HELIA, **19**, Nr. 24, p.p. 17-28, (1996) UDC 631.67.034.3:633.854.78

LEAF AREA DEVELOPMENT OF FIELD-GROWN SUNFLOWER PLANTS (*Helianthus annuus* L.) IRRIGATED WITH SALINE WATER

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Received: March 07.1996.
Accepted: July 19.1996.

SUMMARY

Leaf area development and stomatal resistance of field-grown sunflower plants (hybrid Mimosa) were studied in response to irrigation with saline water. Salt treated plants were irrigated with 1% commercial sea-salt water solution. Irrigation started on the 30th day after sowing when plants had 10 primordia and 2 fully expanded basal leaves.

Midday leaf water potential decreased during the season in both salt-treated (I1) and control plants (I0), with lower values for I1. At anthesis, 65 days after sowing, the difference was 0.12 MPa and LAI of I0 was 3.31 whereas for I1 it was 20% lower. I1 plants showed a remarkable morphological adjustment to salt stress, by modulating leaf area through leaf expansion rate, whereas the role of leaf senescence was negligible. There was no physiological adjustment in terms of stomatal resistance.

In conclusion, field-grown salt-treated sunflower plants showed avoidance mechanisms which are similar to those observed elsewhere in water stress conditions.

Key words: Diffusive leaf resistance, leaf area development, leaf water potential, irrigation, salt stress, sunflower.

INTRODUCTION

The salinity of both soils and water used for agriculture in Mediterranean environments is becoming a serious problem affecting food production because of competing water uses in various sectors and climatic changes. In particular, a few areas of Southern Italy are forced to use very saline water for irrigation.

It is well-known that leaf area development is a major factor affecting crop yield (Passioura et al., 1993) and that both salt and water stress have a detrimental effect on plant growth (Shalhevet and Shao, 1986). Leaf area develop-

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ment has been found to be more affected by water stress compared with leaf photosynthesis (Rawson and Constable, 1980; Curtis and Lauchli, 1986). Leaf expansion of field-grown sunflower plants subjected to water deficit was found to be very sensitive to the lowering of leaf water potential and it stopped at -1.8 MPa (Rawson et al., 1980).

Plants subjected to water stress have to deal with a reduction of water flux that roots supply to leaves, and in order to avoid leaf dehydration (avoidance mechanisms) transpiration loss can be reduced and/or water uptake increased (Jones et al., 1981). For these purposes, plants can rely on physiological and morphological responses, which are namely a reduction in leaf diffusive conductance and a modulation of development of both leaf area and the root system. Leaf area can be modified by acting on leaf number, leaf expansion rate and leaf senescence (Connor et al., 1985).

According to a physiological model (Munns and Termaat, 1986; Munns, 1993) the growth response to salinity can be divided into two phases. Initially, salts act outside the root system by lowering soil water potential and if salt is able to reach the leaves *via* the xylematic stream, the same is compartmentalized in the leaf cell vacuoles. In this phase, the effect of salinity is not salt-specific (water stress-like effect). Afterwards, the vacuoles became "full" and the leaf cells rapidly die due to a very intensive salt toxic effect. At the whole-plant level, this hastens leaf senescence.

As there are very few physiological studies on field-grown sunflower plants subjected to salt stress, the aim of this work was to study whether avoidance mechanisms similar to those observed elsewhere (Rawson et al., 1980; Connor et al., 1985) for water stress may also be observed in salt stress conditions.

MATERIALS AND METHODS

The experiment was carried out at the C.N.R.-ISPAIM experimental station in Vitulazio (CE), Southern Italy, situated 25 km north of Naples (41° 07' N, 14° 12' E, 250 m above sea level). The soil is an alluvial montmorillonite clay loam defined as Mollic Haplaquept according to the USDA soil classification. The other main characteristics of the experimental field soil are reported in Table 1.

Sunflower (hybrid Mimosa) was mechanically sown on May 30th 1995, in 0.75 m row spacing in 2 nearby 24 x 15 m plots and thinned at 21 DAS (days after sowing) to obtain 60,000 plants per hectare. 200 kg N Ha^{-1} and 500 Kg P_2O_5 ha^{-1} were applied to the soil prior to sowing.

