EFFECTS OF WATER STRESS ON SUNFLOWER PHYSIOLOGY.

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ABSTRACT :

Physiological mechanisms of sunflower (Helianthus annuus L., cv Viki) adaptability to water stress were investigated. A constant water deficit (predaw leaf water potential -0,9 MPa, soil humidity 10,5 %) was applied to the plants, from the vegetative stage until the harvest. The drought led to a decrease in leaf area and an increase in stomatal resistance to limit water consumption. The translocation of assimilates from leaves was reduced, but the sink effect of inflorescences was greater in stressed than in well-watered plants. Abscisic acid (ABA) accumulation in roots and leaves and higher ABA and zeatine contents in capitulum were shown in water-stressed plants. Correlations were established between phytohormone contents and physiological responses, and ABA seems to be the main plant signal during the drought.
When the pre-stressed plants recovered a normal water status, the leaves expanded again, specially if the water supply occurred at the beginning of the reproductive cycle. Stomatal opening was similar to those of control plants. ABA levels in roots and leaves decreased whereas ABA and zeatine still accumulated in inflorescence of the rewatered-plants. These two hormones allowed the capitulum to keep its attractive capacity towards the photosynthate.
All these responses ought to have positive repercussion on the yield.

INTRODUCTION :

In their natural environment, sunflower plants are submitted to variable pedoclimatic conditions and to different constraints ; water is often the main limiting factor of yield elaboration. In spite of a large decrease of leaf area which leads to a low production, sunflower has a good adaptability to dry conditions : activities of fundamental enzymes such RuBisCO, saccharose-phosphate synthase and nitrate reductase were not significantly affected (Nicco et al., 1992). Subsequently we have investigated the role of phytohormones in drought resistance. The present study examines the drought effects on leaf area, stomatal closure, ABA and zeatine contents in roots, leaves and inflorescences and the relationships between these data.

MATERIALS AND METHODS :

Sunflower plants (Helianthus annuus L. cv. Viki) were grown in pots on a mixture of clay-slime-sandy soil supplied with mineral elements. The pots were first watered daily to maintain soil water content close to field capacity (30%). The water stress (predawn leaf water potential -0,9 MPa, soil

humidity 10,5%) was applied from the vegetative stage and maintained constant until the harvest. The potential of well-watered plants was - 0,3 MPa and soil humidity 30%. The pots were weighted twice a day, and the amount of water supply was regularly recalculated as a function of the plant development. Some water-stressed plants were rewatered as the control after 19 days of stress application.

The leaf water potential was measured using a pressure chamber (Scholander *et al.*, 1964) before the beginning of the light period.

The experiments were conducted on three leaf stages (lower, median and upper), and the used leaf for analysis was the more representative of each one. All results presented are average values of four replicates with the standard errors of the means, except for root and capitulum analysis.

The relative water content (RWC) of the leaves was measured following Turner (1981). The leaf area was estimated as follows : length x width x 0,7. The stomatal resistance was determined with a diffusion porometer (type Delta T Devices) early in the afternoon.

The hormone was extracted according to Weiler (1980) and Leroux (1984). The abscissic acid (ABA) and zeatine levels were quantified by enzyme linked immunosorbent assay (ELISA), using Phytoscience (France) commercial kit.

RESULTS :

One week after the water stress application, the leaf area was significantly reduced, about 30% (figure 1). After the 15th day, the decrease reached 60% whatever the leaf stage. In rewatered plants, the lower leaves had a sligtly more important area (20 %) than in water-stressed sunflowers ; nevertheless they remained still reduced (50%) compared to controls. The median and upper leaves also expanded again, more specially if the leaf is young when the water supply occurred.

The stomatal resistance in the adaxial (figure 2A) and abaxial (figure 2B) epidermis in leaves of water-stressed plants was increased. However this rise was lower in abaxial epidermis, specially after 30 days. When the pre-stressed plants recovered a normal water status, the stomatal opening in median and upper leaves was similar to those of irrigated plants.

Root ABA concentration increased strongly in plants at low water potentials (figure 3). After rehydration, it was still higher than the control, but lower compared to plants maintained under drought conditions.

ABA content of all leaf stages was at least twice more important than in the control (figure 4). Moreover the ABA level in leaves was higher than in roots. The amount of ABA decreased rapidly in median leaves of reirrigated sunflowers, and reached the control values.

ABA and zeatine contents in capitulum are shown in figure 5. An important accumulation of these hormones was found in inflorescence of drought-treated sunflowers. During the flowering period, the ABA content in water stressed plants increased as well in control as in water stessed plants. In the contrary, the maximum zeatine level was found 30 days after stress

application, which coincides with the intense growth period of the reproductive organ.

