

## Photosynthetic and morphological characters of leaves of the annual and biennial *Salvia sclarea* biotypes

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**ABSTRACT** The leaf anatomy, the CO<sub>2</sub> assimilation rate, the photosynthetic pigment content and the chlorophyll fluorescence has been studied in young and old leaves of the biennial and annual biotypes of *Salvia sclarea* plants. The structure of the mesophyll of all leaves of both biotypes resembled to the „shade plants”. The values of the CO<sub>2</sub> assimilation of young leaves however represented typical light leaf character with significantly higher saturation light intensity than that of the old leaves. According to our preliminary results it proved that the chlorophyll fluorescence characters (Fv/Fm and ETR) indicated moderate damage in the photosystem II of the young leaves. The increased NPQ of the same plant parts represented increased efficiency of heat dissipation and hence, greater photoprotection capacity.

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### KEY WORDS

*Salvia sclarea* biotypes  
leaf age  
leaf anatomy  
photosynthesis

*Salvia sclarea* was one of the traditional medicinal plants of monastery gardens. Its essential oil having characteristic muscate-like flavour are widely used also in our days in cosmetics and aroma therapy. The distribution of this species is the mediterranean area, it prefers the warm environment and the full sun. *S. sclarea* has biotypes with biennial and annual growth habits. For economic consideration the annual biotype should be appropriate for breeding, therefore numerous studies are carried out on the special environmental demand and the quantity and quality of drug- and oil production (Iljeva 1979; Zámboriné and Tétényi 1990; Lawrence 1994; Bodor et al. 2005). Since the biennial biotypes bears only rosette leaves in the first year, and the annual plants have inflorescence and infructescence at the end of the first vegetation period, we supposed, that the photosynthetic characters of the biotypes differ. The aim of this study was to compare the CO<sub>2</sub> fixation, the photosynthetic pigment content and the chlorophyll fluorescence parameters of the leaves of different ages of annual and biennial growth forms. Attempt was made also to find correlation between photosynthetic characters and leaf anatomy.

### Materials and Methods

#### Plant material

Plants originated from the Department of the Medicinal and Aromatic Plants of Corvinus University Budapest. The biennial plants were *Salvia sclarea* L. sort „Akali”. The annual stock has been selected formerly from this biennial sort and was cultivated separately.

Plants were grown in pots in glasshouse in the Botanic Garden of the University of Szeged until May and then were

transferred to natural light intensity for 8 weeks before measurements. Studies were carried out in the years 2003 and 2004. For the measurements we used plants with six leaves and the first (old) leaves and the middle (young) leaves on the stem were applied.

#### Leaf anatomy

The upper and lower leaf epidermis and the mesophyll was viewed by SEM (Hitachi S 2400) following fixation (Karnovsky fixative), chemical dehydration (ethanol and acetone), critical point drying and sputter coating with gold.

#### CO<sub>2</sub> fixation

The light response curve of CO<sub>2</sub> fixation was measured with LCA-3 infrared gas analyser (ADC England) at 340 ppm CO<sub>2</sub>, 21% (v/v) O<sub>2</sub>. Leaf put in the measuring chamber was kept in darkness for 10 min and then was illuminated with gradually increasing light between 30-1450  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD. We detected the CO<sub>2</sub> fixation in each light level after 7 min continuous illumination.

