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RESPONSES OF PHOTOSYNTHESIS AND CARBOHYDRATE ACCUMULATION IN SUNFLOWER LEAVES TO SHORT-TERM WATER STRESS

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SUMMARY

Short-term effects of falling leaf water potential on photosynthesis and carbohydrate accumulation in the leaves of four NS-sunflower hybrids were studied. Water stress was experimentally induced by holding leaf, petiole submerged for 24 hours in 0; 0.05; 0.1; 0.2; 0.5 and 1.0 M sorbitol solutions. Increased concentrations of sorbitol, up to 0.2 M, induced a rapid decrease of relative water content (RWC) in the leaves of sunflower hybrids NS-H-26-RM, NS-Helios, NS-H-43 and NS-H-101. Rapidly applied water stress significantly reduced CO_2 and light-saturated photosynthesis in the leaves already at 0.05 M sorbitol. The decline in net photosynthesis at decreased RWC in leaves was accompanied by a substantial decline in the apparent quantum yield. Primary photochemical reactions and electron transport appeared to be less affected by low RWC in leaves. Accumulation of soluble carbohydrates was increased under the effect of rapid water stress. The increased sucrose content could affect, through a feedback mechanism, the inhibition of photosynthesis. External water deficit induced the largest decline in the RWC and examined photosynthetic processes in NS-H-26-RM leaves, while the changes were the smallest in NS-H-101 leaves.

Key words: Water stress, NS-sunflower hybrids, photosynthesis and water stress, carbohydrates and water stress.

INTRODUCTION

The effects of drought on plants are a function of the intensity and duration of drought, as well as of the genetically determined capacity of species to cope with stress (Chaves, 1991). Long-term effects of drought are manifested as reduced leaf area and decrease in the size of plant canopy. Short-term effects include depression of the CO_2 assimilation rate in leaves in response to a drop in air humidity (Bunce, 1981) or in soil water potential (Gollan et al., 1986). Beside stomatal closure and the associated decrease in the supply of CO_2 as the cause of inhibition (Schulze, 1986), photosynthesis can be appreciably inhibited in water-stressed leaves after eliminating the stomatal influence by removing the epidermis (Dietz and Heber, 1983) or at high CO_2 which penetrates through the epidermis (Ben et al., 1987). By studying the effects of short-term water stress on different aspects of photosynthetic metabolism in spinach leaves, Quick et al (1989) found that activation of sucrose-phosphate synthase was one of the first sites at which spinach leaves respond to a rising water deficit.

Changes in the apparent quantum yield and reduction in the rate of CO_2 and light-saturated CO_2 assimilation were among the earliest and repeatedly reported biochemical responses of leaf photosynthesis to water stress applied to sunflower plants

(Boyer, 1971; Ben et al., 1987). Besides, it was shown that chronic water deficit could induce a significant increase in the sucrose and glucose content in sunflower leaves at the apparent expense of starch reserves (Fredeen et al., 1991). Our fluorescence and quantum yield data show that photochemical activity is preserved under the conditions of slow leaf dehydration. At the same time, the increased level of sucrose in dehydrated leaves, when CO_2 and light-saturated rate of photosynthesis are strongly reduced, indicates the action of a feedback mechanism (Panković et al., 1994).

In this study we have examined the immediate response of photochemical reactions, photosynthesis and carbohydrate accumulation to a fast decrease of RWC in four sunflower hybrids that exhibit different water use efficiency in the field (Plesničar et al., 1993). Water stress was experimentally induced by holding leaf, petiole submerged for 24 hours in sorbitol solutions of different osmolarities.

