INTRODUCTION

The effects of salt stress on rice are highly dependent on plant phenology: young seedlings and plants at the flowering stage appear to be the most sensitive while tillering plants are less sensitive (Heenan, Lewin and McCaffery, 1988; Lutts, Kinet and Bouharmont, 1995). Salinity applied at the seedling stage frequently induces premature senescence of leaves (Sahu and Mishra, 1987; Yeo et al., 1991). Leaf senescence is most often quantified by decreases in protein or chlorophyll concentration (Kurra-Hotta, Satoh and Katoh, 1987; Hashimoto, Kura-Hotta and Katoh, 1989; Chen and Kao, 1991; Chen, Li and Kao, 1991) and by increases in membrane permeability (Dhindsa, Plumb-Dhindsa and Thorpe, 1981). Chlorophyll fluorescence kinetics, and more especially the ratio of the maximal variable fluorescence to the maximum level of chlorophyll fluorescence (Fv/Fm) (which is directly related to PS II photochemical efficiency), may also be modified during ageing although the relationship between chlorophyll fluorescence and naturally-occurring senescence processes is rarely considered (Björkman, 1987).

Specific effects of salt stress on leaf senescence have been related to the accumulation of toxic ions (Na⁺ and Cl⁻) or to K⁺ and Ca²⁺ depletion (Yeo and Flowers, 1983; Leidi, Silberbush and Lips, 1991; Yeo et al., 1991). Magnesium, by comparison, has received little attention, although it could play a central role in senescence-related processes. Mg²⁺ is implicated in the regulation of protein synthesis (Flowers and Dalmond, 1992). A decrease in Mg²⁺ absorption could also be responsible for decreased chlorophyll content (Leidi et al., 1991) and quenching of variable fluorescence due to increased ‘spillover’ of excitation energy from PS II to PS I (Krause and Weis, 1991).

Several studies have examined chlorophyll fluorescence in plants subjected to drought (Scheuermann et al., 1991) or thermal stresses (Badiani et al., 1993; Havaux, 1994) but data concerning effect of salt stress on fluorescence are still fragmentary. In cereals, most studies have been performed on barley (Morales et al., 1992; Maslenkova, Zanev and Popova, 1993; Belkhodja et al., 1994). In rice, Bohra, Dörfling and Dörfling (1995) and Bohra and Dörfling (1993) found that NaCl salinity lowered the Fv/Fm chlorophyll fluorescence ratio after 47 d of stress and also at the flowering stage. However no attempt was made to quantify the progressive change of chlorophyll fluorescence in salt-stressed young seedlings.

The precise relationships between (a) senescence-related parameters on the one hand and (b) mean salt-resistance and senescence-related parameters on the other are not well established in rice. Although Yeo and Flowers (1983) found substantial variation in the relationship between chlorophyll and sodium concentrations in nine salt-stressed rice cultivars differing in salinity resistance, most other studies devoted to NaCl-induced senescence in rice leaves were done using a single cultivar. In the present study, protein, magnesium...
and chlorophyll concentrations, membrane permeability (estimated by solute leakages and malondialdehyde production) and chlorophyll fluorescence-related parameters were simultaneously measured in old and young leaves of non-stressed and salt-treated plants of five rice cultivars differing in salinity resistance. The intention was to determine whether salt-induced senescence of rice leaves results from an acceleration of deteriorative mechanisms which normally occur during leaf ontogeny or also involve other processes not found during normal senescence.

**MATERIALS AND METHODS**

**Plant material and growth conditions**

Seeds of rice were obtained from IRRI (International Rice Research Institute, Philippines) and from WARDA (West African Rice Development Association, Senegal). Cultivars 1 Kong Pao (IKP) and IR 31785 are salt-sensitive while Nona Bokra and IR 4630 are classified as salt-resistant (Yeo et al., 1990). Aiwu, which presents an intermediate behaviour in our experimental conditions, is regarded as ‘moderately’ salt-resistant (Lutts et al., 1995).