During the season, one plot (I0 treatment) was irrigated with normal water (EC_w 0.9 dS m^{-1}), whereas the other plot (I1 treatment) was irrigated with a saline solution (EC_w 15.4 dS m^{-1}), obtained by adding 1% commercial sea-salt to irrigation water. Irrigation started at 30 DAS, when the plants had 10 primordia and two fully expanded basal leaves, adopting a furrow infiltration system, and

Table 1: Main physical and chemical characteristics of 0-0.6 m layer of experimental field soil. Total $CaCO_3$, organic matter and total N were determined by Dietrich-Fruhling, Kjeldhal and Walkley and Black methods, respectively

PHYSICAL CHARACTERISTICS	
Texture	Clay loam
Field capacity	38%
Wilting point	25%
Bulk density	1270 Kg m^{-3}
CHEMICAL CHARACTERISTICS	
pH in H_2O (1:2.5)	7.6
Total $CaCO_3$	1.3%
Organic matter	1.4%
Total N	0.7%

continued with irrigation once every 14 days until DAS 86, three weeks after anthesis. On the basis of soil moisture determined by the gravimetric method and a neutron probe at four soil depths, 0-0.12, 0.12-0.37, 0.37-0.62 and 0.62-0.87 m, watering volumes were calculated in order to re-establish soil water content at field capacity. Irrigation volumes were 219, 512, 712, 376 and 384 m^3 ha^{-1}, from the first to last watering, respectively.

Three weeks after sowing, in the middle of each plot, five plants were chosen to be monitored during the experiment. Measurements started when 6 primordia appeared on the plants. Using a ruler, non-destructive linear measurement of lamina length (Le) and maximal width (Wi) of all individual leaves were carried out almost every three days until DAS 70, when all leaves reached maximal expansion. Leaf senescence was also monitored by visual inspection. On each occasion, up to 540 leaves were measured.

In order to estimate leaf area from linear measurements, on DAS 21, 25, 31 and 63, nine plants per treatment were cut and Le, Wi and lamina leaf area (A) were destructively measured by a Digital Image Analysis System (Delta-T Devices LTD, Cambridge UK, model Dias II). Development stage, leaf position and salt treatment did not affect the relationship between Le, Wi and A (data not shown). Therefore, a single equation was determined by linear regression to estimate leaf area from linear measurements for both treatments ($A = 1.167^{**} + 0.621^{**}$ Le Wi, $R^2 = 0.996$).

During the growing season, on three plants per plot, midday leaf water potential was measured on an expanded leaf in the upper part of the plant, using a Scholander pressure chamber (Soil Moisture Equip. Corp., Santa Barbara, USA, model 3000). Before cutting, leaves were enclosed in aluminum bags and the reading carried out in less than 2 minutes. On the same days, midday leaf resistance to water vapor flux was measured by a diffusion porometer (Delta-T Devices, Cambridge, UK, model Mk3). Measurements carried out on two plants per plot were taken on both abaxial and adaxial surface of two expanded upper leaves per plant. Average total leaf resistance was calculated considering the two surfaces acting in parallel (Jones, 1992). Independent two-sample t-test was used to compare the treatments.