MATERIALS AND METHODS

Sunflower (*Helianthus annuus* L.) hybrids NS-H-26-RM, NS-H-43, NS-Helios and NS-H-101 were grown at the Institute's experimental field at Rimski Šančevi. Fully

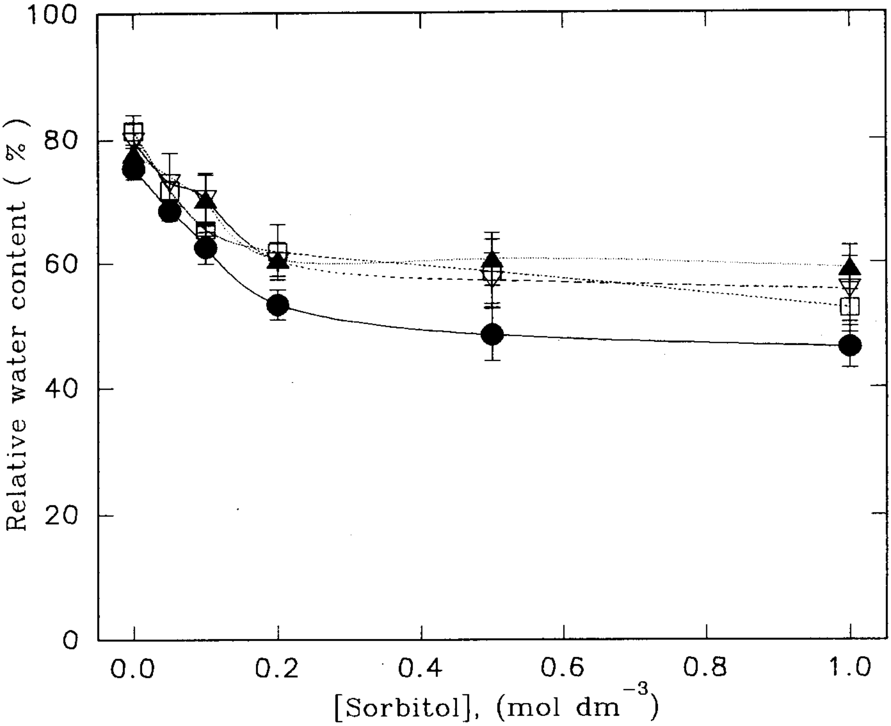

Figure 1. Changes in relative water content in the leaves of sunflower hybrids NS-H-26-RM (●), NS-H-43 (▽), NS-Helios (☐), NS-H-101 (▲) in response to rising external water deficit. Means for each sorbitol concentration are shown with standard errors (n=5).

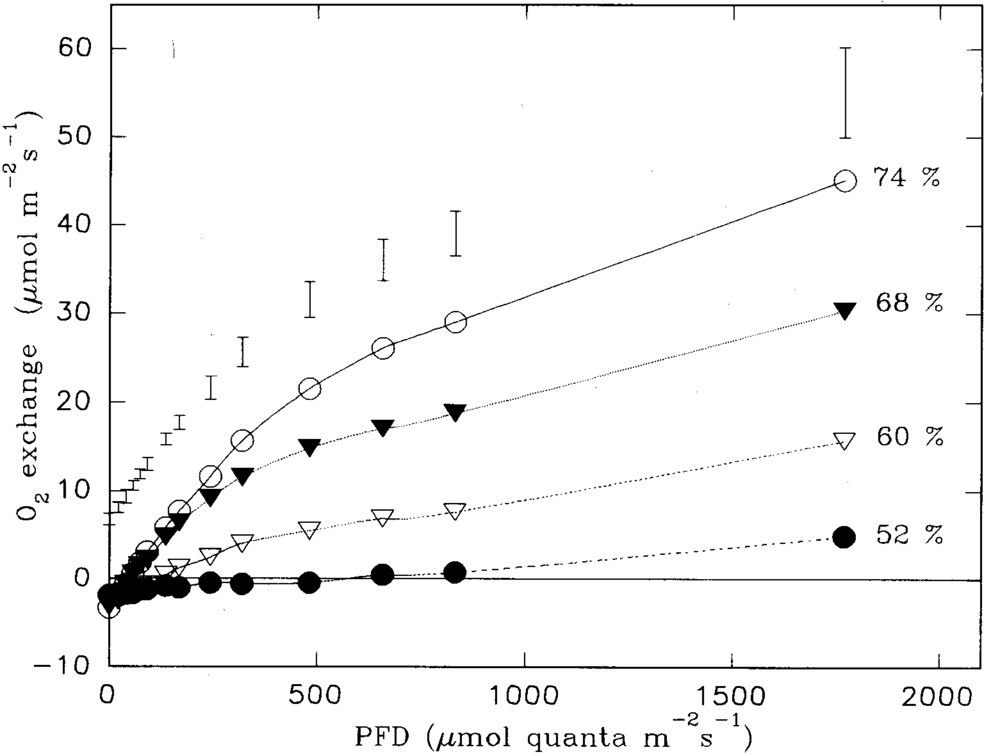

Figure 2. Light response of CO_2-saturated O_2 exchange by NS-H-26-RM leaves in response to increasing water deficit. Numbers by the curves indicate relative water content of the leaves. LSD (P=0.05) values for individual PFDs are presented by bars.

