ABA CONTENT AND SOME KEY ENZYME ACTIVITIES IN RESPONSE TO WATER STRESS.

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ABSTRACT

A modified "Snow and Tingey system" (Plant Physiol., 1985, 77: 602-607) was used to apply constant water deficits (-0,3 to -1,5 MPa) to sunflower (Helianthus annuus L. hybrids cv. Viki and Flamme) plants grown in semi-controlled conditions. The experiments were carried out from flowering ("bud 1 cm.") until seed filling in order to investigate some physiological mechanisms of water stress resistance.

The water deficit had no incidence on root nitrate absorption and no significative effect on nitrate reductase (NR) activities at both leaf and root levels. Initial and potential carboxylase activities of Rubisco (Ribulose-1,5-bisphosphate carboxylase-oxygenase) were stimulated but the activation state of the enzyme was unchanged ; the oxygenase activity of Rubisco was also increased in response to low water potential. Effect of water deficit was also observed on sucrose-phosphate synthase (SPS) activity, which controls the flux of carbon into sucrose and hence photosynthates translocation. No difference could be found between the two cultivars studied. The abscisic acid (ABA) content is now measured in order to investigate the role of hormonal balance on drought adaptative processes.

INTRODUCTION

Limited water supply to sunflower plants results in stomatal closure, loss of turgor, accumulation of abscisic acid and higher concentration of solutes (Graan and Boyer,1990). Water stress also affects plant metabolism and can reduce efficiency of key processes such as photosynthesis, respiration, nitrogen assimilation, lipid and protein synthesis (Navari-Izzo *et al.*, 1990).

In our laboratory, two types of research were carried out ; first, traits of yield were studied (Flenet,1992) ; then, activities of three fundamental enzymes in plant metabolism and the ABA content were investigated.

MATERIALS AND METHODS

A slightly modified Snow and Tingey (1985) system was used for apply water deficit (figure1). Sunflower plants (*Helianthus annuus* L. cv. Flamme and Viki) were grown in a greenhouse, under controlled environmental conditions : 14 h photoperiod, 28/22 °C day/night thermoperiod, 60% relative hygrometry and photon flux density of 1500 µmol m^{-2} s^{-1}. They were fed with a mineral nutrient solution (Poeydomenge, 1992). The seeds were first germinated in plant chambers (volume 1700 ml) filled with vermiculite. When the plants were 34 d old, the plant chambers were inserted over the

columns. After an establishment period of 3 d, three regimes of water availability were imposed, from bud 1 cm stage until seed filling, with nutrient medium column heights of 1, 5 and 6 cm below the Nytal cloth for control, moderate stress and high stress respectively. The leaf water potential was measured using a pressure chamber (Scholander *et al.*, 1964) before the beginning of hemeroperiod ; thus the water potential around roots was measured.

Physiological traits studied, either on the more expanded leaf of high canopy or on roots, were the following : soluble protein content measured by the method of Bradford (1976) ; initial and potential carboxylase activities of Rubisco determined by radiochemical technic (Raghavendra and Das, 1977) ; oxygenase activity of Rubisco assayed by measuring oxygen consumption with a Clark O_2 electrode (Lorimer *et al.* ,1977) ; saccharose-phosphate synthase (SPS) activity determined by the method described by Salerno *et al.* (1979) ; nitrate reductase (NR) activity measured by the *in vivo* (Radin, 1973) and *in situ* assay (Robin *et al.*,1983) ; nitrate content assayed by the method of Cataldo *et al.* (1975) ; abscisic acid (ABA) content determined by radioimmunoassay as described by Weiler (1980).

RESULTS

Results obtained showed no difference between the two cultivars studied, therefore only data relative to cv. Flamme were reported. The amount of soluble leaf protein was slightly higher in sunflowers submitted to a constant water-stress (figure 2). At the end of the growth cycle, the protein content always decreased but in a greater manner in irrigated plants. The water stress did not affect the potential carboxylase activity (figure 3) measured after enzyme activation, and a slight increase was even noted in drought-treated plants. Measurements of initial carboxylase activity led to the same conclusions ; so the activation state of Rubisco (results not reported) was not modified by the drought treatment and varied between 70 and 90 %. The plants grown under water constraints had increased Rubisco oxygenase and SPS activities (figure 4, 5). The amount of nitrate in leaves increased during the growth cycle (figure 6) and was the lowest in plants subjected to water starvation. Moreover, this content was the more decreased as the stress intensity was increased. The nitrate reductase activity measured by the *in situ* and *in vivo* assay led to same type of results. At beginning of the water deficit, the activity (figure 7) was lower in drought-stressed plants than in watered control plants. From the bud development, there was a NR activity recovery and then the drought-stressed plants have a better rate of nitrate reduction. In roots, the nitrate content was twice higher than in leaves and increased also during the growth cycle (figure 8), but in a much greater manner than in leaves. The root NR activity (figure 9) increased at the end of the growth cycle for all treatments but was only the tenth of leaf potentialities. The ABA content was the highest in drougt-stressed sunflowers (figure 10).

