

## Tolerance of gametophytes of *Acrostichum aureum* (L.) to salinity and water stress

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### Abstract

Tolerance of gametophytes of *Acrostichum aureum* to NaCl and dehydration was investigated under controlled conditions following the changes in chlorophyll fluorescence parameters ( $F_v/F_m$ ,  $q_p$ ,  $q_N$ ). Salt tolerance was increased by growing gametophytes in low concentrations of NaCl. However, such treatment could not increase the tolerance of gametophytes to dehydration. Under water stress, a decrease in photochemical quenching ( $q_p$ ) was accompanied by an increase in non-photochemical quenching ( $q_N$ ). Under salt stress,  $q_p$  also decreased, but  $q_N$  did not change significantly in salt-hardened gametophytes.

*Additional key words:* chlorophyll fluorescence; dehydration; fern; NaCl; osmotic potential; salt stress.

### Introduction

One of the most common responses of plants to different environmental stresses is a decrease in photosynthetic carbon assimilation. Salt stress increases drought tolerance in wheat, maize and sorghum under field conditions (Sepaskhah and Boersma 1979, Stark and Jarrel 1980, Richardson and McCree 1985).

*A. aureum* (L.) is commonly found in the landward side of mangrove swamps and brackish waters. Compared to other glycophytes, sporophytes of this fern can grow in soil with relatively high salinity (Singh *et al.* 1989). Our preliminary studies showed that gametophytes of *A. aureum* grew best in 0.2 to 0.5 % NaCl; they could not survive in 2.0 % NaCl. Gametophytes of *A. aureum* have only one layer of cells and

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**Abbreviations:** Chl: chlorophyll;  $F_v/F_m$ : maximum photochemical efficiency of PS2;  $F_v'/F_m'$ : photochemical efficiency of opened PS2 reaction centres;  $(F_m' - F)/F_m' \times PFD$ : relative electron transport rate at steady state; NaCl: sodium chloride; PS2: photosystem 2;  $q_p$ : photochemical quenching;  $q_N$ : non-photochemical quenching.

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stomata are absent on both surfaces. Hence, the effect of salt or drought stress on photosynthesis in *A. aureum* gametophytes could not be attributed to stomatal limitations of CO<sub>2</sub> influx. In this paper, the responses of gametophytes of *A. aureum* to NaCl and water stress were investigated, employing chlorophyll (Chl) fluorescence as a sensitive and rapid probe of photosynthetic functions (cf. Ögren and Öquist 1985).

## Materials and methods

Spores of *A. aureum* were collected and surface-sterilized with 4 % (v/v) *Clorox*<sup>TM</sup>. They were then sown in one tenth-strength Hoagland solution with 0.0 to 1.0 % (m/v) concentrations of NaCl at a density of two to three thousand spores per Petri dish. Petri dishes with spore cultures were kept at 27 °C, a 12 h photoperiod and irradiance of 70  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Upon spore germination, culture solutions were frequently changed. The gametophytes were grown to the cordate stage before experimentation began. All procedures were conducted under sterile conditions.

Salt tolerance of gametophytes was investigated by three experiments. In the first experiment, 17-d-old gametophytes grown in 0.0, 0.2 and 0.5 % NaCl were transferred to 3.0 and 3.5 % NaCl for 2 d; they were then transferred back to their original growth solutions. Five days later, the percentage of surviving gametophytes was recorded. In the second experiment, cordate-shape gametophytes (indicating that all gametophytes were at the same phase of their life cycle) grown in 0.0, 0.5, 0.7 and 1.0 % were transferred to 1.0, 2.0 and 3.0 % NaCl for 2 d. Changes in Chl fluorescence in these gametophytes were determined. In the third experiment, cordate-shape gametophytes grown in 0.0 % NaCl (as the experiment control) and 0.7 % NaCl (as the salt-hardened sample) were transferred to 2.0 % NaCl for 2 d and then transferred back to their original growth solutions for recovery from higher salt stress. The Chl fluorescence of the gametophytes was measured after salt stress and during the recovery.