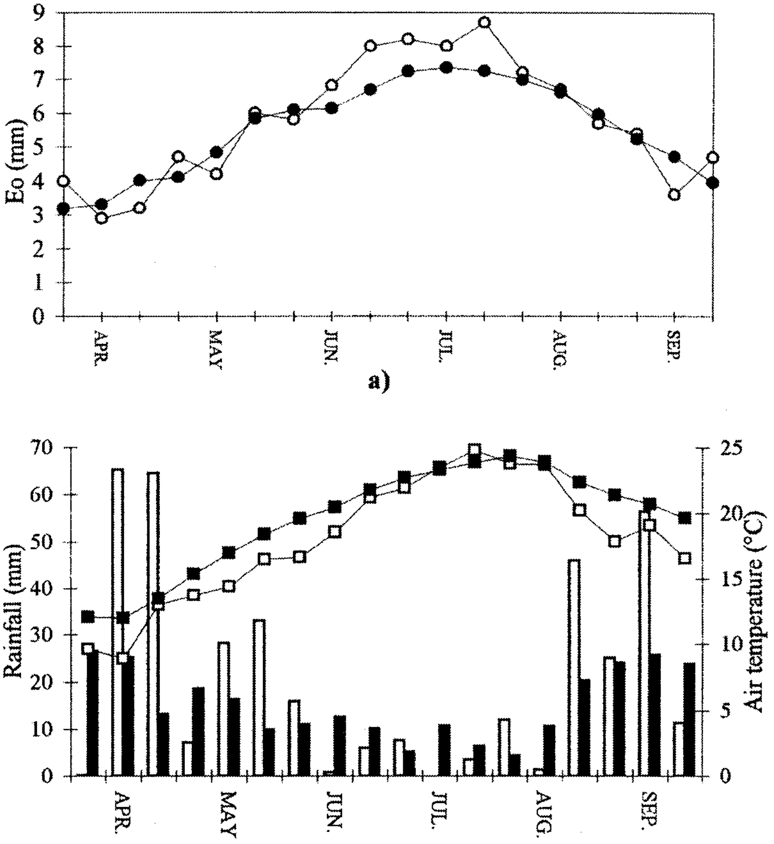

Figure 1. Decadal values for 1995, open symbols, compared with 30-year mean, closed symbols, for (top) class "A" pan evaporation (circles) and (bottom) rainfall (bars) and air temperature (squares) at Vitulazio (CE) meteorological station

RESULTS AND DISCUSSION

Data of climatic conditions from April to September 1995 were obtained from a station located 100 m from the experimental site. The decadal time course of rainfall, air temperature and class "A" pan evaporation compared with 30-year mean values are reported in Figure 1. Rainfall at spring (214 mm) was nearly twice the 30-year mean. As a result, soil water content at sowing was proximal to field capacity (data not shown). From the second decade of June to 20 August, rainfall (31 mm) was half the 30-year mean value. During the summer, the air temperature was quite similar to the 30-year mean, while it was generally lower during the other months. Evaporation was appreciably higher than the mean value from end of June to middle of August.

Table 2: Results of t-test comparison between I0 and I1 treatments for *(a)* plant leaf area, leaf water potential and stomatal resistance during growing season (comparisons before DAS 42 were all statistically non-significant, data not shown) and for *(b)* Amax, growth rate and leaf growing time for leaf positions from 11 to 26. Symbols "*", "**" and "***" indicate statistically significant differences at p values less than 0.05, 0.01 and 0.001, respectively; n.s. indicates non-significant difference

a)

DAS	Plant leaf area	Leaf water potential	Stomatal resistance
42	n.s.	n.s.	n.s.
56	n.s.	-	n.s.
63	*	-	-
66	*	n.s.	**
73	-	*	*
95	-	*	n.s.

b)

Leaf position	Amax	Growth rate	Leaf growing time
11	n.s.	n.s.	*
14	n.s.	n.s.	n.s.
17	n.s.	n.s.	n.s.
20	*	**	n.s.
23	**	*	n.s.
26	***	**	n.s.

Figure 2 shows the time course of plant leaf area of the two treatments. Mean values were practically similar until the date of the second irrigation (DAS 43). Subsequently, I1 plants showed a slowing of leaf area development compared with I0. Mean values were significantly different only from DAS 63 (Table 2). The maximum value for I1 (4444 cm^2 $plant^{-1}$) was reached approximately 9 days earlier than I0 and it was 20% lower. I0 plants reached the plateau value at DAS 63 (subsequent plateau values for I0 are not shown), corresponding to a LAI of 3.31, when both treatments started the anthesis phase (R5 stage, according to Schneiter and Miller, 1981).