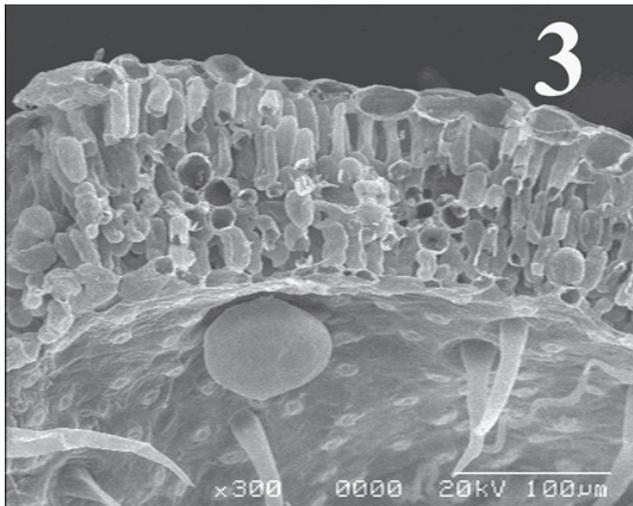
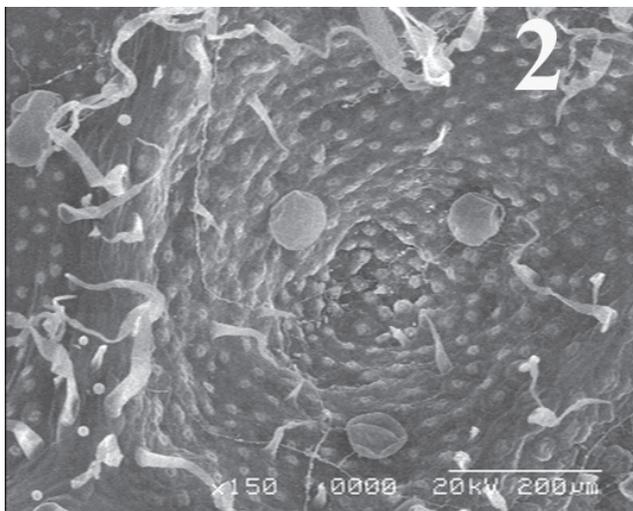
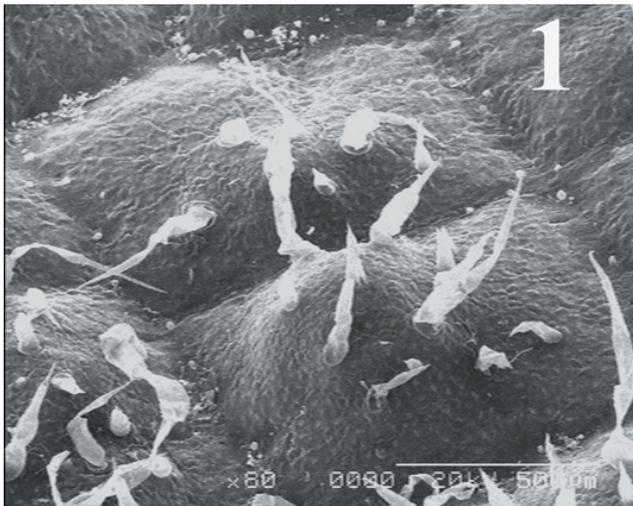
#### Pigment analysis

Chlorophyll a and b and total carotenoid contents were determined by spectrophotometer according to Lichtenthaler (1987) in the leaves used for CO<sub>2</sub> fixation and chlorophyll fluorescence measurements.

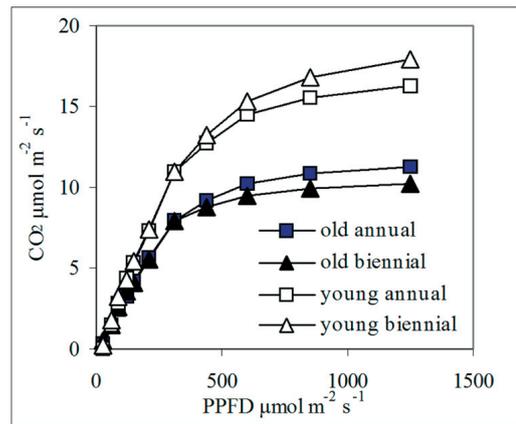
#### Chlorophyll fluorescence measurements

To monitor the differences in light utilization chlorophyll fluorescence quenching was measured by PAM-200 fluorimeter. The following parameters were determined: maximum quantum yield (Fv/Fm), apparent linear electron transport

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**Figure 1.** Leaf anatomy of *Salvia sclarea*. 1: upper epidermis with convex areoles, 2: concave areoles in the lower epidermis with round sessile glandular trichomes and numerous stomata 3: mesophyll with slightly different palisade and spongy parenchyma cells.



**Figure 2.** CO<sub>2</sub> fixation of young and old leaves of the annual and biennial biotypes.

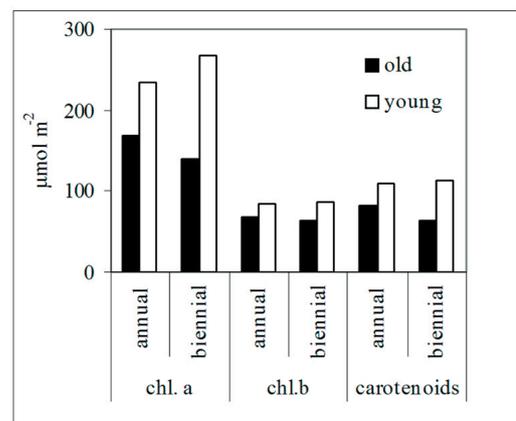
rate (ETR) and light induced non photochemical quenching (NPQ). Calculations were made according to Schreiber et al. (1986) and Genty et al. (1989).