DISCUSSION AND CONCLUSION :

The decrease of leaf number and area is an usual response to limit the water consumption in dry conditions (Rawson *et al.*, 1980 ; Sadras *et al.*, 1993). The stomatal resistance found in irrigated sunflower (150 s.m^{-1}) were lower than in many plants (CETIOM, 1983) ; the important number of stomata and their big size (Sutcliffe, 1979) could explain these data. Limited water supply results in stomatal closure to decrease transpiration. However the drought affects generally the leaf area before the stomatal resistance. Then after 20 days of cessation of watering, the stomatal closure of abaxial epidermis was less important. This may allow CO_2 absorption and maintenance of photosynthetic activity. Such results are reported by Mott and O'Leary (1984) for sunflower, however they can differ according to varieties (Planchon, 1990). This phenomenon could be due to differences in stomatal ABA repartition or stomatal sensitivity to ABA, as suggested by Henson and Turner (1991).
Roots and leaves ABA accumulation in water-stressed plants was reported by several authors (Hubick *et al.*, 1986 ; Masia *et al.*, 1994). It can result from both intense ABA synthesis and hormone redistributions between leaves and roots. At low water potentials, ABA content was higher in older leaves than in young ones.
Some established correlations between leaf ABA content and other data are noted in table 1. The leaf area was significantly and negatively correlated with the ABA concentration and was independant of leaf water status. This is consistent with the ABA role in leaf expansion rate (Zhang and Davies, 1990).
We noted also a significant positive log-linear relationship between leaf ABA concentration and stomatal resistance in the adaxial epidermis. However a more important correlation appeared between the stomatal closure and the relative water content. Usually a strong relationship is obtained between stomatal conductance and ABA in xylem exudate as soil dries (Zhang and Davies 1990), which supports that ABA is a message from the roots controlling stomatal conductance in drying soil (Munns and Sharp, 1993).
In drought conditions, the ABA level increase in inflorescences has been also described by Piquemal *et al.* (1990) ; it has been so reported that ABA is mainly localisated in florets and akenes. ABA increase during the flowering period suggests that capitulum has a strong sink strength for ABA.
Cytokinins play a key role in cellular multiplication ; the important zeatine content in inflorescence of water-stressed plants might explain the rapid development of the capitulum compared to controls. Moreover, ABA and cytokinins play an important role in the metabolites partitioning regulation (Chenesseau, 1984 ; Clifford *et al.*, 1986). During labelling experiments, Flenet (1994) observed that capitulum of drought-treated plants had a

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stronger capacity to attract photosynthate. Therefore ABA should be responsible to improve this assimilates allocation to inflorescence.

The water supply after a deficit period leads to a leaf expansion recovery and to a more important development of upper leaves, while for irrigated or water-stressed plants, the median leaves have always the largest area. Similar data have been reported by Morizet and Merrien (1990).

The stomatal apparatus found again its opening capacity, therefore it has not been affected in irreversible manner by the water deficit. These results are consistent with those obtained by Soldatini and Guidi (1992) ; they showed a 50 % recovery only 3 days after rewatering of pre-stressed (-0,5 MPa) plants.

These phenomena were concomitant with an ABA content decrease in leaves and roots of reirrigated sunflowers. In spite of rewatering, the capitulum kept its sink effect for ABA and zeatine, since their concentrations were still high. This result supports the idea that ABA is responsible to better photosynthate allocation to inflorescence, since this organ attracts still more assimilates after rehydration (Flenet, 1994).

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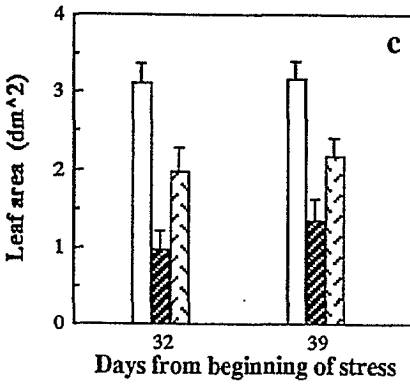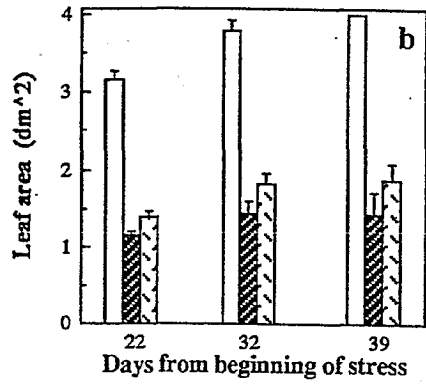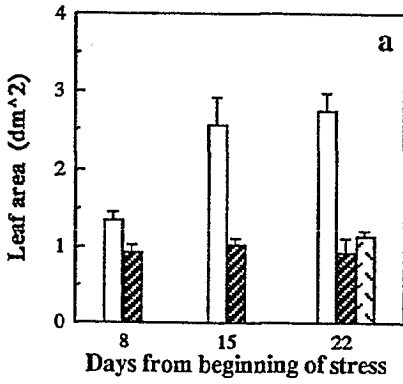
Figure 1 : Area of lower (a), median (b) and upper (c) leaves of (□) well-watered, (▨) drought stressed and (◩) rewatered plants.