Figure 3 reports DAS of leaf appearance and DAS of leaf area at full expansion (Amax), plotted in relation to leaf position. Primordia were considered as

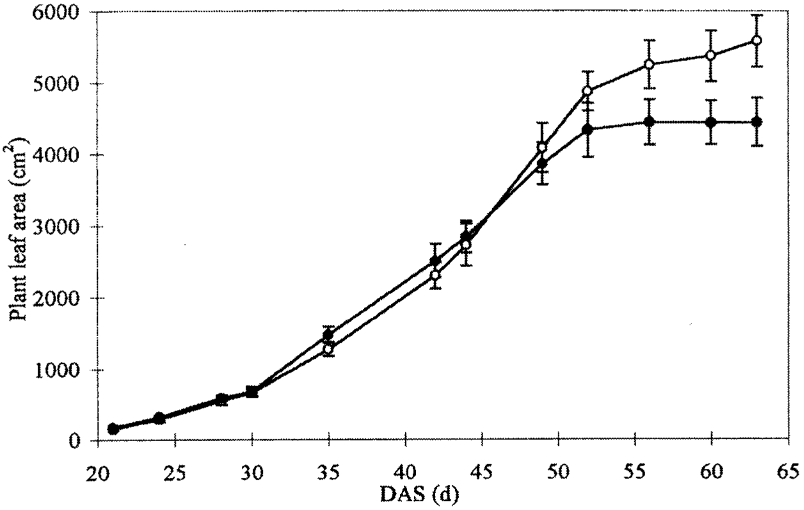

Figure 2. Time course of plant leaf area for control (open circles) and salt treatment (closed circles) from DAS (day after sowing) 21 to 63. Bars indicate s.e. (n=5)

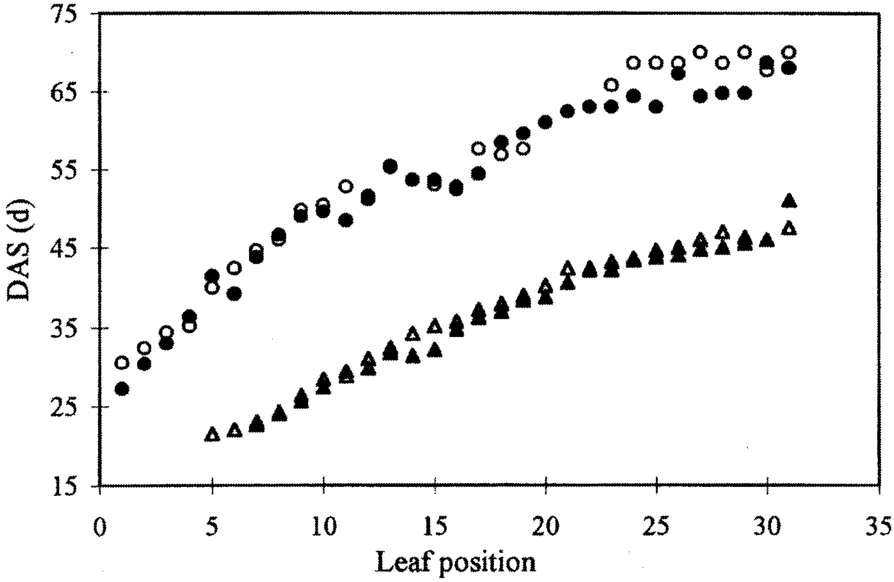

Figure 3. Day after sowing (DAS) at leaf appearance (triangles) and at full leaf expansion (circles) for control (open symbols) and salt treated plants (closed symbols), in relation to leaf position

