

Salt Tolerance in Crop Plants Monitored by Chlorophyll Fluorescence *In Vivo*

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ABSTRACT

The potential of measurements of chlorophyll fluorescence *in vivo* to detect cellular responses to salinity and degrees of salt stress in leaves was investigated for three crop plants. Sugar beet (*Beta vulgaris* L.) (salt tolerant), sunflower (*Helianthus annuus* L.) (moderately salt tolerant), and bean (*Phaseolus Vulgaris* L. cv Canadian Wonder) (salt intolerant) were grown in pots and watered with mineral nutrient solution containing 100 millimolar NaCl. The fast rise in variable chlorophyll fluorescence yield that is correlated with photoreduction of photosystem II acceptors increased in leaves of sugar beet plants treated with salt suggesting stimulation of photosystem II activity relative to photosystem I. In sunflower, this fast rise was depressed by approximately 25% and the subsequent slow rate of quenching of the chlorophyll fluorescence was stimulated. These differences were more marked in the older mature leaves indicating an increasing gradient of salt response down the plant. The salt effect *in vivo* was reversible since chloroplasts isolated from mature leaves of salt-treated and control sunflower plants gave similar photosystem II activities. Unlike in sugar beet and sunflower, leaves of salt-treated bean progressively lost chlorophyll. The rate of slow quenching of chlorophyll fluorescence decreased indicating development of a partial block after photosystem II and possible initial stimulation of photosystem II activity. With further loss of chlorophyll photosystem II activity declined. It was concluded that measurements of chlorophyll fluorescence *in vivo* can provide a rapid means of detecting salt stress in leaves, including instances where photosynthesis is reduced in the absence of visible symptoms. The possible application to screening for salt tolerance is discussed.

Increasing salinity in soil and water and its effect on crop plants has become a vast problem for agriculture in arid and semiarid regions that depend on irrigation. It has been estimated that agriculture in one-third of the land irrigated worldwide is already plagued by excess salinity (9). Further, problems of salinity are not confined to irrigated fields, but now extend to large areas of nonirrigated farmlands across the plains of North American and southern Australia. Although much effort is being expended in water control schemes and engineering projects to improve saline environments, as pointed out by Epstein *et al.* (9), these serve to minimize the problem of salinity but cannot eliminate it and the need to develop salt-tolerant crops at the same time has been emphasized (9, 21).

Visible symptoms, frequently leaf burns, are rather late manifestations of severe salt stress, and except in a few instances, *e.g.* citrus (19), the salt content of leaves or roots is not a reliable guide to salt tolerance. New methods are required to monitor salt stress in physiological studies, to detect adverse effects of salt on field-

grown crops that reduce yield without producing visible symptoms, and in the selection of salt-tolerant strains in plant breeding programs. Recently, we have developed rapid and nondestructive tests for chilling (22) and heat (23) tolerance based on stress-induced changes in the variable component of Chl fluorescence *in vivo*. The utility of Chl as an intrinsic membrane fluorescent probe to follow changes in membrane function in the intact leaf cells in response to salt stress and to indicate degrees of salt tolerance is investigated in this paper. Three plants are compared, sugar beet, which is salt tolerant; sunflower, which is moderately salt tolerant; and bean, which is sensitive (15).

MATERIALS AND METHODS

Sugar beet (*Beta vulgaris* L.), sunflower (*Helianthus annuus* L.) and bean (*Phaseolus vulgaris* L. cv Canadian Wonder), were grown in pots in a greenhouse (air temperatures controlled to 28°C maximum, 18°C minimum) using the same mixtures for soil medium, mineral nutrient solution, and procedures for salt treatment as described by Lessani and Marschner (15). After 10 d of growth, salt treatment was begun by adding NaCl gradually (1 g/L each day) to the nutrient solution up to a final concentration of 100 mM NaCl. Other bean plants were treated with either 50 or 25 mM NaCl. Leaves were harvested 19 to 23 d after treatment with full strength salt. Leaves were harvested at the same time from control plants which were watered with mineral nutrient solution throughout.

To measure leaf Chl fluorescence, a disc (2 cm diameter) was cut from the center of the lefthand side of a leaf and quickly placed abaxial surface up on moist filter paper and covered with a thin plastic film. Harvests were carried out between 11:00 AM and 1:00 PM; no diurnal changes in Chl fluorescence induction curves were discernable during this period. After dark adaptation for 1.5 h, Chl fluorescence induction curves were recorded at 23°C using a portable fluorometer, model SF-10 (Richard Brancker Research Ltd, Ottawa), connected to a DASAR Data Acquisition, Storage and Retrieval System (American Instrument Co., Silver Spring, MD) with readout to an X-Y recorder. The measuring sensor of the SF-10, which was placed on the leaf disc, contained both a light source to irradiate the leaf surface (red light, 15 $\mu\text{E m}^{-2} \text{s}^{-1}$) and a photodiode to detect fluoresced light.