DISCUSSION AND CONCLUSION

Plants at low water potentials have a higher protein content (figure 2) and seem to compensate the reduction of leaf expansion by a prolonged functioning of the assimilative area (Poeydomenge,1992). The carboxylase and oxygenase activities of Rubisco have been studied in order to analyse photosynthetic and photorespiratory potentialities. The carboxylase function is not affected by the water stress as shown by Piquemal *et al.* (1990), and Gimenez *et al.* (1992). Moreover, measurements of net photosynthesis realized *in situ* lead to similar conclusions (Flenet, 1992 and Blanchet *et al.*, 1990). The rise of oxygenase function (figure 4) is logical because plants close their stomata in response to drought conditions (Wookey *et al.*, 1991) ; stomata closure, protecting the plant against

water loss, simultaneously restricts carbon assimilation by the plant (Chaves, 1991) and the photorespiratory process assumes in a large part the chloroplast detoxification against reducing power. However, this increase is low and it cannot influence the photosynthetic yield. With respect to SPS activity, the results were in agreement with the data obtained by Poeydomenge (1992), which suggested that the largest part of sucrose synthesized ensures both a good bud carbon supply and maintenance of a higher osmotic potential in leaves. The water stress has not apparent incidence on nitrate uptake since the nitrate content of the roots is higher ; therefore the accumulated nitrate can be considered as an important solute in terms of osmoregulation (Larsson *et al.*, 1989). The leaf nitrate content decreases in water-stressed plants (figure 6) and this can be explained by a reduced nitrate flux caused by a reduction in both the rate of transpiration and the rate of nitrate delivery to the transpiration stream (Shaner and Boyer, 1976). The leaf NR activity presents the same evolution than that reported by Piquemal *et al.*, (1990).

In conclusion we have observed that enzymatic activities studied are often increased by a water deficit. Therefore the sunflower has a good adaptability to drought conditions. However, plants submitted to severe stress presents a large decrease of leaf area and therefore activities expressed on whole plant basis decreased. Drought-induced increases in ABA levels are observed in leaves of sunflower. This result is in good agreement with data reported in field grown sunflowers (Piquemal *et al.*, 1990) and can represent an adaptative phenomenon. However, the study of other phytohormones variations would give more informations.

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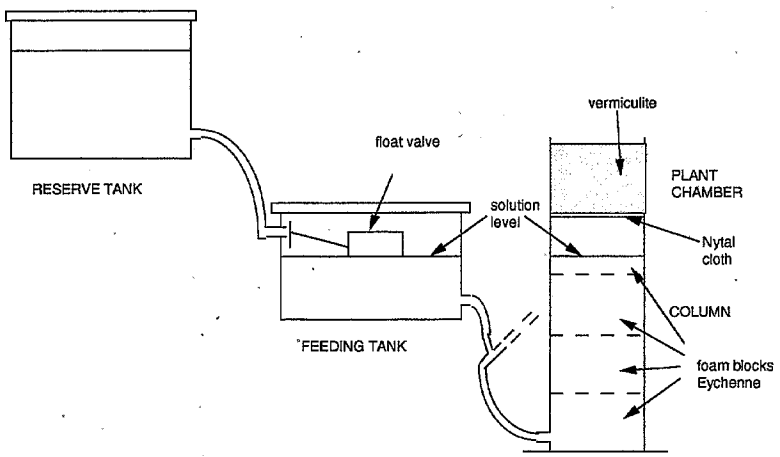

Figure 1 : A cross-section of the modified system for imposing water stress on plants.
The plant chamber was maintened over the top foam column by an adhesive tape. The nutrient solution within the column was adjustable and was maintened by the float valve in the feeding tank, which was supplied by the reserve tank. From a feeding tank, fourteen columns were connected. The both tanks were covered with a black plate in order to prevent the growth of algae.