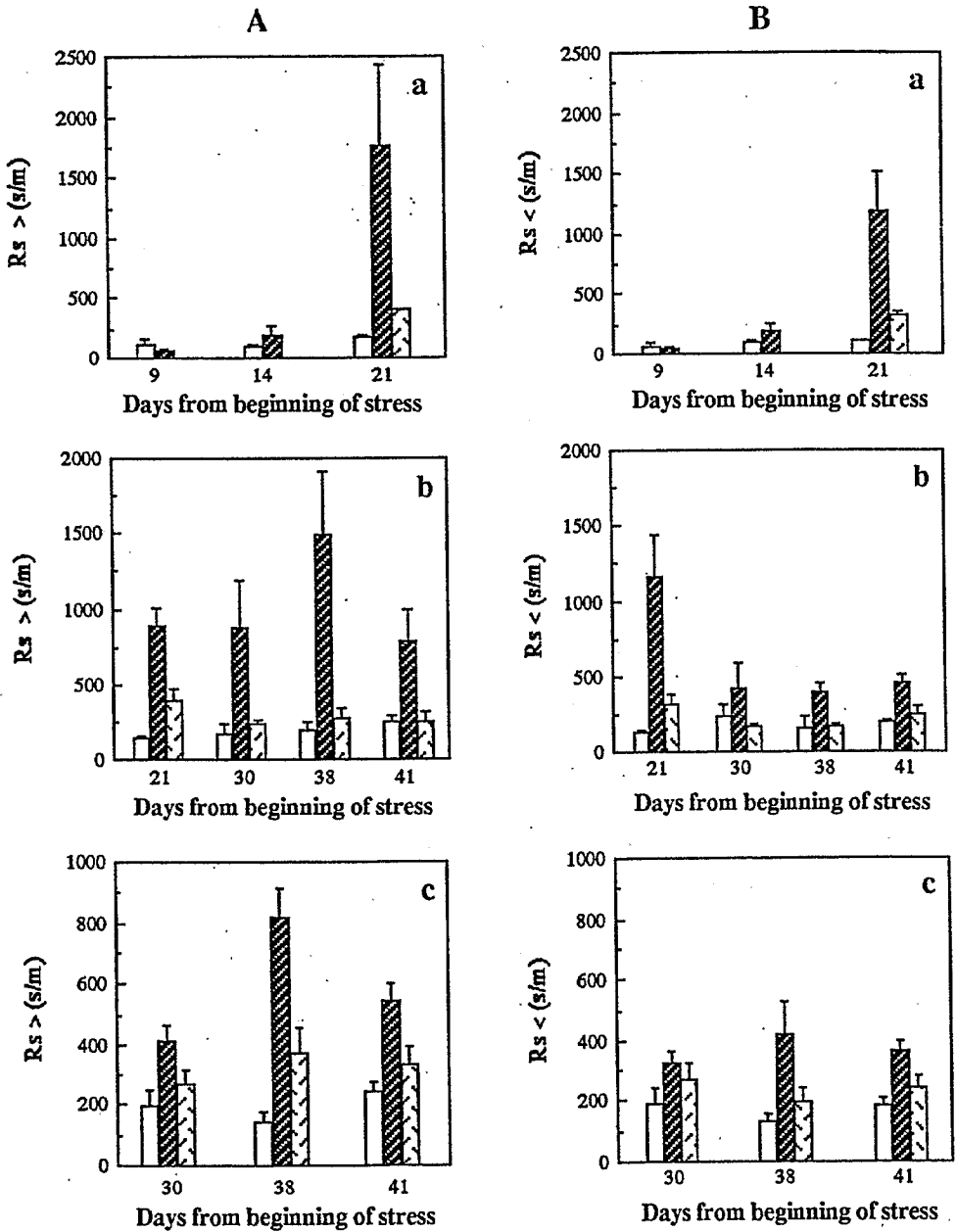**Figure 2 :** Stomatal resistance of adaxial (Rs >, A) and abaxial (Rs <, B) epidermis of lower (a), median (b) and upper (c) leaves of (□) well-watered, (▨) drought stressed and (▣) rewatered plants.
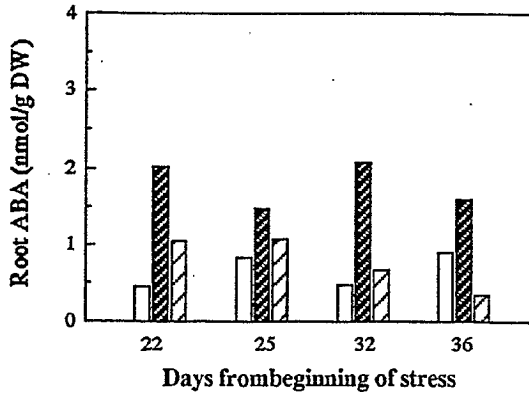
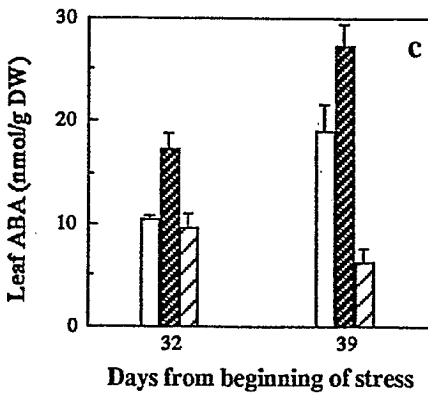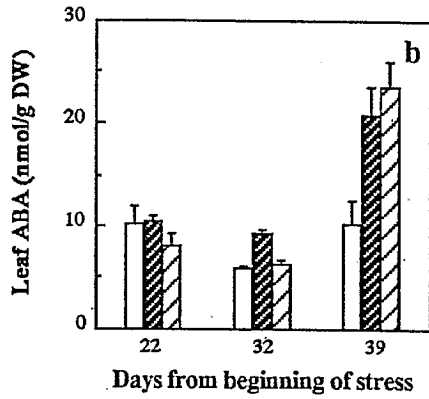**Figure 3 :** Root ABA content of (□) well-watered, (▨) drought stressed and (▨) rewatered plants.
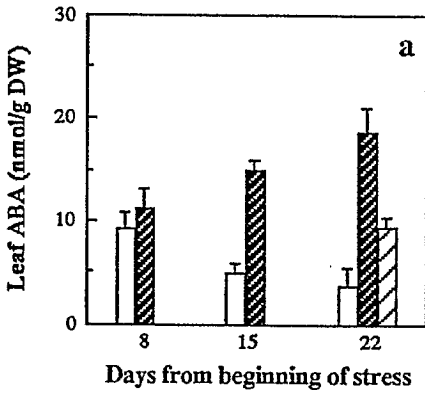