Chloroplasts were isolated as described previously (24) and photochemical activities assayed at 23°C using an Aminco DW-2 spectrophotometer (American Instrument Co.) with saturating red-light side illumination. The assay mixture (1.5 ml) contained chloroplasts (4 $\mu\text{g Chl ml}^{-1}$), 50 mM Sørensen's phosphate buffer (pH 7.5), 50 mM NaCl, 0.05% (w/v) BSA, 0.34 mM potassium ferricyanide, and where indicated, 2 μM gramicidin D, 0.1 mM PD¹, and 1 μM DBMIB.

¹ Abbreviations: PD, *p*-phenylenediamine; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone.

RESULTS

Sugar Beet. Visible differences were not apparent between salt-treated and control plants. The Chl fluorescence induction curves for the sugar beet leaves and for the other two species used in this study were typical for leaves of higher plants (17). In the control sugar beet (Fig. 1), upon irradiating the dark-adapted leaf, the Chl fluorescence yield rose rapidly to the O level (the terminology adopted by Papageorgiou [17] is used throughout). This is Chl fluorescence of constant yield at physiological temperatures. The further rise above O is Chl fluorescence of 'variable' yield, determined by the redox state of PSII acceptors (17). Oxidized in the dark-adapted leaf, the acceptors are reduced in light and this is accompanied by an increase in Chl fluorescence yield. Above O, the Chl fluorescence rose to a plateau I (0.2-s measurement, Fig. 1), indicating that PSII was functional, before the main rise to P (5-s time scale). The I to P rise but not the O to I rise is abolished by specific inhibition on the photooxidizing side of PSII such as by chilling temperatures (in tropical plants) or by UV light (R. M. Smillie, unpublished results). After P, Chl fluorescence was slowly quenched and a second smaller peak of Chl fluorescence was seen before the terminal value was obtained at the steady-state (T). Reoxidation of reduced PSII acceptors by PSI-mediated reactions is probably the main contributing reason for the slow quenching (4) and specific inhibition of electron transfer after PSII, e.g. by DCMU, eliminates the slow decrease in fluorescence after P. This also has the effect of increasing the rate of the rise and magnitude of the I to P Chl fluorescence yield.

The sigmoidal rise in fluorescence after I and the second peak of fluorescence made it difficult to determine half rise and fall times for the I to P rise and quenching after P, respectively;

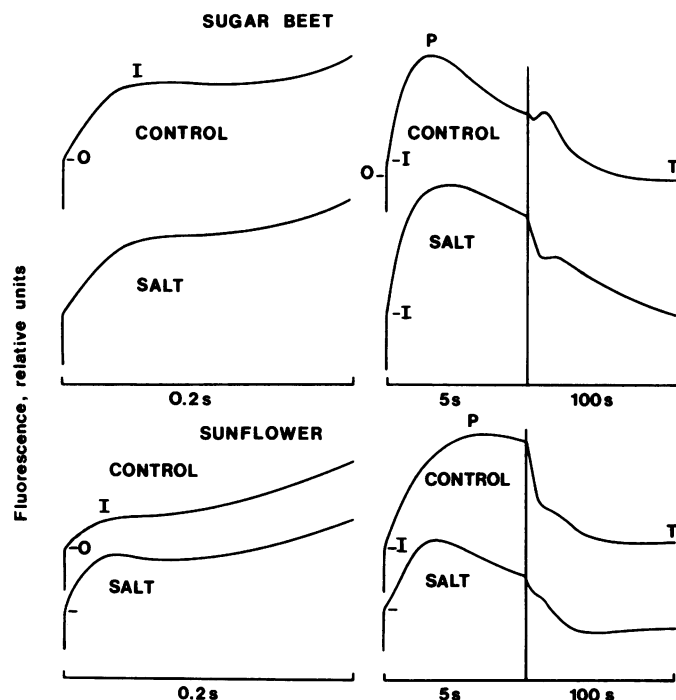


FIG. 1. Chl fluorescence induction curves of sugar beet and sunflower leaves from control plants and plants treated with 100 mM NaCl. Two separate measurements were made on each sample to record leaf fluorescence. Each dark-adapted leaf sample was first irradiated for 0.2 s to record the O-level fluorescence and fast rise to I-level. After 3 min for dark adaptation, a second irradiation was performed for 105 s. A split time sequence allowed recording of rise from I to fluorescence maximum P (1–2 s) and much slower decrease after P to terminal fluorescence T. Curves for the 0.2-s irradiation are expanded 5 times in vertical direction relative to 105-s irradiation.