expanded leaves of the same age were taken off the upper third of a plant once a week, starting with the plants at the stage of anthesis. Leaves were kept, with petioles submerged, for 24 hours in 0; 0.05; 0.1; 0.2; 0.5 and 1.0 M sorbitol, equivalent to the osmotic potentials of about 0; -0.15;-0.3; -0.5; -1.3 and -2.8 MPa, respectively. While in solution, the leaves were illuminated at 300 μmol quanta m^{-2}s^{-1} for 24 hours.

Chlorophyll fluorescence emission was measured from the upper surface of the leaf using the Modulated Fluorescence Measurement System (MFMS, Hansatech, King's Lynn, UK). Dark-adapted leaf (20 min.) was initially exposed to a weak modulated measuring beam (F_o, minimal fluorescence yield), followed by simultaneous exposure to a continuos white light (light intensity as indicated) and the saturating light pulse (F_m, maximal fluorescence yield) to determine photochemical efficiency of photosystem II: F_v/F_m. At steady-state photosynthesis, pulses of saturating light (c. 5000 μmol quanta m^{-2}s^{-1}, 990 ms) were given every 15 s to fully reduce the primary electron acceptor of photosystem II (PSII) and to determine chlorophyll fluorescence quenching coefficients q_P (photochemical) and q_{NP} (non-photochemical) (Schreiber et al., 1986) and quantum efficiency of PSII electron transport Φ_{II} (Genty et al., 1989).