Figure 4 : Leaf ABA content of lower (a), median (b) and upper (c) leaves of (□) well-watered, (▨) drought stressed and (▨) rewatered plants.
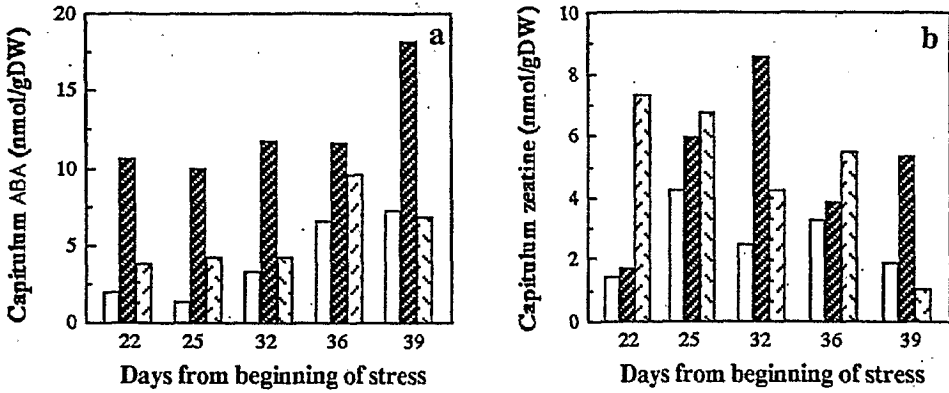
Figure 5 : ABA (a) and zeatin (b) content in capitulum of (▢) well-watered, (▨) drought stressed and (▨) rewatered plants.

Table 1 : Coefficient of correlation between leaf ABA and other data. (curve equations and regression lines)

	RWC	ABA
RWC		-0,657** (y=110,1-33,3.Log x)
Leaf area	0,399NS (y=-10,8+6,76.Log x)	-0,438* (y=4,1-2,2.Log x)
Ln Rs <	-0,459* (y=7,7-0,03 x)	0,378NS (y=4,4+0,07 x)
Ln Rs >	-0,611** (y=10,4-0,06 x)	0,469* (y=4,7+0,09 x)

NS : non significant ; ** : significant at P<0,01 ; * : significant at P<0,05.