To determine the tolerance of gametophytes to dehydration, they were surface-dried and put in a Petri dish on a dry filter paper. The Petri dish was covered and put under normal growth conditions. This was to simulate the process of dehydration of gametophytes under field conditions. After different periods of dehydration, osmotic potential and Chl fluorescence of the gametophytes were determined. These gametophytes were rehydrated with their growth solutions and Chl fluorescence was again determined.

Osmotic potential was determined using a dew point microvoltmeter (*Wescor*, Logan, Utah, USA). Surface-dried gametophytes were sealed in Eppendorf tubes and kept frozen for one day. They were thawed at room temperature for one hour before the determination of osmotic potential.

The Chl fluorescence was measured (cf. Bolhàr-Nordenkampf and Öquist 1993) with the PAM fluorometer (*Walz*, Effeltrich, Germany). For determinations of F<sub>0</sub> and F<sub>m</sub>, measuring irradiance was lower than 0.01  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . The saturating radiation pulse was at 5000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . To determine photochemical (q<sub>p</sub>) and non-

photochemical quenching ( $q_N$ ), actinic radiation was at  $45 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and a saturating radiation pulse of 600 ms was given every 30 s. Relative electron transport rates at steady state were determined by the method of Genty (1989) with the actinic radiation at  $45 \mu\text{mol m}^{-2} \text{s}^{-1}$ . Before measurement, all samples were kept in the dark for 10 min. All calculations were according to Van Kooten and Snel (1990).

All experiments were replicated more than four times and the values presented are means  $\pm$  standard errors.

## Results and discussion

Gametophytes grown in 0.0, 0.2, 0.5, 0.7 and 1.0 % NaCl showed different rates of growth. Seventeen days after sowing, gametophytes grown in 0.7 and 1.0 % NaCl were still in the filamentous stage and minute in size, while those grown in 0.0-0.5 % NaCl were beginning to show two-dimensional planar growth. Thus, only gametophytes grown in 0.0 to 0.5 % NaCl were used for testing gametophytic survival rate. Only 10 % of the 17-d-old gametophytes grown in 0.0 % NaCl survived after 2 d of transfer to 3.0 % NaCl (Fig. 1). Similar transfers of gametophytes grown in 0.2 or 0.5 % NaCl resulted in higher survival percentages of 35 and 45 %, respectively (Fig. 1). Also when gametophytes were transferred to 3.5 % NaCl for

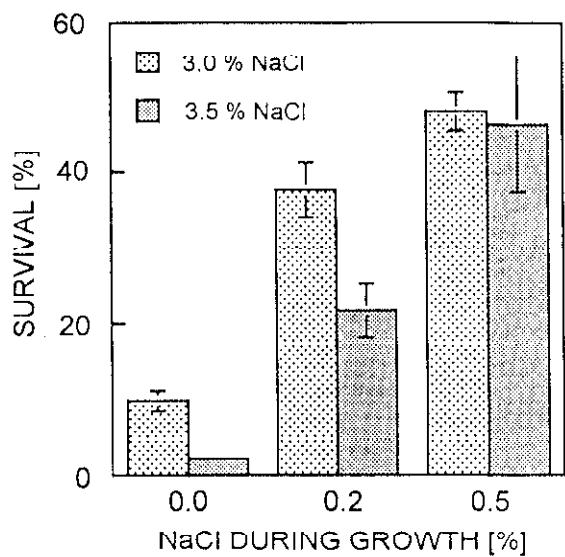


Fig. 1. Survival percentage of gametophytes grown in different concentrations of NaCl after transfer to 3.0 and 3.5 % NaCl for two days.

2 d, the per cent survival was lowest for those grown in 0.0 % NaCl (Fig. 1). Thus the young gametophytes of *A. aureum* were able to tolerate low concentrations of NaCl and could be hardened to withstand higher NaCl concentrations. This tolerance to salt

stress might play an important role in the early establishment of the gametophytes and, hence, the survival of the fern in their natural habitat.