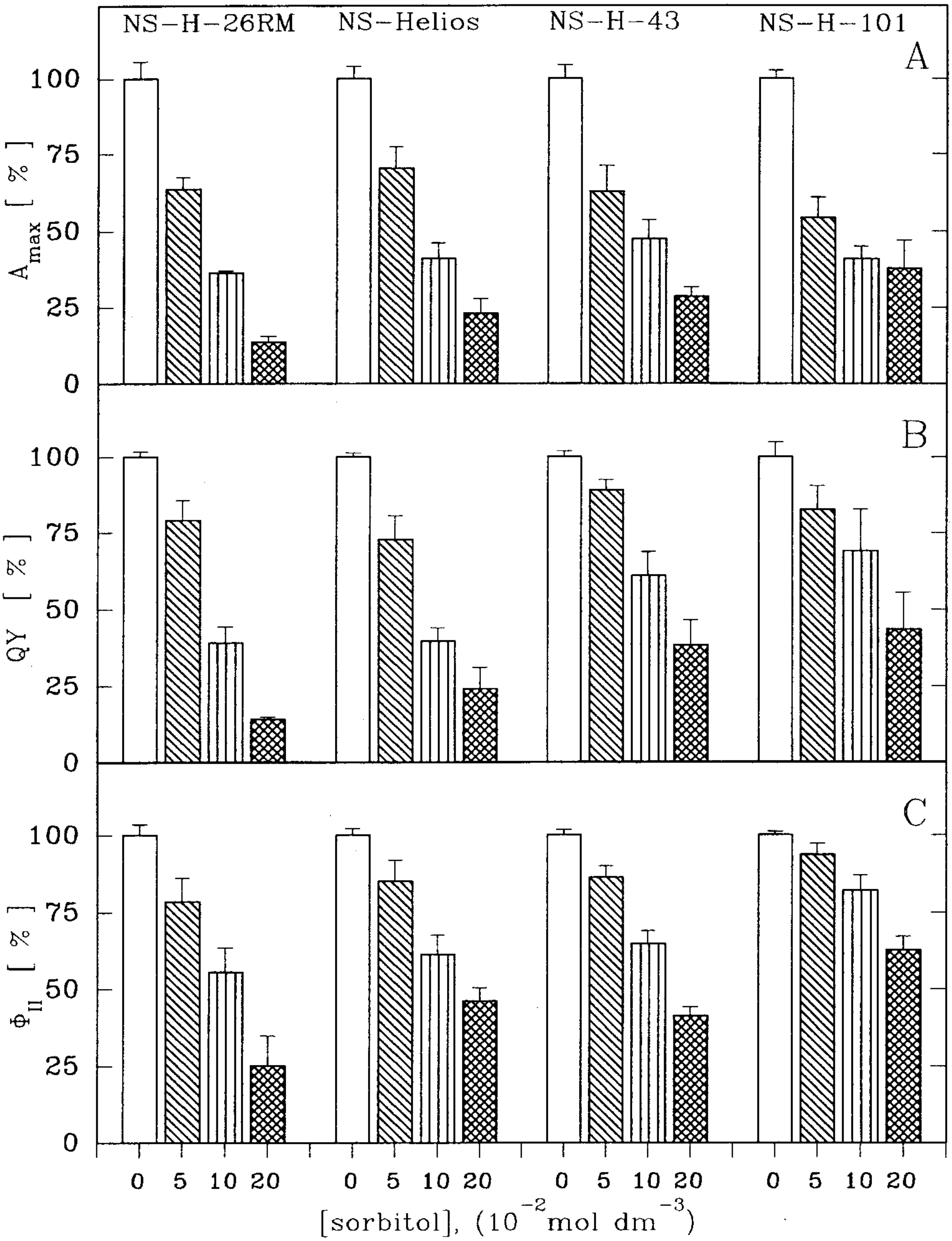

Figure 3. The effect of increasing external water deficit on A) CO_2 and light-saturated O_2 evolution, B) apparent quantum yield and C) quantum yield of linear electron transport in detached leaves of 4 sunflower hybrids at 1770 μmol quanta $m^{-2}s^{-1}$. The estimates are normalized on the results at 0 sorbitol (control) = 100. The results are the mean ±SE of 5 separate experiments.

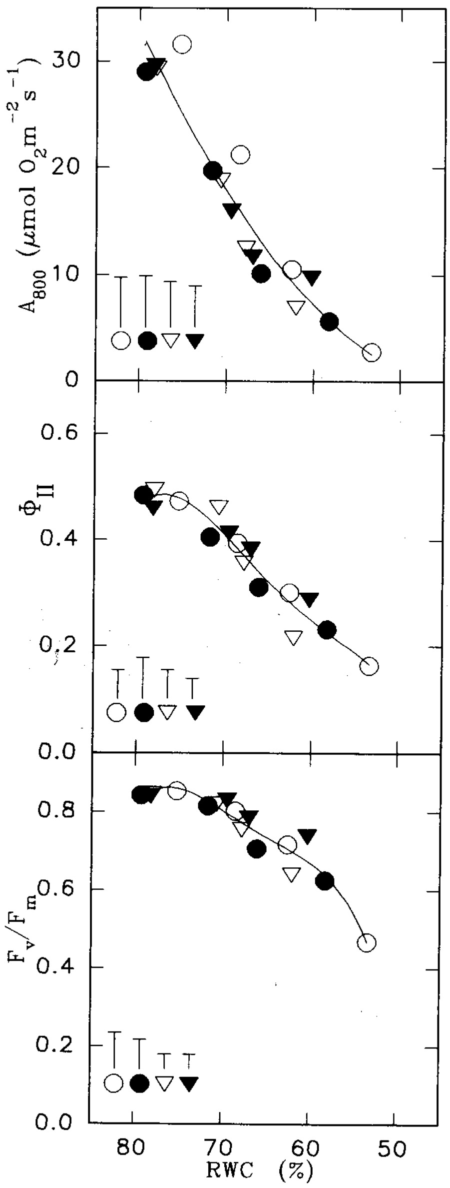

Figure 4. Responses of A) CO_2-saturated photosynthesis and B) quantum yield of linear electron transport at 800 μmol quanta $m^{-2}s^{-1}$ and C) photochemical efficiency of photosystem II, F_v/F_m, to acute water stress in detached leaves of NS-H-26-RM (○), NS-Helios (●), NS-H-43 (▽) and NS-H-101 (▼). LSD (P=0.05) values for each hybrid are presented by bars.