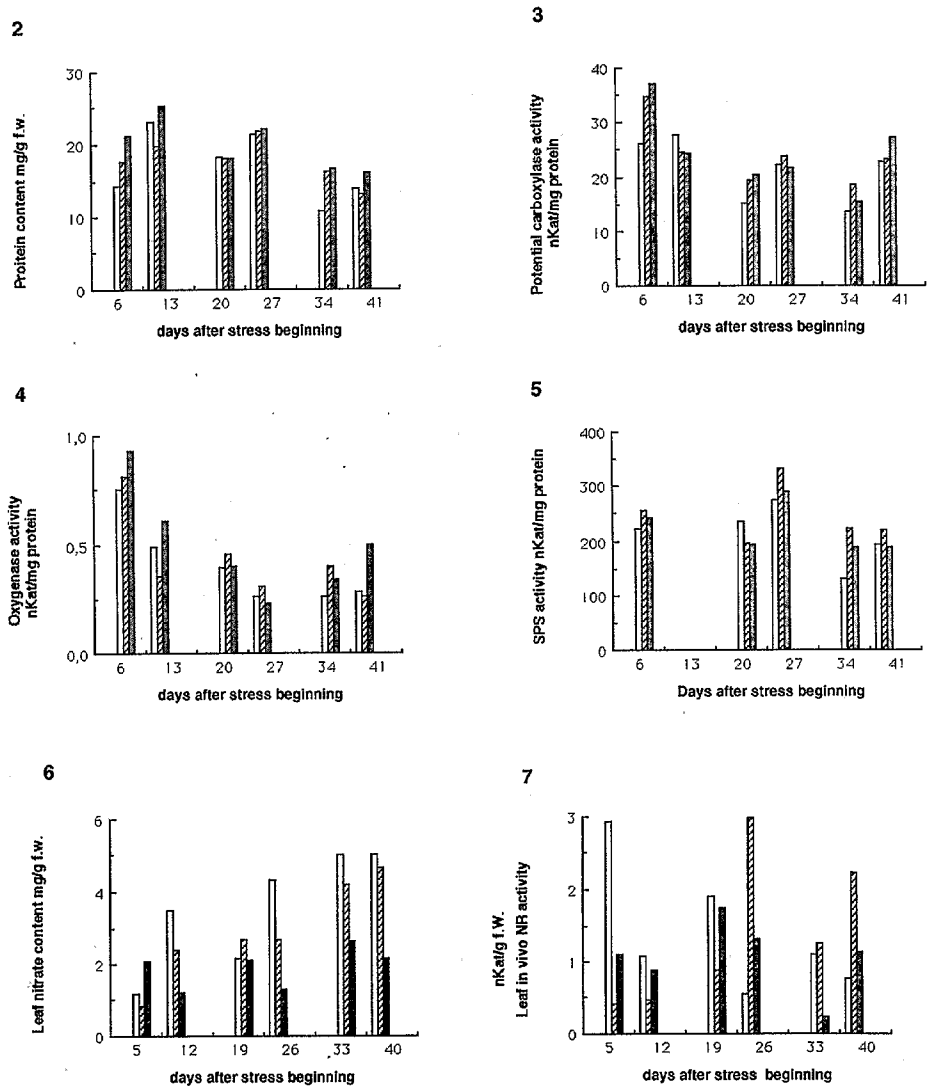

Figure 2 : Leaf soluble protein content, during different water treatments.
Well-watered plant (control) : □ ; drought-stressed plants : moderate stress (from -0.45 to -0.80 MPa.) : ◪ ; high stress (from -0.80 to -1.4 MPa.) : ■ .

Figure 3 : Potential carboxylase activity of rubisco. □ , □ , ■ : see figure 2.

Figure 4 : Oxygenase activity of rubisco. □ , ▨ , ■ : see figure 2.

Figure 5 : Saccharose-phosphate synthase activity. □ , □ , ■ : see figure 2.

Figure 6 : Leaf nitrate content. □ , □ , ■ : see figure 2.

Figure 7 : Leaf *in vivo* nitrate reductase activity. □ , □ , ■ : see figure 2.

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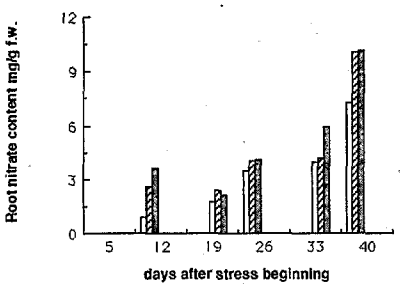

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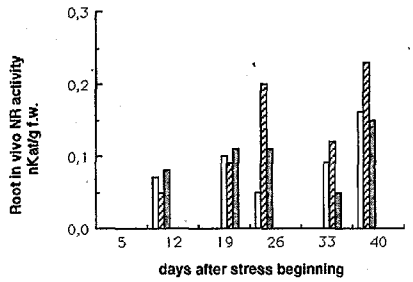

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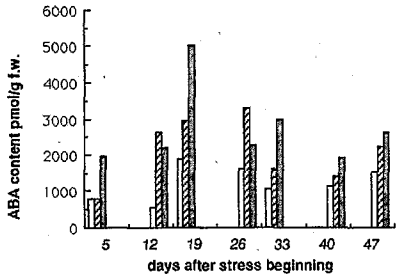

Figure 8 : Root nitrate content. □ , ▨ , ■ : see figure 2.

Figure 9 : Root *in vivo* nitrate reductase activity. □ , □ , ■ : see figure 2.

Figure 10 : ABA content in leaves. □ , ▨ , ■ : see figure 2.

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