Table I. Chl Fluorescence Parameters for Different Regions of Leaves from Control and Salt-Treated Sugar Beet

The table shows the magnitude of the fluorescence rise from O to the I level ($F_I - F_O$), the rise from I to P ($F_P - F_I$), and the maximal rates of the O to I rise, the I to P rise (r_1), and the decrease immediately after P (r_2). Relative fluorescence units are in millivolts and rates in volts per second. Each value is the mean obtained from samples from four different leaves.

Leaf	$F_I - F_O$	$F_P - F_I$	O-I rate	r_1	r_2
Control					
Tip	108	1236	4.23	1.66	0.22
Middle	125	1284	4.88	1.71	0.27
Base	109	1262	4.55	1.50	0.25
Mean	114	1261	4.55	1.62	0.25
Salt					
Tip	126	1557	4.44	1.80	0.18
Middle	151	1468	4.27	1.64	0.18
Base	145	1417	4.65	1.97	0.20
Mean	141	1481	4.45	1.80	0.19

instead, the maximal rates for the I to P increase (r_1) and the maximal rate of quenching immediately after P (r_2) were determined. In screening applications based on stress-induced changes in the I to P rise, the Chl fluorescence parameter, r_1 , is useful since it is unnecessary to prolong the measurement until P is reached, thus reducing the time taken to measure each sample.

Differences between the Chl fluorescence induction curves of leaves from treated and control plants were fairly small (Fig. 1). In leaves from salt-treated plants the variable fluorescence (O to I rise) tended to be larger, r_1 was slightly faster, and the subsequent rate of decline (quenching) of the fluorescent yield (r_2) was a little slower. This is better seen in the data given in Table I, in which a comparison is also given of readings taken near the tip, the middle, and basal regions of each leaf. No consistent differences were noted for Chl fluorescence parameters derived from readings taken at different regions of the leaf in either control or salt-treated plants.

Sunflower. The most obvious effect of salt on sunflower was decreased productivity, the above ground portion of the plant and foliage being reduced by 20% to 30% in size compared with control plants. Leaf color appeared normal and there was no visible evidence of premature senescence of the lower leaves.

Figure 1 (bottom two curves) shows Chl fluorescence induction curves of two mature leaves of sunflower, one from a control plant and one taken from the corresponding leaf position of a salt-treated plant. The effect of salt on the transient Chl fluorescence rise to P in sunflower was quite different in sugar beet. In sunflower, the magnitude and rate of rise of Chl fluorescence yield between O and I was higher in salt-treated than in control plants, whereas the magnitude and rate of rise between I and P was suppressed. The fluorescence decay r_2 was faster in the salt-stressed leaf. Significant differences were not found between Chl fluorescence induction curves measured on different regions of a leaf, but differences were noted between leaves depending on their point of insertion in the stem. To determine the extent to which the curves shown in Figure 1 were representative of the test of the foliage of each plant, Chl fluorescence was measured on all individual leaves of a salt-treated and a control plant.

Two experiments were carried out. Salt-treated and control plants were grown until just beginning to flower in a greenhouse in March and April 1980. Chl fluorescence measurements were then made down the stem on all of the alternate leaves over 5 cm in length and on one leaf from each of the next two sets of older paired leaves. Paired leaves lower on the plant had already senesced and died. The experiment was repeated using plants

grown during August and September 1980. The trends in Chl fluorescence parameters with leaf age were similar in both experiments and only the results for the second experiment are shown in Figure 2. Mean values of r_1 and parameters shown in Figure 2 for all nonsenescing leaves (see legend to Fig. 2) are given in Table II.

All leaves sampled from the salt-treated plant were smaller than corresponding leaves from the control plants (Fig. 2). The constant or F_0 -fluorescence yield was similar in control and salt-treated leaves, both showing a slight upward trend with increasing leaf age. However, the variation in F_0 -fluorescence from leaf to leaf was large and when compared with the total variable Chl fluorescence (O to P rise), no correlation between the two was found, some leaves with a high F_0 showing a below average variable Chl fluorescence and vice versa. For this reason, values of F_1-F_0 and F_P and F_1 are given directly instead of expressing them as a ratio of the F_0 value, as is frequently done in other studies of the variable Chl fluorescence.