Photosynthetic oxygen evolution was measured polarographically on a leaf disc (10cm^2) in a closed chamber (LD2, Hansatech, King's Lynn, UK) at 25°C and 5% CO_2 (Delieu and Walker, 1981) over a range of 14 photon flux densities (PFDs). The "Leaf Disc" computer program allows O_2 exchange rate to be plotted against PFD and also permits determination of the dark respiration rate (the intercept on the vertical axis), light compensation point (LCP, the intercept on the horizontal axis), the apparent quantum yield (QY) by least-squares regression analysis and photosynthetic capacity of a leaf. Actual light utilization capacity (ALUC) is the area under the rate versus PFD curve, expressed as a percentage of the area beneath the initial slope, the horizontal drawn through the rate of O_2 evolution at 800 μmol quanta $m^{-2}s^{-1}$, the horizontal axis and the vertical at 800 μmol quanta $m^{-2}s^{-1}$ (Walker, 1990).

Soluble carbohydrates were determined from the acetonitrile extract of a leaf disc by the application of the HPLC method (HPLC, Optilab 5931, Sweden) (Sakač et al., 1992).

Samples (30 cm^2) for gravimetric determination of relative water content (RWC) were excised from the leaves before photosynthesis measurements.

The data presented are the means of 5 separate experiments. Experimental results were analysed by analysis of variance and the LSD (P=0.05; P=0.01) test was used to compare different drought treatments.

RESULTS

Similar leaves of four NS sunflower hybrids, that were kept with petioles in 0; 0.05; 0.1; 0.2; 0.5 and 1.0 M sorbitol for 24 hours, were sampled for relative water content determination. Increased concentrations of sorbitol, up to 0.2 M, induced rapid decrease of relative water content in the leaves of the four sunflower hybrids, while further increases in sorbitol concentration decreased relative water content of leaves for a few