For each cultivar, 340 seeds were germinated on two layers of Whatman No. 2 filter paper moistened with sterile deionized water; germination rates were always >90%. Young seedlings were acclimated in a phytotron under controlled temperature conditions (29/26 °C, day/night). Illumination was provided by Sylvania fluorescent tubes (F96T12/CW/VHO) for 12 h d⁻¹ at a photon flux density (PAR) of 300–350 µmol m⁻² s⁻¹ at canopy level. Daytime humidity was between 60 and 80%. Seedlings used for malondialdehyde determination were acclimated in greenhouses during summer; natural lighting was supplemented by Philips fluorescent lamps and other conditions (temperature, humidity) were kept close to those prevalent in the phytotron. Plants were distributed among nine tanks containing 50 l of Yoshida solution (Yoshida et al., 1976) circulated by an Eheim ultra small filter (2007) circulating on a fresh matter basis to facilitate comparisons between these parameters and chlorophyll fluorescence or solute leakage which were also recorded on fresh tissues. The relative behaviour of the five cultivars studied (i.e. the ‘ranking order’) did not change when measurements were expressed in other ways, e.g. on a dry weight basis.

**Protein and malondialdehyde concentrations**

Five plants for each treatment (cultivar x dose) were harvested at the start of stress imposition and again after 1, 2 or 4 weeks of stress. Old and young leaves were ground separately in a chilled mortar and pestle in the presence of liquid nitrogen. Soluble proteins were extracted from 2.0 g fresh matter in 10 ml of 0.1 m Tris-HCl (pH 7.5) after centrifugation at 40 000 g for 30 min at 4 °C. The precipitate was incubated for 2 h at room temperature in Tris buffer containing 10% SDS to extract insoluble proteins (Hashimoto et al., 1989). Protein levels were estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard. For malondialdehyde (MDA) determination, a 0.5 g leaf sample was homogenized in 5 ml 0.1% trichloroacetic acid and centrifuged at 10 000 g for 10 min. The amount of MDA in the supernatant was estimated by the thiobarbituric reaction according to Dhindsa and Matowe (1981). All measurements were made in triplicate.

**Chlorophyll concentration and fluorescence parameters**

Chlorophyll concentration was estimated on three individual plants per treatment at the time of stress imposition and after 1, 2 or 4 weeks of stress. A median segment (1 cm-length) was collected on each unfolded leaf; weighed segments from leaves belonging to the same level (old or young leaves) were ground together in 10 ml 80% cold acetone. The absorbance of the extract was estimated at 645 and 663 nm and chlorophyll a and b concentrations were estimated according to MacKinney (1941). Chlorophyll fluorescence was monitored every 3 d from the start of stress imposition for 30 d on the median and the upper unfolded leaf of the main tiller from six individual plants per treatment. All measurements were made between 1100 and 1300 h. Prior to fluorescence measurements, a circular surface of the upper face was dark-adapted for 30 min. The basal non variable chlorophyll fluorescence level (F₀) and the maximal fluorescence induction (Fₘ) at 692 nm were determined by a Plant Efficiency Analyser (Hansatech Instruments Ltd., Norfolk, UK) by illuminating the leaves with a beam of saturating light (3000 µmol m⁻² s⁻¹) of peak wavelength (650 nm) obtained from light-emitting diodes. The variable fluorescence (Fᵥ = Fₘ – F₀) and the ratio Fᵥ/Fₘ were then estimated.

**Membrane permeability (solute leakage)**

Leaves of three plants per treatment were harvested 1, 2 and 4 weeks after stress imposition and cut in 1 cm segments. Samples were washed with three changes of deionized water to remove surface-adhered electrolytes according to Blum and Ebercon (1981). Leaf segments were placed in stoppered vials containing 10 ml of deionized water and incubated at different pH levels (3.0, 6.0 or 9.0). After 4 h of incubation, the suspensions were centrifuged at 10 000 g for 10 min and the absorbance of the supernatant was estimated at 232 nm.
25 °C on a rotary shaker (100 rpm). Electrical conductivity of the bathing solution (\( L_t \)) was determined after 24 h. Samples were then autoclaved at 120 °C for 20 min and a last conductivity reading (\( L_f \)) was obtained upon equilibration at 25 °C. The electrolyte leakage was defined as \( L_t/L_f \) and expressed as percent.