Further experiments were done with gametophytes at the cordate-shape stage. Gametophytes grown in 0.0, 0.5, 0.7 and 1.0 % NaCl were transferred to 1.0, 2.0, and 3.0 % NaCl for 2 d.  $F_v/F_m$  [which indicated the maximum photochemical efficiency of photosystem 2 (PS2)] of gametophytes grown in 0.0 to 1.0 % NaCl was about 0.7. (Although in higher plants and in sporophytes of this fern this ratio was about 0.83, such values of  $F_v/F_m$  could not be obtained in gametophytes.) It did not change when gametophytes grown in 0.0 % NaCl were transferred to 0.5 % NaCl (values not shown) or from 0.7 to 1.0 % NaCl (Fig. 2). The  $F_v/F_m$  decreased when gametophytes grown in 0.0 or 0.5 % NaCl were transferred to 1.0 % NaCl (Fig. 2). Gametophytes grown in 0.0 to 1.0 % NaCl showed decreases in  $F_v/F_m$  when transferred to 2.0 and 3.0 % NaCl for 2 d. However, the decrease in  $F_v/F_m$  was lower in gametophytes grown at higher NaCl concentrations (Fig. 2).

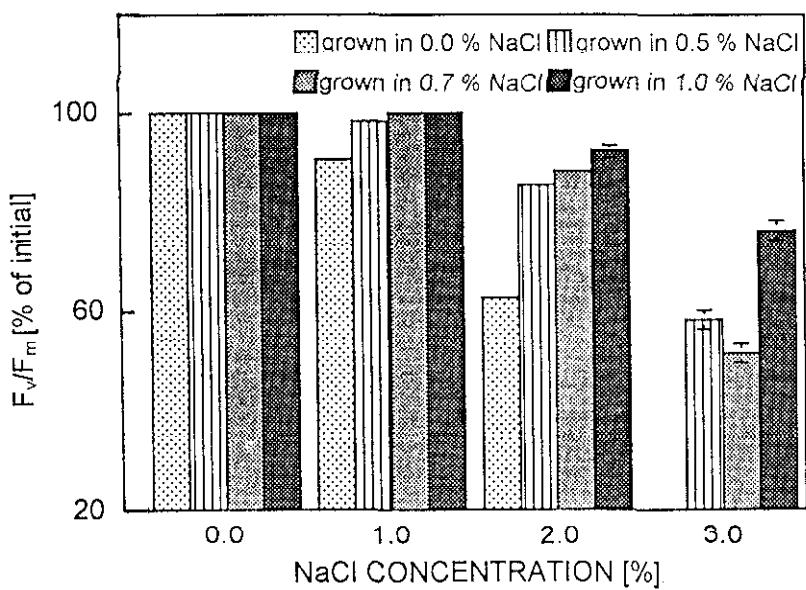


Fig. 2. Effect of NaCl stress (2 d treatment) on  $F_v/F_m$  of gametophytes grown in different NaCl concentrations.

For more detailed studies of salt tolerance, gametophytes grown in 0.0 % NaCl (control) and 0.7 % NaCl (as salt-hardened samples) were transferred to 2.0 % NaCl. After 2 d in 2.0 % NaCl,  $F_v/F_m$  decreased from 0.703 to 0.506 (28 % decrease) in control and from 0.699 to 0.572 (18 % decrease) in 0.7 % NaCl-grown gametophytes (Fig. 3). These results indicated that gametophytes grown in 0.7 % NaCl were more tolerant to higher salt stress than those grown in 0.0 % NaCl. After 1 and 2 d of transfer of gametophytes from 2.0 % NaCl back to their original growth solutions, all gametophytes recovered from salt stress and there was no significant difference in

the speed of recovery between gametophytes grown in 0.0 and 0.7 % NaCl as shown by changes in  $F_v/F_m$  (Fig. 3).