Most Chl fluorescence parameters for the control plant showed slight changes only with leaf age (Fig. 2). The exception was r_2 which showed an upward trend with increasing leaf age (F_1-F_0 also increased in senescing leaves). It was not possible on the basis of the Chl fluorescence measurements to distinguish between expanding leaves (leaves 1 to 6) and mature leaves. However, consistent differences were evident in values describing variable Chl fluorescence between leaves from salt-treated and control plants. In salt-affected leaves, F_P-F_1 was smaller and r_2 larger, the difference between the salt-treated and control plants becoming more pronounced with increasing leaf age. The increase in F_1-F_0 seen in Figure 1 in the salt-stressed leaf is typical only of the older expanded leaves. F_1-F_0 was slightly lower in the expanding salt-stressed leaves and about the same in recently expanded leaves compared with leaves of the control plant.

The rate of quenching immediately after P (r_2) was faster in all of the salt-stressed leaves compared with the corresponding control leaves and the extent of the quenching at the steady-state was

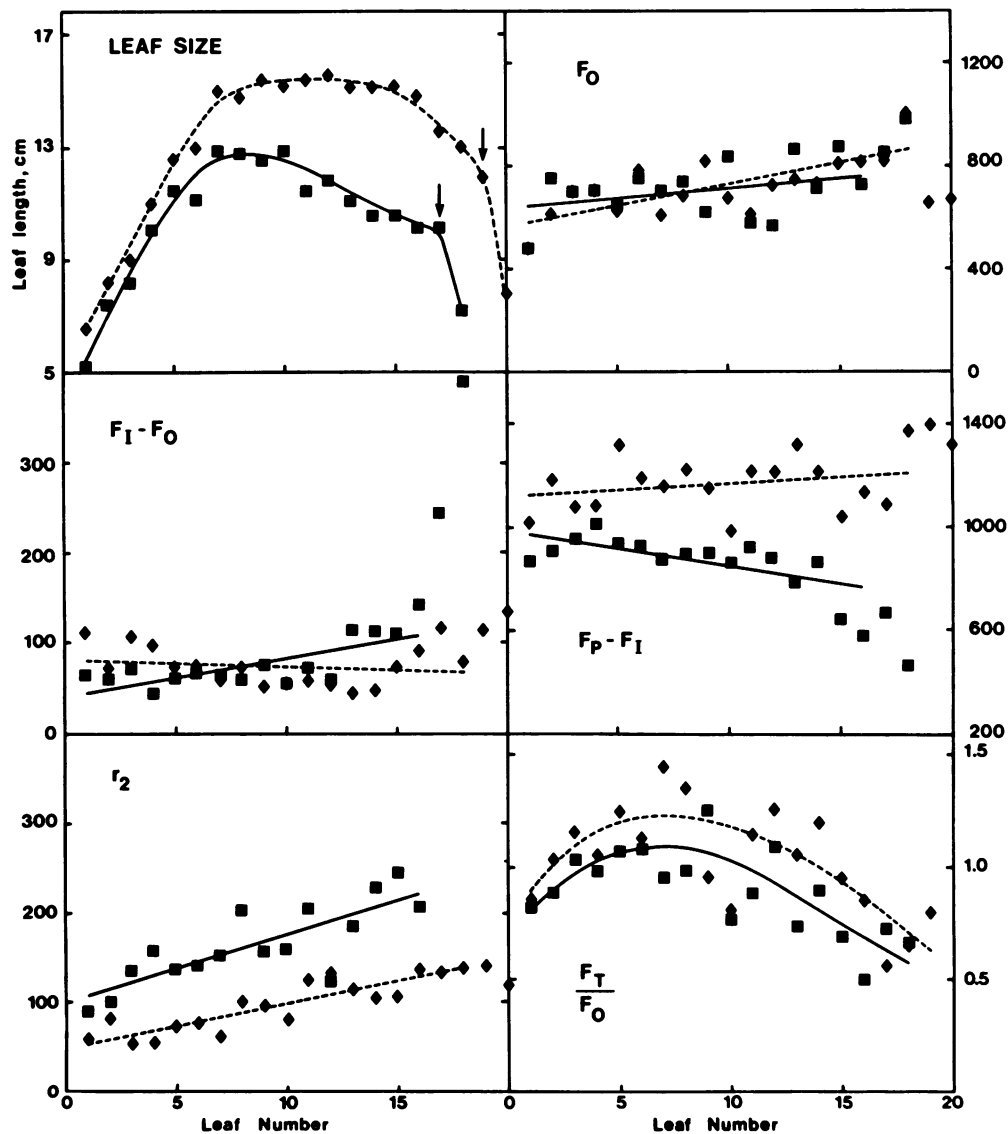


FIG. 2. Changes in leaf size and Chl fluorescence values of sunflower leaves of different ages. Dashed line, control plant; solid line, plant treated with 100 mM NaCl. Fluorescence was measured as described in Figure 1. First leaf down from tip over 5 cm in length was designated leaf number 1. All lower alternate leaves were sampled down to first paired leaves (marked by an arrow in top left figure, leaf 19 in control plant and leaf 17 in salt-treated plant), after which one of each leaf pair was sampled. Leaf pair 19 (control) and 17 (salt-treated) were highest leaves on plant to show signs of senescence (paler green than leaves above), whereas leaf pair 20 (control) and 18 (salt-treated) were senesced and yellow-green in color. Relative fluorescence values are in millivolts and maximal rate of fluorescence decrease after P (r_2) in volts per second. Regression analysis was used where straight lines were fitted, excluding data points for two oldest and visibly senescing leaves.

Table II. Mean Values of Fluorescence Parameters of Sunflower Leaves
Mean values are of the measurements on nonsenescent leaves of control and salt-treated plants shown in Figure 2.

Fluorescence Parameter	Treatment	Mean	±SE
$F_I - F_0$	Control	74.4	5.1