The reliability of electrolyte leakage technique suffers from two limitations: (a) the direct effect of salt-stress on excised leaf segments cannot be determined by adding NaCl to incubating solutions since it would interfere with electrolyte leakage measurements and (b) an apoplastic accumulation of toxic ions in salt-stressed leaves will contribute to electrolyte leakage although they are not involved in cellular efflux. For these reasons membrane permeability was also quantified by the leakage of UV-absorbing substances (UVAS) according to Redmann, Haraldson and Gusta (1986). Leaf samples collected as described above, were incubated in the presence of 10 ml of either deionized water or 100 mM NaCl. A 2-5 ml aliquot of the bathing solution was removed from the flasks after 24 h incubation and the absorbance was estimated spectrophotometrically at 280 nm (\( A_{280} \)). This 2-5 ml aliquot was then added back to the original solution and the flasks cooled to −30 °C for 4 h to break the cells. A final absorbance measurement (\( A_{280} \)) was recorded after thawing and the relative leakage ratio (RLR) of the UVAS was calculated as \( RLR = A_{280}/A_{280} \).

**Magnesium concentration**

Leaves of three individual plants per treatment were harvested 1, 2 and 4 weeks after stress imposition, weighed and oven-dried at 70 °C for 48 h. Digestion of dried tissues was accomplished in a 3:1 nitric:perchloric acid mixture and analysis was conducted using an inductively coupled argon plasma emission spectrophotometer (Jobin-Yvon JY 48).

**RESULTS**

Salinity had no effect on the rate of leaf emergence: thus, at a given genotype always had the same number of leaves, although salinity clearly shortened the leaves.

**Protein concentration**

In non-stressed plants, soluble protein concentration was higher in young than in old leaves and no significant difference was recorded among cultivars. NaCl induced a progressive decrease in soluble protein concentration of

![Figure 1](https://example.com/figure1.png)  
**Fig. 1.** Effects of the duration of salt-stress treatment on soluble protein concentration (mg g\(^{-1}\) f. wt) in old (OL) and young (YL) leaves of rice cultivars IKP, salt-sensitive (A); Aiwu, moderately resistant (B) and Nona Bokra, salt-resistant (C). Plants were exposed to 0 or 50 mM NaCl for 0, 7, 14 and 28 d (means of five replicates). [(———) OL-0 mm, (— —) YL-0 mm, (——△——) OL-50 mm, (——▲——) YL-50 mm.]

![Figure 2](https://example.com/figure2.png)  
**Fig. 2.** Effects of the duration of salt-stress treatment on insoluble protein concentration (mg g\(^{-1}\) f. wt) in old (OL) and young (YL) leaves of rice cultivars (IKP and IR 31785, salt-sensitive; Aiwu, moderately resistant; Nona Bokra and IR 4630, salt-resistant). Plants were exposed to 0 or 50 mM NaCl for 7 (A) or 28 (B) d (means of five replicates). [(——-OL-0 mm; (■) YL-0 mm; (○) OL-50 mm; (□) YL-50 mm.]
salt-sensitive cultivars which was more marked in young than in old leaves. This is shown in Fig. 1 for the cultivar IKP exposed to 50 mM NaCl. Consequently, soluble protein concentration became similar in old and young leaves by the end of stress exposure. Moderate salinity applied to Aiwu for a short period (7 d) had no effect on protein concentration but 50 mM NaCl progressively decreased it. In salt-resistant cultivars, concentration of soluble protein was always higher in young than in old leaves although it increased transiently in all leaves after only 7 d exposure to salinity.

Insoluble protein fraction was not affected by salinity after 7 d stress in salt-resistant Aiwu, Nona Bokra and IR 4630 (Fig. 2). Insoluble protein decreased in salt-sensitive genotypes but at a slower rate than soluble proteins. After 7 d stress, insoluble protein concentration was reduced only in old leaves of cultivars IKP and IR 31785. However, by 28 d, NaCl strongly reduced insoluble protein concentration in all leaves of salt-sensitive cultivars and no differences were found between leaves of the two levels (Fig. 2). In contrast 28 d stress decreased insoluble protein concentration mainly in old leaves of salt-resistant genotypes and to a lesser extent in IR 4630 than in Nona Bokra.