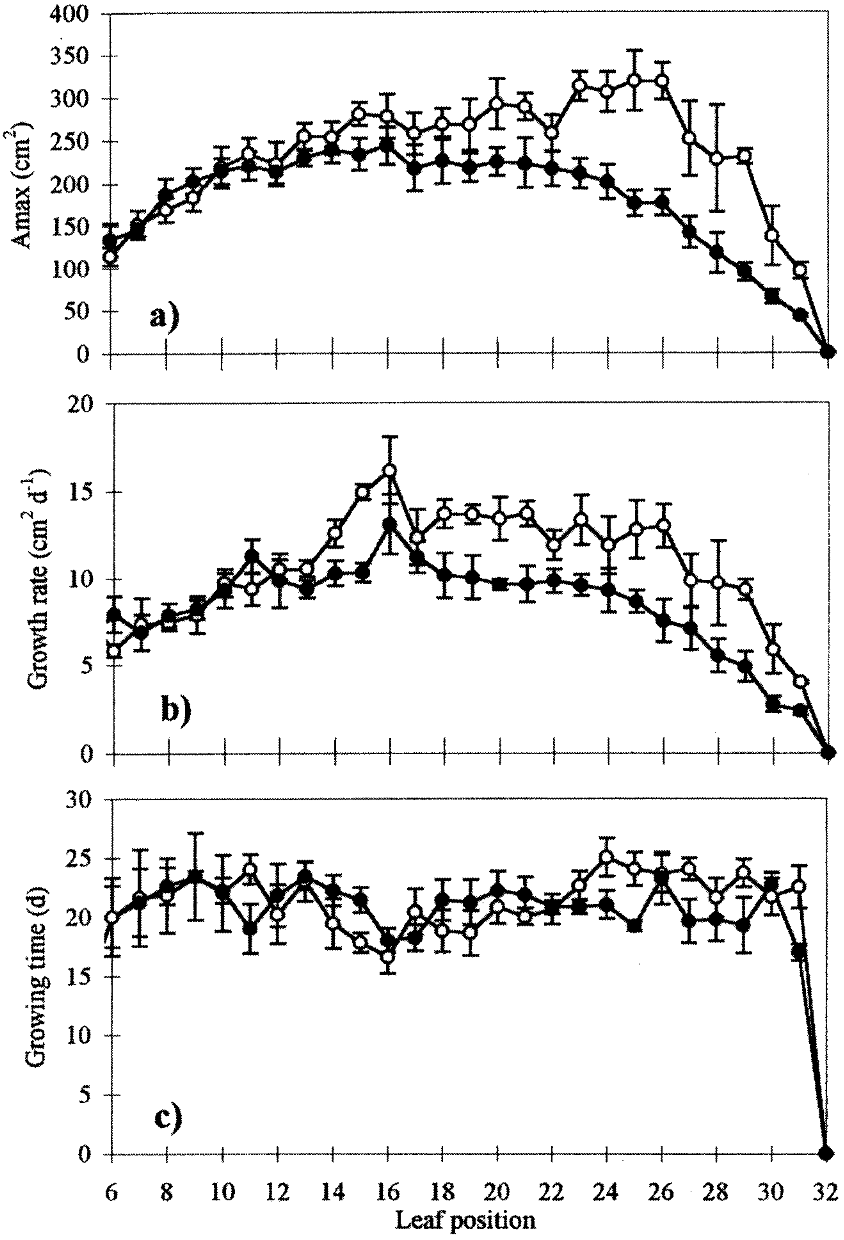

Figure 4. Leaf area at full expansion, Amax (a), average growth rate (b) and growing time from leaf appearance to full expansion (c) in relation to leaf position for control (open symbols) and salt treated plants (closed symbols). Bars indicate s.e. (n=5)

appeared leaves when the product Le x Wi had reached approximately 5 cm^2. From these data it emerges that final leaf number per plant, and the time course of both leaf appearance and Amax were not affected by salt treatment. The first saline irrigation was applied at 30 DAS when 12 leaves had appeared (40% of final leaf number) with only the first 2 fully expanded. At the 2nd irrigation (DAS 43), 23 leaves had appeared, with 7 basal ones fully expanded. All leaves had appeared when the 3rd irrigation was applied. At that time (DAS 57), the first 18 leaves were fully expanded whereas the above 12 were still growing. In conclusion, except for the two basal ones, the growth of all leaves was potentially influenced by saline irrigation.

Amax in relation to leaf position is reported in Figure 4a. Treatments appear to be different starting from leaf 15 with a difference of 47 cm^2 . Starting from leaf 20 differences are statistically significant (Table 2), with maximum difference being observed for leaf 25 (143 cm^2).