	Salt	76.6	6.7
$F_P - F_I$	Control	1170	25
	Salt	869	44
r_1	Control	817	36
	Salt	652	36
r_2	Control	96.3	7.2
	Salt	165	11
F_T/F_0	Control	1.04	0.05
	Salt	0.92	0.05

Table III. Activities of Chloroplasts Isolated from Leaves of Salt-Stressed and Control Sunflower Plants

Chloroplasts were isolated from mature leaves (four lowest alternate leaves) of three control and three salt-stressed plants. Experiment 1, plants grown in March and April 1980, experiment 2, plants grown in August and September 1980.

Additions	Rate of Ferricyanide Reduction	
	Control	Salt-stressed
	$\mu\text{mol h}^{-1} \text{mg}^{-1} \text{Chl}$	
Experiment 1:		
None	143	151
PD + DBMIB	199	196
Experiment 2:		
None	104	106
PD + DBMIB	154	142

slightly higher (decrease in the ratio of F_T to F_0 [Fig. 3], this ratio was used because the steady-state variable Chl fluorescence [$F_T - F_0$] was small relative to F_0).

Table II lists mean values for parameters of Chl fluorescence on all nonsenescent leaves. Mean values for $F_P - F_I$ and r_1 were decreased by 26% and 25%, respectively, in salt-stressed leaves, whereas r_2 showed a 71% increase. Mean values for $F_I - F_0$ were similar and F_T/F_0 was slightly higher in control plants.

To ascertain whether the differences observed in the Chl fluorescence of intact leaves of control and salt-treated plants extended to the isolated chloroplasts, PSII activities of chloroplasts isolated from salt-treated and control plants were measured. Mature leaves toward the base of the plant were chosen for this experiment as these showed the largest differences in fluorescence induction kinetics between salt and control treatments (Fig. 2). PSII activity (ferricyanide photoreduction in the presence of PD and DBMIB) in chloroplasts isolated from the salt-treated plants was almost as high as in chloroplasts from control plants (Table III).

Bean. The bean plants were severely affected by salt. Expansion of new leaves was partially suppressed as was previously observed by Nieman (16) and new and existing leaves progressively lost Chl and by harvest time leaf color ranged from a slightly paler green than leaves from control plants to yellow or yellow-brown, the oldest leaves being the most affected. Consequently, changes in Chl fluorescence induction kinetics have been related to leaf color. In leaves uniform in color, differences were not observed in the Chl fluorescence kinetics of different areas of the leaf.