Chlorophyll concentration

NaCl decreased total chlorophyll concentration in both leaf types, as illustrated in Fig. 3 for young leaves. Highly significant differences were recorded among cultivars, especially after 28 d of stress exposure. Salt-sensitive cultivar IR 31785 had the smallest chlorophyll concentration and IR 4630 the largest. Slight differences were also recorded between the two salt-resistant genotypes. At the highest stress intensity, total chlorophyll concentration in the young leaves of IR 4630 was 33 % higher than in the young leaves of cv. Nona Bokra. No differences in chlorophyll concentration of young leaves were recorded between the two doses of NaCl in IR 4630, regardless of stress duration, while significant differences were recorded in Nona Bokra. Chlorophyll a and b levels were not equally sensitive to salt stress. Indeed, Chl b concentration was not reduced after 7 d of exposure in any of the cultivar tested at any NaCl dose. In contrast, Chl a concentration decreased markedly at this time. Consequently, Chl a/Chl b ratios (Table 1) exhibited a minimal value after 7 d of stress and then increased, as a consequence of a more marked decrease in Chl b as the stress was prolonged. By the end of stress exposure, the ratio recorded approximately returned to non-stressed controls. The same trend was observed in all genotypes and the five rice cultivars did not differ in the relative sensitivity of Chl b and Chl a to salt.

Magnesium concentration

In the absence as well as in the presence of salt, Mg²⁺ was evenly distributed among leaves of different ages. Mg²⁺ concentration in controls was constant over time in both old and young leaves. Leaf Mg²⁺ concentration was not
significantly affected by salt stress after 1 week. After two weeks of stress, Mg" decreased significantly in the cultivar IKP exposed to 50 mM NaCl and in IR 31785 exposed to 30 and 50 mM NaCl (Table 2). After 28 d of stress, however, Mg" concentration was reduced by NaCl in all leaves and in all cultivars (data not shown).

**Chlorophyll fluorescence parameters**

The $F_{v}/F_{m}$ ratio decreased in response to salt treatment from day 9 in median leaves and from day 12 in youngest ones. In all treatments, the $F_{v}/F_{m}$ ratios were slightly lower in median (overall mean of 0.803 ± 0.026) than in youngest leaves (overall mean of 0.815 ± 0.029) and NaCl did not modify the mean difference in $F_{v}/F_{m}$ ratios between leaves of different ages. No significant differences in $F_{v}/F_{m}$ ratio were recorded among genotypes until day 18 of stress (Table 3) and even after that time, differences among cultivars were always significant at $P = 0.05$ but not at $P = 0.01$. After 30 d exposure to 50 mM NaCl, the $F_{v}/F_{m}$ ratio fell to a minimal value of 0.729 in median leaves of cultivar IR 31785 while it remained at 0.771 and 0.781 in Nona Bokra and IR 4630, respectively.

The main effect of NaCl upon chlorophyll fluorescence parameters consisted in increased $F_{m}$ values as shown in Fig. 4A for the median leaf. The various cultivars differed mainly in $F_{m}$ values (Fig. 4B), which decreased in salt-stressed plants after 12 d in cultivars IKP, IR 31785 and Aiwu and after 18 d in Nona Bokra. In salt-resistant IR 4630, $F_{m}$ values remained constant throughout the experiment and a decrease in $F_{v}/F_{m}$ ratios could therefore be mainly ascribed to an increase in $F_{m}$ in this latter cultivar.

**Electrolyte leakage**

In the absence of stress, electrolyte leakage did not vary among cultivars and was always slightly higher in old than in young leaves. The presence of NaCl in the nutritive solution induced an increase in subsequent electrolyte leakage, as shown in Fig. 5 for salt-sensitive cultivar IKP and salt-resistant Nona Bokra. Leakage increased with the duration of stress exposure (data not shown). In the presence of NaCl, electrolyte leakage was higher in salt-sensitive IKP and IR 31785 than in salt-resistant Nona Bokra and IR 4630. At the end of the second week of stress, significant differences were recorded among these two latter genotypes, Nona Bokra being less affected than IR 4630. In salt-resistant cultivars, electrolyte leakage remained higher in old than in young leaves up to the end of the stress treatment (Fig. 5). In contrast, electrolyte leakage was unexpectedly higher in young than in old leaves of salt-sensitive genotype IKP and IR 31785 stressed for 2 or 4 weeks.