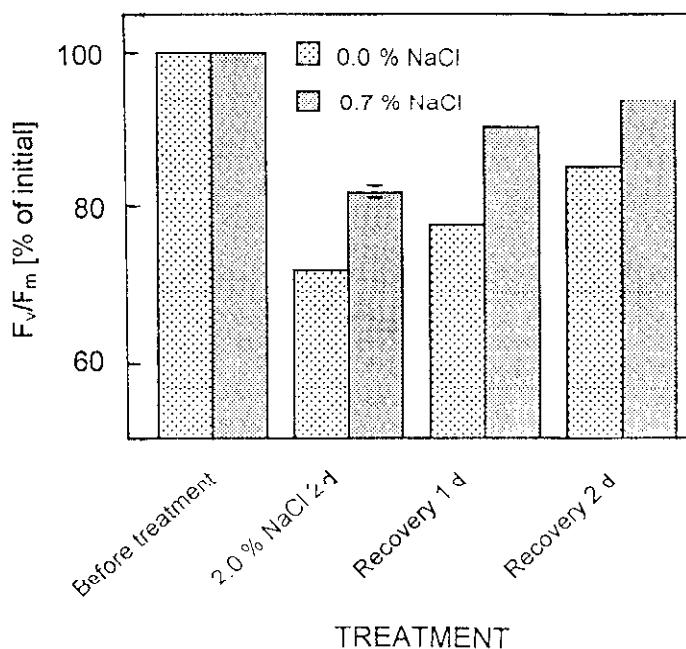


Fig. 3. Changes in  $F_v/F_m$  in unhardened (0.0 % NaCl-grown) and salt-hardened (0.7 % NaCl-grown) gametophytes following transfer to 2.0 NaCl and subsequent recovery.

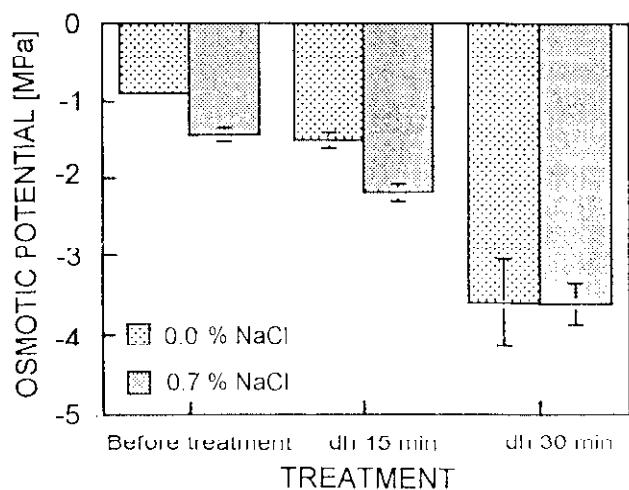


Fig. 4. Osmotic potential of gametophytes grown in 0.0 or 0.7 % NaCl after different durations of dehydration (dh).

Gametophytes grown in 0.7 % NaCl exhibited more negative osmotic potential than those grown in 0.0 % NaCl (Fig. 4). After 30 min dehydration, osmotic potentials of gametophytes grown in 0.0 and 0.7 % NaCl were at the same level. This indicated that water loss was faster in gametophytes grown in 0.0 % than in 0.7 % NaCl. All gametophytes were rehydrated with their respective growth solutions after 30 min dehydration. In gametophytes grown in 0.0 % NaCl,  $F_v/F_m$  decreased following dehydration; it increased as the gametophytes were rehydrated (Fig. 5). Following dehydration,  $F_v/F_m$  of gametophytes grown in 0.7 % NaCl decreased to a smaller extent compared with gametophytes grown in 0.0 % NaCl; it decreased continuously in the first 1 h of rehydration. However, rehydration of the 0.7 % NaCl-grown gametophytes for 1 d brought the  $F_v/F_m$  value up to that of the dehydrated samples (Fig. 5).