The two components of Amax, the time of growth from leaf appearance to full expansion and the corresponding average growth rate in relation to leaf position, are reported in Figures 4b and 4c. Growing time was not affected by salt treatment, whereas the major factor affecting Amax was the average expansion rate. Also for this parameter, differences were statistically significant starting from leaf 20 (see Table 2). Although average growth parameters of leaves in positions 8 to 19 were not statistically affected by salt treatment (see Table 2), their role in the control of plant leaf area was not negligible. In fact, at 2nd irrigation (DAS 43), these leaves had not completely expanded (see Figure 3). For I1 plants, the ratio of leaf area to Amax was 98%, 57% and 19% for leaf position 8, 15 and 19,

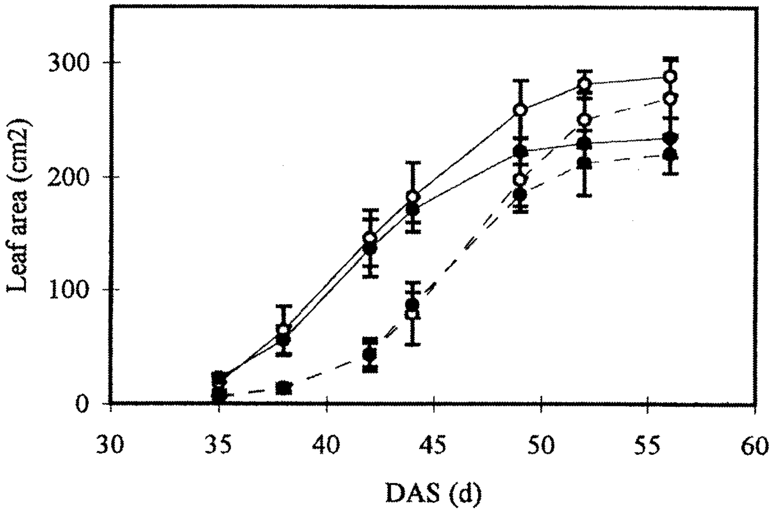

Figure 5. Time course of leaf area for leaf position 15 (solid line) and 19 (dashed line) of control (open symbols) and salt treated plants (closed symbols). Bars indicate s.e.