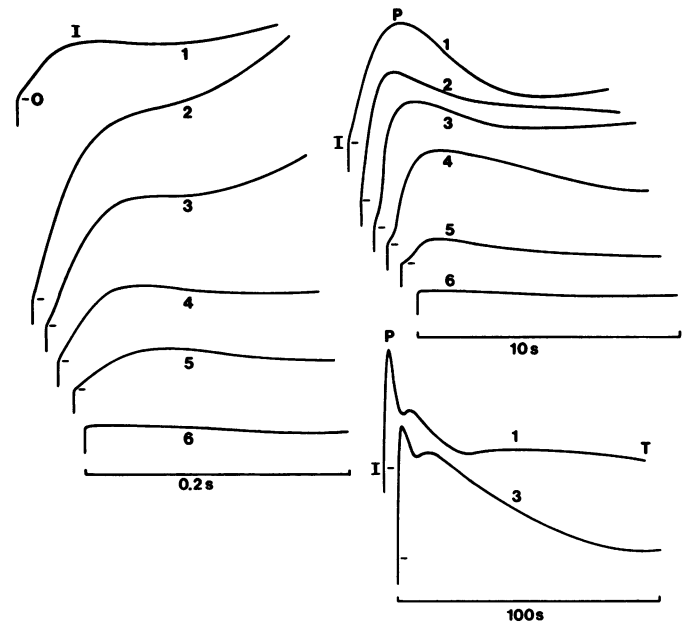


FIG. 3. Chl fluorescence induction curves of bean leaves of plants treated with 100 mM NaCl. Three sequential measurements were made on each sample to record leaf fluorescence. First irradiation was for 0.2 s to record O-level fluorescence and rise to I level. After 3 min for dark adaptation, sample was irradiated for 10 s to record rise from I to P. After 6 min for dark adaptation a final irradiation of 100 s was made to record slow quenching between P and T. Curves for 0.2-s irradiation are expanded 5 times in vertical direction relative to 10- and 100-s irradiations. 1, Green control leaf. Remaining leaves were from salt-treated plants and ranged in color from pale green to yellow (see text); 2, GGY; 3, GY; 4, GYY; 5, GYYY; 6, yellow.

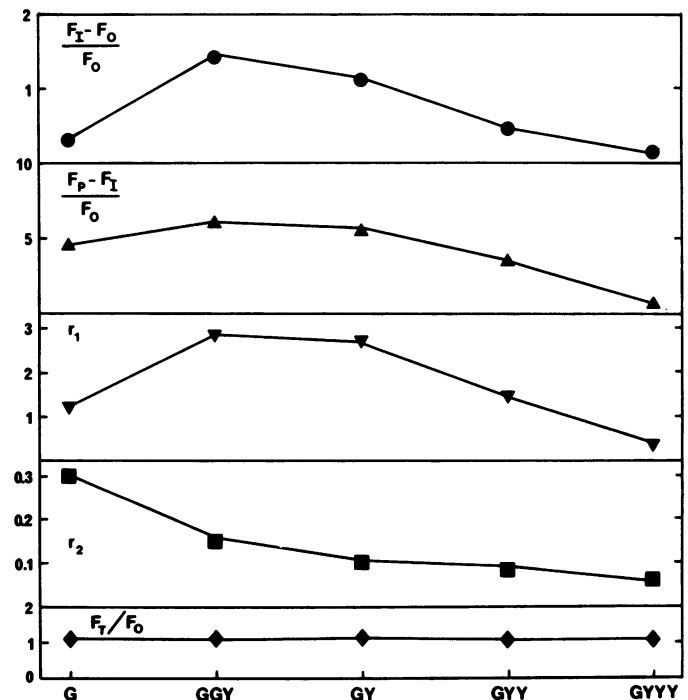


FIG. 4. Changes in parameters of relative Chl fluorescence in bean leaves from plants treated with 100 mM NaCl. Fluorescence values are in millivolts and rates in millivolts per second. G refers to green control leaves and GGY, GY, GYY, and GYYY to leaves at different stages of degreening from salt-treated plants (see text).

Table IV. Changes in Chl Fluorescence Values in Leaves of Salt-Treated Plants

Plant	F_1-F_0	F_P-F_1	I-P Rise (r_1)	Quenching after P (r_2)
			<i>rate</i>	
Sugar beet	Small increase	Small increase	Small increase	Small decrease
Sunflower	Slight change	Decrease	Decrease	Increase
Bean, moderately stressed	Increase	Increase	Increase	Decrease
Bean, severely stressed	Decrease	Decrease	Decrease	Decrease

Figure 3 shows Chl fluorescence induction curves for bean leaves variously affected by salt. Control green leaves are designated as G in the figure legend. Leaves from salt-treated plants ranged from slightly pale green (GGY), to pale green (GY), to yellow-green (GYY), to yellow with traces of green (GYYY), to yellow. A clear pattern of Chl fluorescence changes can be seen. The O to I Chl fluorescence rise at first increased (0.2 s irradiation), but as more Chl was degraded the yield decreased, eventually to zero. Similarly, there was an initial rise in the rate of the I to P Chl fluorescence rise (r_1) and the magnitude of the rise (F_P-F_1). Changes in Chl fluorescence parameters are shown in Figure 4 and since bean leaves, unlike salt-treated sugar beet and sunflower, showed substantial decreases in Chl concentration, the Chl fluorescence values have been expressed as a ratio of the F_0 value. There was an initial increase in $F_1-F_0:F_0$, $F_P-F_1:F_0$, and r_1 . These values then declined but only after most of the Chl had been lost indicating that PSII activity was resistant to the effects of salt.