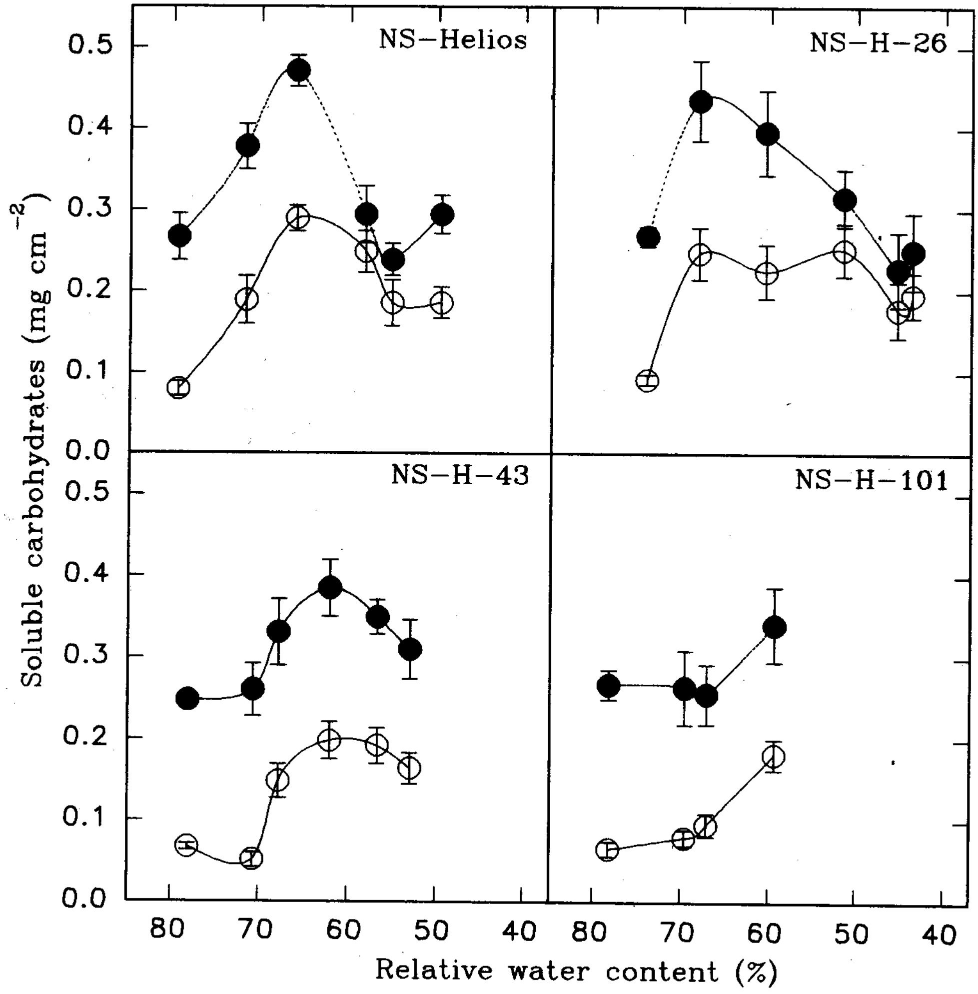

Figure 5. Changes in the contents of soluble carbohydrates (●) and sucrose (○) in response to acute water stress in detached leaves of 4 NS sunflower hybrids. Standard errors are shown for each mean (n=9).

Table 1. Effects of water deficit on the parameters of photosynthetic light response curve.

Genotype	Sorbitol M	RWC %	ALUC %	Respiration μmol O_2 $m^{-2}s^{-1}$	Light Compensation Point μmol quanta $m^{-2}s^{-1}$
NS-H-26-RM	0.00	75.25	88.48	-3.31	50.79
	0.10	62.51	78.84	-3.08	113.60
	0.20	53.33	46.82	-2.03	199.62
$LSD_{0.01}$		7.05	16.34	1.56	45.95
$LSD_{0.05}$		5.27	10.78	1.03	31.58
NS-Helios	0.00	79.37	81.98	-3.34	46.99
	0.10	66.02	74.46	-3.18	111.61
	0.20	58.16	66.06	-2.40	213.45
$LSD_{0.01}$		10.64	25.69	0.64	155.80
$LSD_{0.05}$		7.90	16.96	0.42	107.10
NS-H-43	0.00	78.10	83.82	-4.32	58.94
	0.10	67.77	77.98	-3.26	82.01
	0.20	62.04	71.19	-2.72	112.98
$LSD_{0.01}$		11.15	9.82	1.07	61.97
$LSD_{0.05}$		8.28	6.48	0.70	42.59
NS-H-101	0.00	78.25	81.93	-4.00	54.19
	0.10	67.00	82.58	-3.29	69.70
	0.20	59.26	77.37	-2.83	117.78
$LSD_{0.01}$		9.32	11.75	1.03	92.10
$LSD_{0.05}$		6.92	7.75	0.68	63.22

percents only (Figure 1). Leaves of NS-H-26-RM were the most responsive to the acute water stress.

Studies in the O_2 electrode system showed that the rapidly applied water stress of sunflower leaves produced inhibition of CO_2 saturated photosynthesis in all NS hybrids. The effect of acute water stress on the light response curve for photosynthetic oxygen evolution at CO_2 saturation in NS-H-26-RM leaves is presented in Figure 2. The decline in CO_2 and light-saturated photosynthesis at decreased relative water content in leaves was accompanied by a substantial decline in the apparent quantum yield of O_2 evolution (Figures 2 and 3). The decline in the apparent quantum yield in NS-H-26-RM and NS-Helios leaves followed the same kinetics as the decline of maximum photosynthesis at relative water contents was lower than 70%. The apparent quantum yields of NS-H-43 and NS-H-101 leaves in sorbitol solution decreased less than the corresponding rates of light and CO_2 saturated photosynthesis and than the apparent quantum yields of NS-H-26-RM and NS-Helios leaves. Photosynthetic O_2 evolution could not be detected in most leaves held at 0.5 and 1.0 M sorbitol for 24 hours. Dark respiration rates were diminished, while light compensation points were increased in the leaves of all hybrids under the influence of drought stress (Table 1). Water deficit affected actual light utilization capacity of leaves by diminishing it. The inhibition was again more pronounced with NS-H-26-RM and NS-Helios than with NS-H-43 and NS-H-101 leaves.