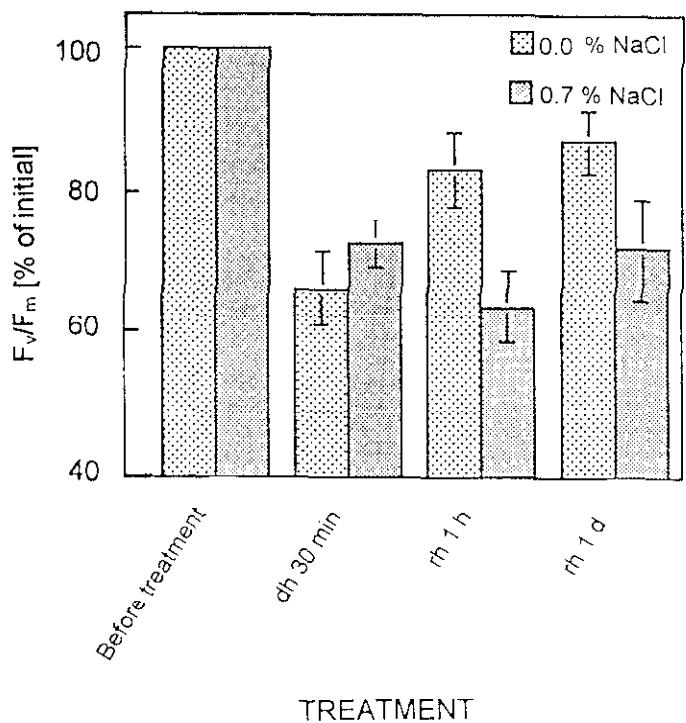


Fig. 5. Changes in  $F_v/F_m$  in unhardened (0.0 % NaCl-grown) and salt-hardened (0.7 % NaCl-grown) gametophytes following 30 min dehydration (dh) and different durations of rehydration (rh).

Comparing salt and dehydration stress, it seemed that salt hardening could increase salt tolerance of the gametophytes. However, it was not able to increase their tolerance to dehydration. This could imply that the mechanisms of tolerance to salt and dehydration were different in the gametophytes. By measuring changes in  $F_v/F_m$ , the ability of *A. aureum* gametophytes to tolerate salt stress was detected upon exposure of the gametophytes to higher NaCl concentration. Salt-tolerant

gametophytes (grown in 0.7 % NaCl) showed smaller decreases in  $F_v/F_m$  under salt stress. Tolerance of the gametophytes against water stress was detected as changes in  $F_v/F_m$  only after rehydration. Gametophytes grown in 0.7 % NaCl showed less decrease in  $F_v/F_m$  after water stress but their recovery was much slower than that of unhardened gametophytes after rehydration.

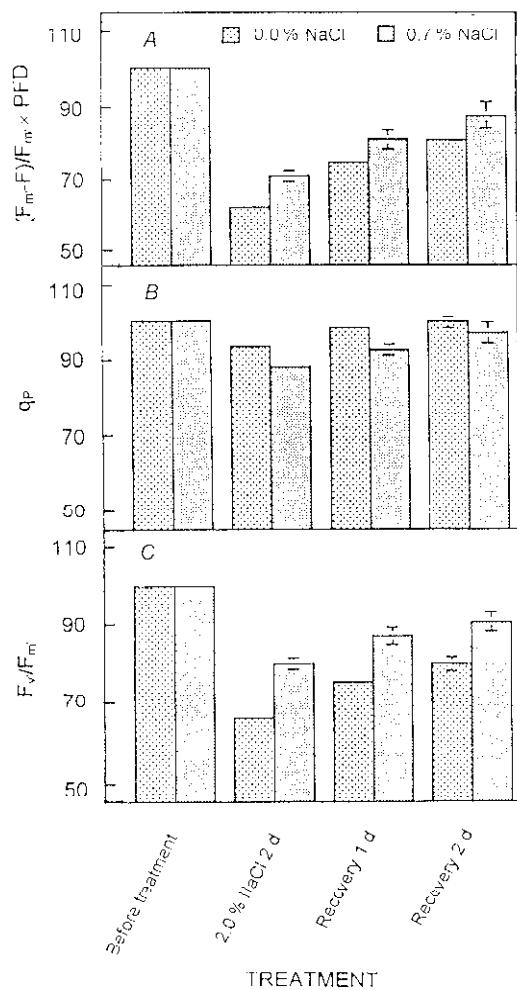


Fig. 6. Changes in (A)  $(F_m' - F)/F_m' \times PFD$ , (B)  $q_p$ , and (C)  $F_v/F_m'$  [% of initial levels] in unhardened (0.0 % NaCl-grown) and salt-hardened (0.7 % NaCl-grown) gametophytes following a transfer to 2.0 % NaCl and subsequent recovery.