**UVAS leakage**

Relative leakage ratio (RLR) did not vary among cultivars in non-stressed conditions. In the presence of NaCl, RLR increased markedly during the first 2 weeks of stress.

**Table 1. Chlorophyll a/b ratios in old and young leaves of rice plants after 0, 7, 14 and 28 d of exposure to 0, 30 and 50 mM NaCl. Data were pooled for five rice cultivars (means of 15 replicates ± s.e.). Plants were 32-d-old at the start of the experiment.**

<table>
<thead>
<tr>
<th>Dose of NaCl (mM)</th>
<th>Old leaves (d of stress exposure)</th>
<th>Young leaves (d of stress exposure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 ± 0.07</td>
<td>0 ± 0.10</td>
</tr>
<tr>
<td>30</td>
<td>2 ± 0.04</td>
<td>2 ± 0.06</td>
</tr>
<tr>
<td>50</td>
<td>2 ± 0.04</td>
<td>2 ± 0.06</td>
</tr>
</tbody>
</table>

**Table 2. Mg" concentrations (µmol g⁻¹ f. wt) in young leaves of salt-sensitive cultivars of rice (IKP and IR 31785) and salt-resistant cultivar (Nona Bokra) exposed during 2 weeks to 0, 30 and 50 mM NaCl (means of three replicates ± standard deviation). Plants were 32-d-old at the start of the experiment.**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>NaCl dose (mM)</th>
<th>0 ± 2.5</th>
<th>30 ± 2.9</th>
<th>50 ± 3.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>IKP</td>
<td>49 ± 2.5</td>
<td>51 ± 2.9</td>
<td>34 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>IR 31785</td>
<td>75 ± 4.1</td>
<td>38 ± 2.9</td>
<td>35 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Nona Bokra</td>
<td>61 ± 6.6</td>
<td>58 ± 3.5</td>
<td>52 ± 3.9</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. Two way variance analysis of $F_{v}/F_{m}$ ratios recorded in the median leaf and youngest unfolded leaf in salt-stressed cultivars of rice after 0, 6, 12, 18, 24 and 30 d of exposure to 0, 30 or 50 mM NaCl. F-ratios are given for the two main levels of classification (cv. and NaCl dose) as well as for interactions (ns, non-significant; *, significant at $P = 0.05$; **, significant at $P = 0.01$). Plants were 32-d-old at the start of the experiment.**

<table>
<thead>
<tr>
<th>Duration of exposure (d)</th>
<th>Median leaf</th>
<th>Youngest leaf</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.28*</td>
<td>2.03**</td>
</tr>
<tr>
<td>6</td>
<td>2.84**</td>
<td>2.18**</td>
</tr>
<tr>
<td>12</td>
<td>3.97**</td>
<td>3.71**</td>
</tr>
<tr>
<td>18</td>
<td>5.78**</td>
<td>5.78**</td>
</tr>
<tr>
<td>24</td>
<td>10.32**</td>
<td>12.13**</td>
</tr>
<tr>
<td>30</td>
<td>14.17**</td>
<td>19.71**</td>
</tr>
</tbody>
</table>

**Table 4. Chlorophyll a/b ratios in old and young leaves of rice plants after 0, 7, 14 and 28 d of exposure to 0, 30 and 50 mM NaCl. Data were pooled for five rice cultivars (means of 15 replicates ± s.e.). Plants were 32-d-old at the start of the experiment.**

<table>
<thead>
<tr>
<th>Dose of NaCl (mM)</th>
<th>Old leaves (d of stress exposure)</th>
<th>Young leaves (d of stress exposure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 ± 0.07</td>
<td>0 ± 0.10</td>
</tr>
<tr>
<td>30</td>
<td>2 ± 0.04</td>
<td>2 ± 0.06</td>
</tr>
<tr>
<td>50</td>
<td>2 ± 0.04</td>
<td>2 ± 0.06</td>
</tr>
</tbody>
</table>
Fig. 4. Effects of the duration of salt-stress treatment on basal non variable chlorophyll fluorescence level ($F_0$) (A) and maximal fluorescence induction ($F_m$) at 692 nm (B) in the median leaf of the main tiller. In (A), data are pooled for the five rice cultivars exposed to 0 (---■---), 30 (---□---) and 50 (---△---) mM NaCl and each point is the mean of 30 replicates. In (B), data are pooled for the two highest doses of NaCl (30 and 50 mM) and are given separately for each cultivar: IKP (---■---), salt-sensitive; Aiwu (---□---), moderately resistant; Nona Bokra (---△---) and IR 4630 (---△---), salt-resistant. Each point is the mean of 12 replicates.