Quantum yield of linear electron transport Φ_{II}, calculated from chlorophyll fluorescence measurements of control and water stressed *Helianthus* leaves, was less inhibited by water deficit than the corresponding quantum yield of photosynthesis (Figure 3).

External water deficit induced the largest decline in the RWC and examined photosynthetic processes in NS-H-26-RM leaves, while the changes were smallest in NS-H-101 leaves. However, each examined photosynthetic process showed the same dependence on the relative water content in the leaves of all four NS hybrids (Figure 4). After 24 hours in sorbitol solution, all leaves with 66 - 68% relative water content showed a 50% decline in CO_2 and light saturated photosynthesis. Quantum yield of noncyclic electron transport was 50% inhibited at 60% RWC, while photochemical efficiency of photosystem II, Fv/Fm, was 50% inhibited at 50 - 60% RWC in leaves (Figure 4).

The content of soluble carbohydrates increased as the relative water content in the leaves decreased from 80 to 60% (Figure 5). The effect was more pronounced in NS-Helios and NS-H-26-RM leaves , where the content of soluble carbohydrates was significantly increased already at 70% RWC. Contribution of sucrose to the content of soluble carbohydrates was also increased in the leaves with diminished relative water content.

DISCUSSION

Our results confirm that with *Helianthus* the primary effect of rapidly applied water stress on leaves is on maximum photosynthetic activity (Ben et al., 1987) and on the apparent quantum yield (Boyer, 1971). We observed significant depression of CO_2 and light-saturated photosynthetic oxygen evolution already at an external water deficit of -0.15 MPa. Quick et al. (1989) concluded, however, that there was no large inhibition of CO_2-saturated photosynthesis in spinach leaf discs up to an external water deficit of -1.2 MPa, achieved by floating leaf discs overnight in 0.5 M sorbitol solution.

Photosynthetic efficiency is usually defined in terms of quantum yield but, in environmental terms, it also concerns the convexity of the light response curve (Terashima and Saeke, 1985) and what has been referred to elsewhere as light utilisation capacity (Walker, 1990). In our experiments quantum yield was depressed to the same extent as the net photosynthesis at the external water deficit of -0.5 MPa, in difference to the results by Ben et al (1987), who obtained QY depression only by severe water stress subsequent to wilting of leaves. On the other hand, actual light utilisation capacity was reduced much less than the apparent quantum yield, indicating a significant light utilisation capacity of droughted leaves at higher light intensities.

Studies of chloroplasts isolated from water stressed *Helianthus* leaves (Keck and Boyer, 1974) indicated that electron transport and photophosphorylation were very sensitive to water stress. In contrast to this, recent data indicate that primary photochemical reactions and electron transport either in isolated protoplasts (Sharkey and Badger, 1982) or in leaves (Cornic et al., 1989) do not appear to be much affected by low water potential. Our experiments with sunflower leaves indicate that electron transport is less sensitive and that primary photochemical reactions are even tolerant to the external water deficit (Figure 4; Panković et al., 1994). These and other results confirm that the reduction in the efficiency of photosystem II photochemistry observed in plants growing under water stress at high irradiance levels, probably reflects a response that protects the PSII reaction centers from over-excitation rather than reflecting a damage to these centers (Björkman, 1987).

Kaiser (1987) suggested that photosynthetic metabolism is inhibited by a general effect of rising ion concentrations on many different enzymes as the volume of dehydrated cells decreases. There are data that show increased sucrose-phosphatesynthase activity and sucrose synthesis under the influence of short-term water stress (Quick et al., 1989). Our data indicate that the increased sucrose concentration is probably the result of decreased export from leaves kept in sorbitol solutions. Different sensitivities of sunflower genotypes to external water deficit (Figs. 1 and 3) could be explained by differences in anatomy and morphology of leaves and petioles. Most probably, there is a different uptake of sorbitol from the solution (Kaiser, 1982). Different concentrations of sorbitol in the intercellular space might prevent sucrose export to a different extent, which could be the cause of differences in the accumulation of sucrose in the examined hybrids. The accumulated sucrose is in part responsible for inhibition of photosynthesis induced by a rapid water stress. When compared on the basis of the same RWC, there are no differences among hybrids in the sensitivity of photochemical reactions and photosynthesis to water deficit. However, the higher sensitivity of NS-H-26-RM and NS-Helios leaves to the external water deficit induced by sorbitol, as compared with the leaves of NS-H-43, is in agreement with the lower water use efficiency of the same sunflower hybrids grown in the field (Plesničar et al., 1993).