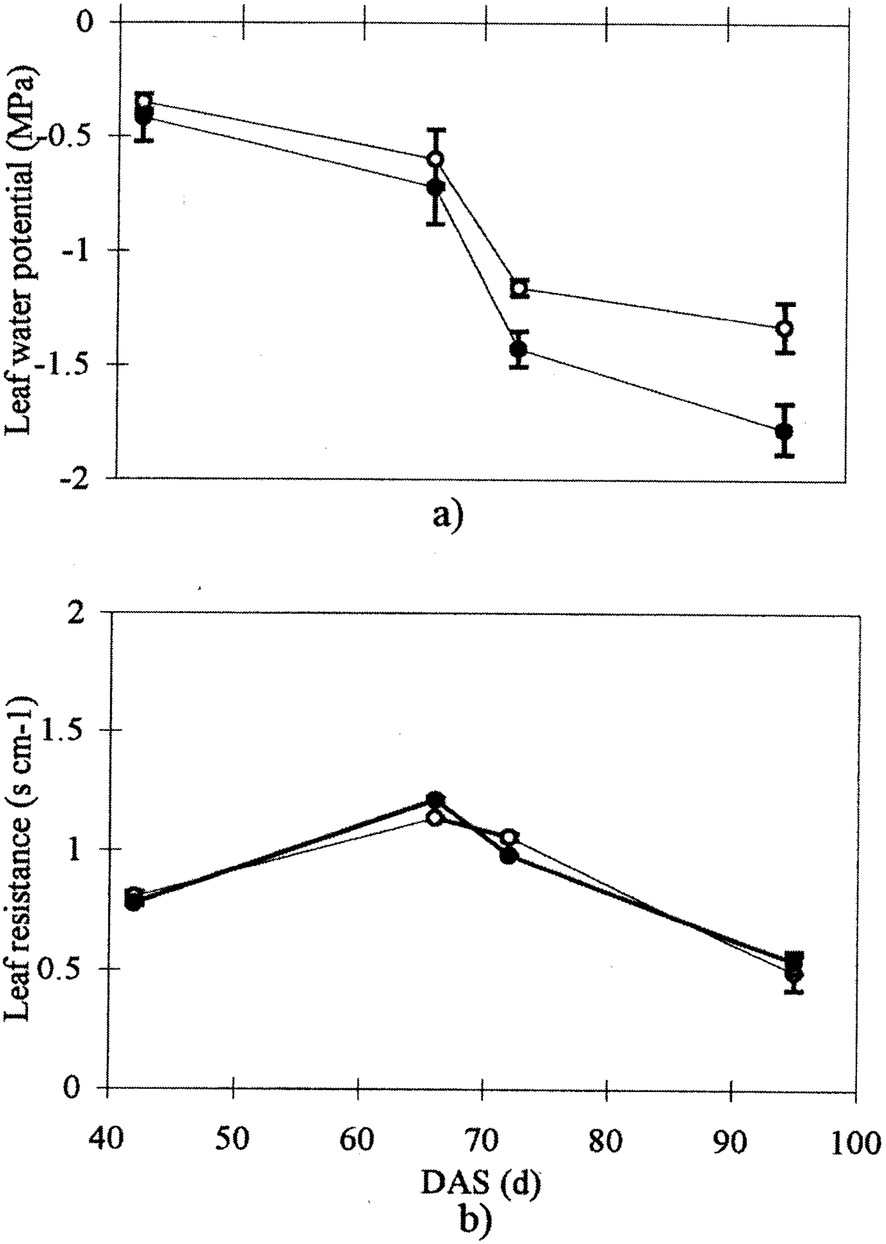

Figure 6. Time course along season of midday leaf water potential (a) and leaf resistance (b) for control (open symbols) and salt treated plants (closed symbols). Bars indicate s.e. (n=3 for leaf water potential and n=4 for leaf resistance)

respectively. Figure 5 reports the time course of leaf area for both treatments for leaf positions 15 and 19. It clearly shows that the development of leaf area for both positions was greatly affected by the 2nd saline irrigation (DAS 43). As a consequence, leaves 8 to 19 contributed to the total reduction of plant leaf area by ca. 17%, compared with 83% for leaves 20 to uppermost (data not shown).

The effect of saline irrigation on leaf senescence was negligible and statistically non-significant. At anthesis, a week after the 3th irrigation (DAS 63), I1 plants had an average of 8.6 dead or senescing leaves, compared with 3.4 of I0 (data not shown). The area of such leaves would have represented only a small fraction of plant leaf area at anthesis (3% for I1 and 1.8% for I0). Therefore, up to the anthesis phase, leaf senescence did not contribute to modulated plant leaf area development.

Midday leaf water potential (see Figure 6a) decreased during the season in both treatments, with lower values for I1. The difference between the treatments was 0.07 MPa at DAS 42, 0.12 MPa at DAS 66 and 0.27 MPa at the end of anthesis (DAS 73). Although only the latter value was statistically significant (see Table 2) there was a generally high sensitivity of leaf expansion rate to leaf water potential. At anthesis, a difference of 1.2 MPa produced a considerable reduction for I1 plants in the area of leaves still growing (from 23 to uppermost leaf position, see Figure 3). The relative Amax (Amax (I1) / Amax (I0) x 100) decreased in relation to leaf position, dropping to only 40% for uppermost leaf (data not shown).

For midday leaf stomatal resistance, there was a statistical significant difference at anthesis (see Table 2). However, this can be considered physiologically immaterial. In fact, during the season (Figure 6b) values ranged between 1.3 and 0.6 s cm-1, showing that stomata were essentially open (Connor and Jones, 1985).

CONCLUSIONS

As reported above, leaf area development of sunflower is known to be very sensitive to a moderate reduction in leaf water potential. Rawson and Munns (1984), from short-term measurement of relative leaf expansion rate on sunflower plants grown in solution culture (100 mol NaCl m-3), estimated an 85% reduction in plant leaf area at anthesis. This estimate is considerably higher than the reduction observed in our experiment. It must be noted that only at DAS 56, when nearly all leaf area had already formed, volumetric soil moisture (0-0.6 m) felt down to 0.28 m3 m-3, corresponding to 74% of field capacity (data not shown). This may have mitigated the effect of saline irrigation on soil osmotic potential.

The response of leaf area development to leaf water potential from our data is more comparable to what was observed by Rawson et al. (1980) on 5 field-grown sunflower cultivars subjected to water stress. Compared with our data, they found, approxymately, an average twice reduction in leaf area at anthesis with a double stress rate.