reaching a plateau at the end of the second week (Fig. 6). Highly significant differences were observed between the RLR values recorded after incubation in deionized water on the one hand and 100 mM NaCl on the other hand, suggesting strong differences in membrane sensitivity to hypo and hyper-osmotic shocks. RLR was always higher after an hyper-osmotic shock, whatever the cultivar, stress intensity in nutritive solution, or the age of the leaf. Therefore, the nature of the bathing solution used during incubation did not modify the ranking order of the various cultivars in respect to cell membrane stability. IKP and IR 31785 gave the highest values and Nona Bokra the lowest one (Table 4). A slight (although statistically significant) difference was recorded between the two salt-resistant genotypes at the end of the second week of stress. The RLR values were always higher in old than in young leaves of salt-resistant genotypes, whatever the NaCl dose in the nutrient solution. However, after 2 weeks of stress, no significant differences among leaves of different ages were recorded in IKP, IR 31785 and Aiwu.

$MDA$ accumulation

Accumulation of malondialdehyde (MDA) strongly paralleled changes in RLR by showing a sharp increase during the two first weeks of stress and then stabilizing or even slightly decreasing at the end of stress exposure in salt-
sensitive genotypes. This is shown in Fig. 7A for IR 31785. In the presence of salt, MDA concentration was least in salt-resistant cultivars (as shown in Fig. 7B for Nona Bokra) and greatest in salt-sensitive genotypes. The MDA concentration of young leaves was lower than in old ones, except in salt-stressed IKP and IR 31785 after 2 and 4 weeks of stress. When data are pooled for all treatments, a highly significant positive correlation was found between MDA concentration and RLR and this relationship was similar for old ($r = 0.89$; significant at $P < 0.01$) and young leaves ($r = 0.92$; significant at $P < 0.01$), suggesting a direct relationship between malondialdehyde generation by lipid peroxidation and the level of UVAS leakage (Fig. 7C). A significant positive correlation also existed between MDA and the electrolyte leakage for both old ($r = 0.71$) and young ($r = 0.63$) leaves, although the statistical correlation was weaker.

## DISCUSSION

### Naturally-occurring vs. stress-induced senescence

In the absence of stress, strong differences were recorded throughout the experiment between old and young leaves in every cultivar. Protein and chlorophyll concentrations, Chl $\alpha$/Chl $b$ and $F_a/F_m$ ratios and $F_m$ were higher in young leaves while electrolyte leakage, RLR and MDA concentration were higher in old leaves.

It appears that many NaCl-induced changes in rice leaves represent a hastening of naturally-occurring senescence processes in relation to photoresynthesis (Dwivedi, Kar and Mishra, 1979; Hashimoto et al., 1989; Chen et al., 1991), alteration of photosynthetic apparatus (Kura-Hotta et al., 1987, 1990) and membrane permeability properties (Dhindsa et al., 1981; Irigoien, Emerich and Sánchez-Díaz, 1992). Nevertheless, we demonstrated that some of the modifications induced by salinity are not observed during naturally-occurring senescence. Several were recorded in all cultivars under salt stress, such as increases in $F_a$ and in Chl $\alpha$/Chl $b$ ratios (at the end of stress exposure), or a decrease in Mg$^{2+}$ concentration. One other modification, a decrease in MDA concentration after a long-term exposure to the highest stress intensity (Fig. 7A), was seen only in salt-sensitive cultivars. This phenomenon can be linked to an overall inhibition of plant metabolism. Finally, some specific changes were recorded only in salt-resistant genotypes, e.g. a transient increase in soluble protein concentration after 1 week of growth in the presence of salt (Fig. 1).