Under salt stress and its recovery,  $(F_m' - F)/F_m' \times PFD$ , which reflected the relative electron transport rate at steady state, exhibited similar changes as  $F_v/F_m$  with salt-hardened gametophytes showing a smaller decrease (Fig. 6A). Relative electron

transport rate at steady state was determined by two components, the concentration of open PS2 reaction centres ( $q_p$ ) and the photochemical efficiency of these open reaction centres ( $F_v'/F_m'$ ) (Genty *et al.* 1989). The effects of salt stress on these two components were different between unhardened and salt-hardened gametophytes. In salt-hardened gametophytes,  $q_p$  decreased to a larger extent, while  $F_v'/F_m'$  decreased to a smaller extent compared with unhardened gametophytes (Fig. 6B,C). Dehydration resulted in a decrease in relative electron transport rate to a greater extent in gametophytes grown in 0.7 % NaCl (Fig. 7A). Their recovery was relatively slower than that of the 0.0 % NaCl-grown gametophytes (Fig. 7A); this could be due

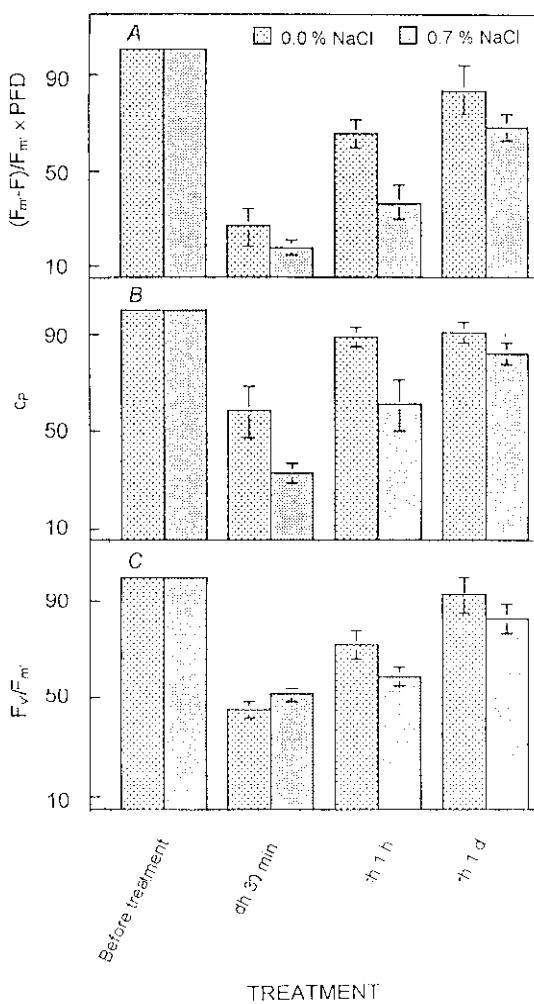


Fig. 7. Changes in (A)  $(F_m' - F)/F_m' \times PFD$ , (B)  $q_p$ , and (C)  $F_v'/F_m'$  [% of initial levels] in unhardened (0.0 % NaCl-grown) and salt-hardened (0.7 % NaCl-grown) gametophytes following 30 min dehydration (dh) and different durations of rehydration (rh).

to the slower recovery of  $F_v'/F_m'$  (Fig. 7B,C). When gametophytes were dehydrated for 15 min, similar changes in  $F_v/F_m$  and relative electron transport rate were observed (values not shown). The results also indicated that under salt stress, the decrease in relative electron transport rate was mainly due to the decrease in photochemical efficiency of open PS2 reaction centres (Fig. 6); under water stress both the concentration of opened PS2 reaction centres and the photochemical efficiency of these reaction centres contributed to the decrease in relative electron transport rate (Fig. 7).

