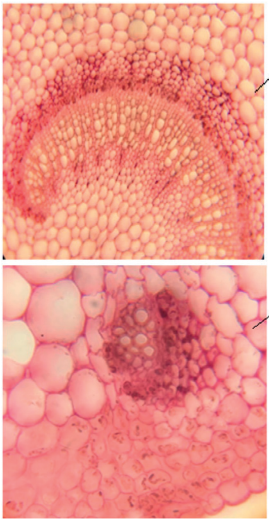

Figure 3. Multicellular hairs in *Amaranthus retroflexus* species

Stomata are specialized structures, in the form of small openings or pores, located at the level of the epidermis, having a role in gas exchange between the plant and the external environment, as well as in the release of water vapor (transpiration). Through the stomata, the carbon dioxide necessary for assimilation and the oxygen necessary for respiration enters the plant body, as well as the elimination from the plant body of various gaseous substances (oxygen and carbon dioxide) formed during the processes of respiration, respectively transpiration. Stomata help reduces water loss by closing when conditions dictate. Most of the stomata are located on the underside of the leaves, which reduces their exposure to heat and drafts.

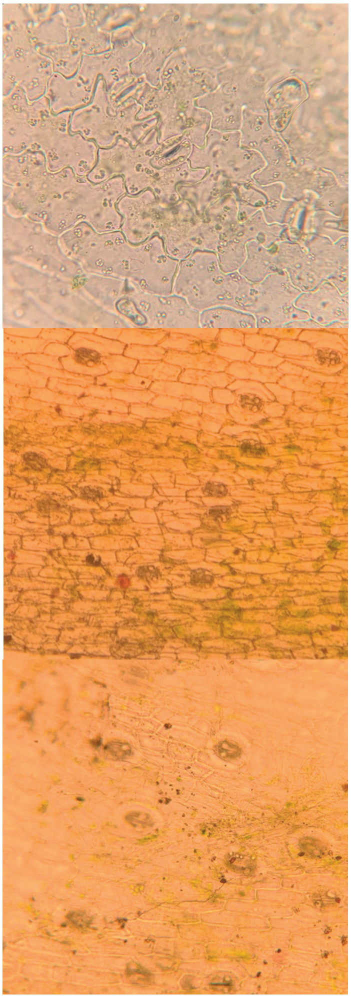

Figure 4. Appearance of stomata in the species *Amaranthus retroflexus* (40x)

In the process of transpiration, water is eliminated into the atmosphere mostly through the stomata and less through the cuticle of the epidermis. Cuticular transpiration is reduced in mature leaves and even more intense in young leaves, whose epidermal cells have a thin cuticle.

Transpiration through the stomata is about 10 times more intense than through the cuticle. The stomata perform opening and closing movements, which are the main means of regulating the intensity of transpiration. The amount of water removed in this way is proportional to the degree of opening of the stomata, and the speed of water vapor diffusion is directly proportional to the surface and the diameter of the ostiole.

Stomata open in the morning under the influence of light (photoactive reaction), when the stomatal cells have a higher degree of turgor than the accessory cells. The opening degree reaches a maximum at midday, in sunny conditions, when the penetration of CO_2 necessary for photosynthesis is more intense. These phenomena cause the leaves to transpire at a high intensity.

CONCLUSIONS

In conclusion, the study of the internal structure of *Amaranthus retroflexus* leaf revealed that it consists of two epidermises, one upper and one lower, as well as mesophyll. Stomata in this species open in response to light in the morning due to a photoactive reaction, and their degree of opening is maximum in sunny conditions when the penetration of CO_2 is intense. This mechanism facilitates the process of photosynthesis, but it also leads to the transpiration of the leaves at a high intensity. The fixation and preservation techniques used in this study enabled the observation of the internal structure of the leaf in detail. This knowledge contributes to a better understanding of the anatomy and physiology of this species, which can be useful in various fields such as agriculture, botany, and ecology.

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