Even when the nature of metabolic modifications induced

### Table 4. Relative leakage ratio values (in %) of old and young leaves in five cultivars of rice maintained for 14 or 28 d in the absence (0 NaCl) or in the presence of salt (+ NaCl; data pooled for 30 mm and 50 mm NaCl). Leaf segments were incubated for 24 h in 100 mm NaCl (mean of six replicates ± s.e.)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Stress duration (d)</th>
<th>Old leaves</th>
<th>Young leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 NaCl</td>
<td>+ NaCl</td>
<td>0 NaCl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dura</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14±0.9</td>
<td>38±2.5</td>
</tr>
<tr>
<td>IKP</td>
<td>28</td>
<td>16±2.1</td>
<td>39±3.1</td>
</tr>
<tr>
<td>IR 31785</td>
<td>14</td>
<td>12±1.2</td>
<td>41±3.0</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>19±2.3</td>
<td>45±2.3</td>
</tr>
<tr>
<td>Aiwu</td>
<td>14</td>
<td>14±1.3</td>
<td>30±2.4</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>15±1.1</td>
<td>33±1.3</td>
</tr>
<tr>
<td>Nona B.</td>
<td>14</td>
<td>13±1.4</td>
<td>23±1.2</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>15±1.1</td>
<td>25±1.1</td>
</tr>
<tr>
<td>IR 4630</td>
<td>14</td>
<td>13±0.4</td>
<td>31±3.1</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>14±0.9</td>
<td>31±1.9</td>
</tr>
</tbody>
</table>

### Fig. 7. Effects of the duration of salt-stress treatment to rice on malondialdehyde (MDA) concentration of old (OL) and young (YL) leaves of salt-sensitive cultivar IR 31785 (A) and salt-resistant Nona Bokra (B). (■ OL-0 mm; (––) YL-0 mm; (—) OL-30 mm; (——) YL-30 mm; (——) OL-50 mm; (——) YL-50 mm.) In (C), the mean correlation coefficients between RLR values and MDA concentration are given separately for old (●, $r = 0.89$) and young (○, $r = 0.92$) leaves (data pooled for all treatments).
by salt stress appeared similar to naturally-occurring senescence processes, the extent of those modifications could be quite different, especially in relation to leaf position. In salt-sensitive genotypes IKP and IR 31785, NaCl imposed during 2 or 4 weeks masked the differences between the leaves of different levels, in soluble (Fig. 1) and insoluble (Fig. 2) protein concentrations, RLR values (Table 4) and MDA concentrations (Fig. 7A). Salt stress even induced a higher electrolyte leakage in young than in old leaves (Fig. 5) hereby reversing the usual situation. In salt-resistant cultivars, in contrast, differences between old and young leaf properties were maintained throughout the experiment (the only exception being in chlorophyll concentration in cultivar Nona Bokra). This result was not unexpected, since a previous study showed that Na⁺ and Cl⁻ accumulation in salt-stressed rice plants occurred mainly in oldest leaves of salt-resistant genotypes Nona Bokra and IR 4630. Such a compartmentation was not observed to the same extent in salt-sensitive cultivars (Lutts, Kinet and Bouharmont, 1996). This reinforces the earlier suggestion (Yeo et al., 1991) that long term effect of salinity on rice leaves is mainly due to the pathological consequences of toxic ions accumulation and should be distinguished from the initial growth reduction due to a limitation of water supply.

First symptoms of NaCl-induced senescence

A time-course analysis of metabolic modifications in stressed leaves suggests that the alteration of membrane permeability is one of the first symptoms of stress-induced senescence. Indeed, a highly significant increase in electrolyte leakage, RLR and MDA concentration was recorded in all leaves of all cultivars after 7 d of exposure to salt stress, while chlorophyll fluorescence or Mg²⁺ concentration were not modified and protein concentration showed variable trends according to genotype and leaf age. It is noteworthy that the strong NaCl-induced decrease in cell membrane stability was not directly linked to a decrease in insoluble protein concentration while Hashimoto et al. (1989) demonstrated that most of the insoluble proteins obtained after extraction with SDS are membrane-bound proteins. The high correlation recorded between RLR values (and to a lesser extent electrolyte leakage) and MDA also favours the hypothesis that membrane alteration recorded after short-term exposure could be ascribed to peroxidation of unsaturated fatty acids rather than to membrane protein modifications. Dhindsa et al. (1981) previously demonstrated that there are similar changes in the level of lipid peroxidation and in electrolyte leakage during senescence of tobacco leaves. Irigoyen et al. (1992) also observed an increased MDA content in response to slight drought stress in alfalfa.