Under salt stress,  $q_P$  decreased while  $q_N$  increased in unhardened gametophytes. However, in salt-hardened gametophytes, although  $q_P$  decreased after salt stress,  $q_N$  did not change significantly. Dehydration caused a decrease in  $q_P$  and an increase in  $q_N$  in gametophytes grown in both 0.0 and 0.7 % NaCl (values not shown). In plants, the first response to any environmental stress is an increase of non-radiative energy dissipation resulting in an increase in  $q_N$  (Schreiber *et al.* 1994). In this way, plants protect themselves against damage resulting from a lack of balance between radiant energy absorption and utilization. Under water stress, with the decrease in relative water content,  $q_P$  was relatively stable but  $q_N$  increased at a faster rate in the dehydration-resistant line of maize (Jovanović *et al.* 1990). In contrast, under salt or dehydration stress,  $q_N$  in salt-hardened gametophytes of *A. aureum* did not increase to the same extent as that in unhardened gametophytes. This indicated the possibility of another mechanism to dissipate the excessive energy absorbed in salt-adapted photosynthetic apparatus. The dissipation of excessive energy by other mechanisms was also suggested by Brugnoli and Björkman (1992) in study on cotton under salinity stress.

Thus, the results suggested that gametophytes of *A. aureum* might employ different mechanisms to overcome salt and dehydration stresses.

## References

Bolhár-Nordenkampf, H.R., Öquist, G.: Chlorophyll fluorescence as a tool in photosynthesis research. - In Hall, D.O., Scurlock, J.M.O., Bolhár-Nordenkampf, H.R., Leegood, R.C., Long, S.P. (ed.): Photosynthesis and Production in a Changing Environment. A Field and Laboratory Manual. Pp. 193-206. Chapman & Hall, London - Glasgow - New York - Tokyo - Melbourne - Madras 1993.

Brugnoli, E., Björkman, O.: Growth of cotton under continuous salinity stress. influence on allocation pattern, stomatal and non-stomatal components of photosynthesis and dissipation of excess light energy. - *Planta* **187**: 335-347, 1992.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. - *Biochim. biophys. Acta* **990**: 87-92, 1989.

Jovanović, L., Janjić, V., Veljović, S.: The effect of drought on chlorophyll fluorescence in two maize lines. - In: Baltscheffsky, M. (ed.): Current Research in Photosynthesis. Vol. IV. Pp 725-728. Kluwer Academic Publishers, Dordrecht - Boston - London 1990.

Ögren, E., Öquist, G.: Effects of drought on photosynthesis, chlorophyll fluorescence and photoinhibition susceptibility in intact willow leaves. - *Planta* **166**: 380-388, 1985.

Richardson, S.G., McCree, K.J.: Carbon balance and water relations of sorghum exposed to salt and water stress. - *Plant Physiol.* **79**: 1015-1020, 1985.

Schreiber, U., Bilger, W., Neubauer, C.: Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. - In Schulze, E.-D., Caldwell, M.M. (ed.): *Ecophysiology of Photosynthesis*. Pp. 49-70. Springer-Verlag, Berlin 1994.

Sepaskhah, A.R., Boersma, L.: Elongation of wheat leaves exposed to several levels of matric potential and NaCl induced osmotic potential of soil water. - *Agron. J.* **71**: 848-852, 1979.

Singh, N.T., Mongia, A.D., Ganeshamurthy, A.N.: Soils of brackish water marshes of South Andaman. - *J. Indian Soc. Soil Sci.* **37**: 355-362, 1989.

Stark, J.C., Jarrel, W.M.: Salinity-induced modifications in the response of maize to water deficits. - *Agron. J.* **72**: 745-748, 1980.

Van Kooten, O., Snel, J.F.H.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. - *Photosynth. Res.* **25**: 147-150, 1990.