In our experiment, no relationship was found between midday leaf water potential and stomatal resistance (data not shown, cf. Figures 6a and 6b) as observed elsewhere for water stress studies (e.g., Rawson, 1979, Connor and Jones, 1985). Leaf transpiration and VPD in both irrigated and rainfed field-grown sunflower plants in the experiment of Rawson and Constable (1980) had a linear relationship. This also means that in this species, stomatal conductance is quite a conservative parameter. Our experiment confirmed that stomata remain open when leaf water potential is higher than -1.8 MPa.

We deduce that for sunflower plants grown in the field under salt stress, the control of plant transpiration is strongly dominated by leaf area, through the modulation of growth rate, and not by stomatal closure. As observed elsewhere in water stress conditions, this implies the existence of mechanisms to avoid leaf dehydration which rely essentially on morphological adjustment allowing leaves to maintain high diffusive conductance.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Agata Rascio (Istituto Sperimentale per la Cerealicoltura in Foggia, Italy) for critical reading of the manuscript and to the technical staff of C.N.R.-ISPAIM experimental station in Vitulazio (CE) for their kind assistance.

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DESARROLLO DEL ÁREA FOLIAR EN PLANTAS DE GIRASOL CULTIVADAS EN CAMPO (*Helianthus Annuus* L., cv. Mimosa) REGADO CON AGUA SALINA

RESUMEN

El desarrollo del área foliar y resistencia estomática en plantas de girasol (cv Mimosa) cultivadas en campo fueron estudiadas en respuesta al riego con agua salina. Las plantas tratadas con sal fueron regadas con 1% de una solución acuosa con 1% de sal de mar comercial. El riego comenzó el día 30 después de la siembra cuando las plantas tenían 12 hojas con solo las dos primeras hojas expandidas.

El potencial de agua en las hojas a medio día decreció durante la estación en ambas plantas tratadas (I1) y controles (I0), con valores más bajos para I1. En anterior, 65 días después de la siembra la diferencia fue 0.12 Mpa y el IAF de I0 fue 3.31 mientras que para I1 fue 20% más bajo. Las plantas I1 mostraron un notable ajuste morfológico al estrés salino, modulando el área foliar mediante la tasa de expansión de hoja, mientras que el papel de la senescencia de la hoja fue despreciable. No hubo ajuste fisiológico en término resistencia estomática.

En conclusión las plantas de girasol cultivadas en campo tratadas con sal mostraron mecanismo de evitación que son los observados para cualquier condición de estrés hídrico.

DÉVELOPPEMENT FOLIAIRE DE PLANTES DE TOURNESOL DE PLEIN CHAMP (*Helianthus annuus* L. cv. Mimosa) IRGUÉES AVEC DE L'EAU SALÉE

RÉSUMÉ

Le développement de la surface foliaire et la résistance stomatique de plantes de tournesol cultivées au champ (cv. mimosa) ont été étudiés en réponse à une irrigation avec de l'eau salée. Les plantes traitées au sel ont été irriguées avec une solution d'eau contenant 1% de sel de mer. L'irrigation a débuté le 30^0 jour aprés le semis sur des plantes au stade 12 feuilles, présentat seulement les deux premières feuilles totalement étalées.

Le potentiel hydrique de la feuille en milieu de journée a diminué durant la saison à la fois dans le traitement salin (I1) et chez les plantes témoins (IO), avec des valeurs plus faibles pour I1. A la floraison, 65 jours après le semis, la différence était de 0.12 Mpa et la LAI de IO était de 3.31, tandis qu'elle était réduite de 20% dans le traitement I1. Les plantes de I1 ont montré un ajustement morphologique remarquable au stress salin, en modulant la surface foliaire par la vitesse d'expansion foliaire, alors que le rôle de la sénescence foliaire était négligeable. Il n'y a pas eu d'ajustement physiologique en termes de résistance stomatique.

En conclusion, le traitement salin de tournesols cultivés en plein champ a montré des mécanismes d'évitement, similaires à ceux observés par ailleurs dans conditions de stress hydrique.