The relationships between parameters involved in senescence processes, and more especially membrane stability, protein, chlorophyll and Mg²⁺ concentrations, are still unclear. In senescence studies, it is commonly assumed that chlorophyll loss is linked to protein degradation (Kurahotta et al., 1987; Hashimoto et al., 1989). In our study, however, an important loss of chlorophyll a was recorded in young leaves before any substantial decrease in insoluble proteins concentration. Our results are therefore consistent with the observation of Chen et al. (1991) who found no direct relationship between the chlorophyll and the protein concentrations of leaf ageing tissues. When data are pooled for all treatments, significant positive correlations were recorded between Mg²⁺ concentrations on the one hand and total protein or chlorophyll concentrations on the other. However, decreases in chlorophyll always preceded decreases in Mg²⁺. Therefore, it seems unlikely that the first is the consequence of the second as suggested by Leidi et al. (1991). Mg²⁺ concentration recorded in the present study was always higher than the optimum Mg²⁺ concentration for in vitro protein synthesis in glycophytes, which ranged between 2 and 4 mm (Flowers and Dalmond, 1992).

Bohra and Dörfling (1993) and Bohra et al. (1995) have already reported that the deleterious effect of NaCl on potential photosynthetic activity in Oryza sativa could be attenuated by potassium fertilization or exogenous application of abscisic acid but these studies did not reveal the cause of the stress-induced modification of chlorophyll fluorescence. In our work, we show that a decrease in the chlorophyll a concentration did not immediately lead to a concomitant modification of Fm/Fo ratios, thus confirming previous results obtained on barley (Morales et al., 1992) or sorghum (Sharma and Hall, 1992). It is noteworthy that in our study the decrease in Fo/Fm ratios coincided with a decrease in chlorophyll b concentration, although only chlorophyll a is involved in fluorescence. Since chlorophyll b is mainly associated with PS II antenna, a decrease in its concentration could reflect a structural modification of antenna which would explain the NaCl-induced increase in Fo (Fig. 4) occurring before any decrease in Fo .

Criteria for stress tolerance and resistance

Chlorophyll concentration in stressed tissue can be construed as an index of tissue tolerance to NaCl. Although the primary cause of chlorophyll loss is still unknown, the two salt-resistant cultivars differed with respect to chlorophyll concentration. IR 4630 always gave a smaller decrease in chlorophyll concentration than did Nona Bokra. Our results thus corroborate previous findings by Yeo et al. (1990) who observed that the internal leaf Na⁺ concentration required to decrease the chlorophyll content by 50% was higher in IR 4630 than in Nona Bokra. Chlorophyll concentration, however, is not necessarily an exhaustive index of tissue tolerance. Indeed, Yeo, Caporn and Flowers (1985) demonstrated that in some rice genotypes, photosynthesis was reduced by half at a sodium concentration in the leaf which did not depress chlorophyll levels. We are led to suggest that cell membrane stability of stressed tissues could also be considered as an index of tolerance and it should be emphasized that, in the present work, as far as electrolyte leakage and RLR values are concerned, Nona Bokra appeared less affected than IR 4630.

The Fo/Fm ratio did not appear as a reliable index of early identification of salt-resistant genotypes, since a significant cultivar effect was not recorded before day 18 of stress.
Most rice cultivars differing in salt resistance differed in $F_m$ values. This is considered to reflect a reduction in the amount of the primary electron acceptor $Q_a$. A decrease in $F_m$ values in stressed plants could result either from photochemical quenching, from non-radiative energy dissipation in the pigment bed or from transfer to PS I (Björkman, 1987; Krause and Weis, 1991). It is possible that differences among the cultivars arose from differences in the thylakoid membrane properties. Other parameters related to chlorophyll fluorescence should be analysed in our rice cultivars to bring complementary information on the nature of constraints acting on photosynthetic processes.

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LITERATURE CITED


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