In contrast, r_2 was much reduced even in the slightly pale green leaves and remained so in more severely salt-stressed leaves. This was consistent with the development of a partial block after PSII in salt-affected leaves. As shown in Figure 3 (100-s irradiations), the Chl fluorescence was still quenched to the same final extent as in a control leaf, but at a slower rate; in fact, this was the case for all stages of degreening as evidenced by the constancy of $F_1:F_0$ (Fig. 4).

Bean plants treated with 50 mM NaCl showed reduced visible symptoms and Chl fluorescence changes, but the same pattern of changes in fluorescence was seen over a more extended period. At 25 mM NaCl, visible symptoms were absent and there was a small increase in r_1 in the oldest leaves.

DISCUSSION

The three plants used in this study differ in salt tolerance. Sugar beet having halophytic ancestors (11) is salt tolerant and its growth can even be stimulated by NaCl (8). Sunflower exhibits moderate salt tolerance, whereas bean is sensitive (15). Lessani and Marschner (15), whose growth procedures and salt treatment were adopted for the work described in this paper, found that these three plants when watered with nutrient solution containing 100 mM NaCl gave dry weight yields of 106%, 67%, and 37% to 44%, respectively, compared with control plants. These yields were not correlated with salt contents of the leaves.

The measurements of Chl fluorescence emitted by the leaves of salt-treated and control plants provide information on the mechanisms of salt action *in vivo*. Relevant to the future development of salt-tolerant crops, Chl fluorescence also appears to offer a sensitive and rapid means of comparing the responses of different genotypes to salinity. These two aspects will be discussed in turn.

Mechanisms of Salt Action. Although the relative tolerances of sugar beet, sunflower, and bean to salinity can be assessed from the plant yields (15), Chl fluorescence measurements furnish the additional information that the photosynthetic metabolism of the three plants has been affected by salinity in quite different ways (Table IV). In sugar beet, the general increase in size of parameters describing the variable Chl fluorescence rise coupled with the slower quenching after P (Fig. 1; Table I) point to an increased rate of reduction of PSII acceptors relative to their reoxidation by

PSII-mediated reactions. This could result from a partial block in electron transfer after PSII, but stimulation of PSII activity could be a contributing cause. In isolated chloroplasts, the photoreduction of ferricyanide is stimulated progressively with increasing concentrations of NaCl up to 30 to 50 mM (2, 24), an effect possibly related to a cation-dependent alteration in membrane ultrastructure that changes the distribution of absorbed light energy in favor of PSII at the expense of PSI (3). Since PSII is the rate-limiting photosystem at nonsaturating light intensities, it is interesting to speculate whether the enhanced growth of sugar beet in the presence of salt is related to an increase in the production of photosynthate.

In contrast to sugar beet, the rate of the I to P rise in Chl fluorescence (r_1) and magnitude of this rise (F_P-F_1) were depressed in leaves of salt-stressed sunflower (Fig. 2; Table II). Similar changes have been observed in algae exposed to saline media (6, 14). A possible explanation for the salt effect in sunflower may be found in studies of the responses of isolated chloroplasts to high salinity (2). Concentrations of NaCl above 100 mM inhibited photoreduction of ferricyanide uncoupled from photophosphorylation in isolated spinach thylakoids. Also, the rise time for the Chl fluorescence induction curve in the presence of DCMU was increased indicating a decrease in the rate at which the primary acceptor for PSII was reduced, although the primary photochemical capacity of PSII was unchanged. However, the effective size of the secondary acceptor (plastoquinone) pool was diminished and it was thought that the decreased capacity for reduction of secondary acceptors could account for the depressed rate of the primary photochemistry of PSII and consequently the decrease in the rate of electron flow to ferricyanide. The Chl fluorescence induction curves given by salt-stressed sunflower leaves are consistent with a similar action of salt *in vivo* on the sunflower chloroplasts. There were only marginal changes in the O to I rise but a definite decrease in the I to P rise. The affect of salinity *in vivo* on PSII was reversible since chloroplast thylakoids isolated from salt-stressed and control sunflower leaves showed similar PSII activities (Table III).

It was noteworthy that the rate of quenching of Chl fluorescence after P (r_2) was markedly increased in the salt-stressed sunflower leaves and at the steady-state value, quenching was slightly greater than in the control leaves (Fig. 2; Table II). These changes are to be expected with decreased PSII activity but they also indicate the absence of significant inhibition by salt on reactions of photosynthesis after PSII, otherwise quenching ought to have decreased (4). This suggests that the action of salt *in vivo* on the chloroplast system of sunflower is mainly confined to PSII reactions. Similar explanations may apply to the reported decrease in photosynthesis in salt-stressed grape leaves, one not attributable to increased stomatal resistance (7).