### Results and Discussion

On the upper surface of the leaves the hairy areoles are convex (Fig. 1.1). On the lower surface these areas are concave and there are glandular hairs and numerous stomata on them (Fig. 1.2). In the thin mesophyll the shape of the palisade and spongy parenchyma cells are almost the same (Fig. 1.3).

The mesophyll structure resembled the shade adapted leaves did not differed in the two biotypes.

The light response curves of CO<sub>2</sub> assimilation rate (Fig. 2) of the old leaves showed typical „shade” plant shape with low CO<sub>2</sub> fixation (about 10 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>) at saturation light intensity) in comparing with the young ones (16 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>). The leaf age influenced the CO<sub>2</sub> assimilation at a greater



**Figure 3.** Pigment content of young and old leaves in two biotypes.

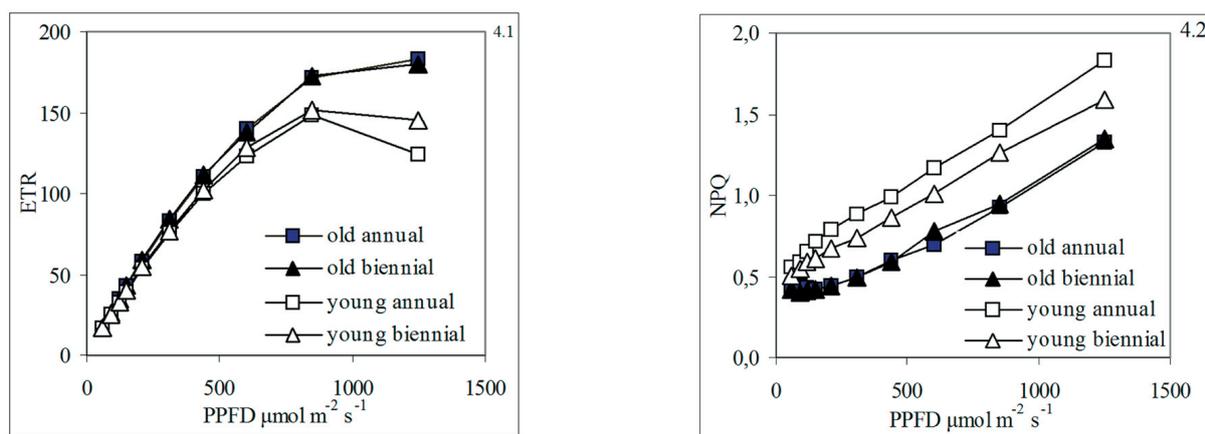


Figure 4. Chlorophyll fluorescence characters. 4.1: electron transport rate (ETR), 4.2: non photosynthetic quenching (NPQ).

extent than the biotypes from where the leaves originated. The difference between the  $\text{CO}_2$  fixation of leaves of different age is significant ( $p < 0.1$ ) above  $600 \mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD.

The importance of leaf age was reflected also in the accumulation of the photosynthetic pigment fractions, in chlorophyll a, chlorophyll b and carotenoids (Fig. 3). Old leaves of both biotypes were characterized by lower pigment amount and lower chlorophyll a/b ratio (at an average 2.45) suggesting also the shade character of the leaves. In the young leaves the content of pigments exceeded the old ones, especially the chlorophyll a fraction were high. Consequently the chlorophyll a/b ratio attained the 3.0, characteristic to the high light acclimated leaves. Chlorophyll a/b differed significantly ( $p < 0.5$ ) in the leaves of different ages.

The measurements on  $\text{CO}_2$  fixation and pigment content suggested, that the age of the leaves had greater influence on these characters, than the life forms (biotypes). Similar conclusion could be drawn from chlorophyll fluorescence measurements. The average of maximum quantum yield (Fv/Fm) was 0.81 in old leaves, 0.78 in young ones irrespectively of the biotypes. The slight decline of the Fv/Fm of the young leaves indicated some damage in the photosystem II. The difference in the photosynthetic electron transport rate (ETR) and the NPQ ( Figs. 4.1 and 4.2) was also independent to the biotypes. NPQ capacity is an important factor for acclimation of plants to high light in natural conditions. Higher values of NPQ in younger leaves represented increased efficiency of heat dissipation and hence, greater photoprotection capacity.

Our results presents, that the intensive growth of the annual biotype of *Salvia sclarea* is not the consequence of the increased photosynthetic effectivity. The annual habit is supposed to be the result of the high apical meristem activity together with the quicker leaf development. Nevertheless this study focused our attention on the need of further examination of the change of photosynthetic and photoprotective processes in the course of the leaf development.

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