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RESPUESTAS DE LA FOTOSÍNTESIS Y ACUMULACIÓN DE CARBOHIDRATOS EN HOJAS DE GIRASOL SOMETIDAS A ESTRESES HÍDRICOS CORTOS

RESUMEN

Los efectos en fotosíntesis y acumulación de carbohidratos en las hojas de reducciones, durante corto tiempo, del potencial de agua en cuatro NS-híbridos de girasol fueron estudiados. El estrés hídrico fue inducido experimentalmente en hojas con peciolos sumergidos durante 24 horas en soluciones de sorbitol 0, 0.05, 0.1, 0.2, 0.5 y 1.0 M. Al incrementarse las concentraciones de sorbitol por encima de 0.2 M indujeron un decrecimiento rápido de contenido relativo de agua (RWC) en las hojas de los híbridos de girasol, NS-H-26-RM, NS-HELIOS, NS-H-43 y NS-H-101. El estrés hídrico aplicado rápidamente redujo significativamente el CO_2 y fotosíntesis en las hojas a 0.05 M. El declive en fotosíntesis a niveles bajos de RWC en las hojas estuvo acompañado de un declive sustancial en el rendimiento. Las reacciones fotoquímicas primarias y el transporte de electrones pareció ser menos afectado por bajo RWC en las hojas. La acumulación de los carbohidratos solubles se incrementó bajo el efecto del estrés hídrico rápido. El incremento de contenido de sucrosa podría actuar como un mecanismo de retroalimentación sobre la inhibición de fotosíntesis. El déficit externo de agua indujo el declive mas largo en el RWC y procesos fotosintéticos examinados en las hojas NS-H-26-RM, mientras los cambios fueron menores en las hojas de NS-H-101.

RÉPONSE À COURT TERME DE LA PHOTOSYNTHÈSE ET DE L'ACCUMULATION DE SUCRES DANS LES FEUILLES DE TOURNESOL, CONSECUTIVE À UN STRESS HYDRIQUE

RÉSUMÉ

Les effets à court terme de la baisse du potentiel hydrique foliaire sur la photosynthèse et l'accumulation des sucres dans les feuilles de 4 hybrides de tournesol NS ont été étudiés. Le stress hydrique a été induit expérimentalement en maintenant les feuilles, avec leurs pétioles submergés durant 24 heures dans des solutions à 0, 0.05, 0.1, 0.2, 0.5 et 1.0 M de sorbitol. L'augmentation des concentrations en sorbitol à 0.2 M induit une décroissance rapide de la teneur en eau relative (RWC) des feuilles des hybrides de tournesol; NS-H-26-RM, NS-HELIOS, NS-H-43 et NS-H-101. Le stress, hydrique rapide appliqué a significativement réduit le CO_2 et la photosynthèse des feuilles en phase lumineuse, dès la concentration de 0.05 M de sorbitol. La baisse de la photosynthèse à faible RWC dans les feuilles est liée a une importante réduction du rendement quantique apparent. Les réactions photochimiques primaires et le transport des électrons apparaissent moins affectés par le faible RWC des feuilles. L'accumulation des sucres solubles s'accroît sous l'effet du stress hydrique rapide. L'augmentation de la teneur en saccharose pourrait agir selon un mécanisme de feed-back sur l'inhibition de la photosynthèse. Le déficit hydrique externe provoque la plus forte baisse du RWC et des processus photosynthétiques étudiés dans les feuilles de NS-H-26-RM, alors que les modifications ont été les plus faibles dans les feuilles de NS-H-101.