One possible effect of high salinity is the formation of a water deficit and consequent depression of growth (11). The faster rate of quenching after P (Fig. 2) suggests that this is not an important factor in the salt-stressed sunflower leaves since water stress inhibits the quenching after P (see below).

In bean, the older leaves were affected first by salinity. A distinct salt gradient is known to occur in beans grown in saline conditions, the older leaves having higher Cl^- concentrations than

younger leaves (10). Older leaves lost Chl and with loss of Chl the variable Chl fluorescence decreased (Fig. 4; Table IV). The younger leaves showed the opposite effect for Chl fluorescence with increases in the magnitude and rate of both the O to I and I to P rises (Figs. 3 and 4; Table IV). However, the rate of quenching after P in these leaves was still decreased. These changes indicate development of a block after PSII and possible stimulation of PSII activity. A similar pattern of changes was found in droughted bean leaves (R. M. Smillie, unpublished results). As wilting took place without loss of Chl, the I to P rise increased and r_2 decreased. However, there was a major difference between salt-stressed and droughted bean leaves. In the former, photosynthetic electron transfer after PSII was only partially blocked and the Chl fluorescence yield always returned to the same F_T as in control leaves, even in severely stressed leaves which had lost most of their Chl (Fig. 4). In wilted bean leaves the block was almost complete and F_T was only slightly lower than F_P deficit. Severe dehydration eventually leads to loss of photosystem activity (13) and abolition of the fluorescence rise to P (12) before loss of Chl. Although photosystem activity was lost in salt-stressed bean leaves, this could be attributed mostly to Chl degradation as some PSII activity remained, as evidenced by the persistence of a Chl fluorescence rise to P, as long as some Chl remained in the leaves (Fig. 4).

Potential for Screening for Salt Tolerance. Strains of several species of plants have been selected for salt tolerance on the basis of their growth performance when cultured in salinized solution media (9). However, it has proved difficult to devise screening tests for salt tolerance based on biochemical and physiological measurements as no single physiological factor has been correlated with salt tolerance (21). In spite of this, some progress has been made with a few species. Salt-tolerant lines have been selected after salt-stressing from high leaf K/Na ratios in sorghum (18), Chl loss and Cl^- uptake in citrus (19) and tall wheatgrass (20), and proline accumulation in leaves of cereals (21). The time involved in quantifying these changes renders them unsuitable as rapid screening techniques. Measurements of Chl fluorescence have the advantages that they are nondestructive to the plant tissue take only a few seconds to record (e.g. 1 to 2 s for r_1), and portable measuring equipment is available commercially.

Chilling, heat, and high visible light stresses applied to susceptible plants preferentially inhibit on the photooxidizing side of PSII thus inhibiting the I to P Chl fluorescence rise and screening procedures based on changes in r_1 have been developed to measure tolerance to chilling, heat, and to photoinhibitory light (5, 22, 23, and unpublished results). Changes in parameters of Chl fluorescence in response to salinity are less specific and not as dramatic. As shown in this paper they nonetheless are of obvious value to ascertain whether leaf metabolism has been affected by salinity and to establish whether gradients of response to salinity have developed in the plant (e.g. Fig. 2). Measurements of Chl fluorescence on leaves showing visible symptoms of salt-stress may provide a means of quantifying damage but the utility of such measurements is likely to lie more in comparative studies of salinity in which visible symptoms such as leaf burn are not expressed although growth is depressed, e.g. in grape (1). In sunflower the most useful indices of salinity were F_P-F_I which decreased and r_2 which increased and it is suggested that these may discriminate between salinity responses of comparable sets of leaves of plants of the same genotype exposed to different levels of salinity or different genotypes exposed to the same saline environment. These parameters also appear to be useful as indicators of early effects of salinity in sensitive plants like bean. Although further study is necessary to evaluate fully the feasibility of Chl fluorescence measurements for screening for salt tolerance, the results obtained are sufficiently encouraging to suggest that it

is worthwhile to test the method on varieties or closely related species that are known to differ in salt tolerance.

The measurements of Chl fluorescence reported in this paper were made on dark-adapted leaf material. Another possible experimental approach would be to measure the steady-state Chl yield (F_T-F_0) in photosynthesizing plants. However, this approach does not appear to be a promising one for detecting salt stress, although it may be for certain other stresses such as dehydration. This is because F_T deviated little from control values in salt-affected leaves (Figs. 2 and 4), even in the most salt-stressed bean leaves.

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