**Growth and Water Relations of Sun-cured Tobacco Irrigated with Saline Water**

by

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**SUMMARY**

We have investigated the effects of saline irrigation on growth and water relations of two sun-cured tobacco genotypes, Xp102 and Px107, which belong to the Xanthia and Perustitza tobacco ecotypes, respectively. We compared three commercial sea salt concentrations of the irrigation water (0.25%, 0.5%, and 1% w/v) plus a non-salinized control, corresponding to an electrical conductivity (ECw) of 4.4, 8.5, 15.7, 0.5 dS m⁻¹ and osmotic potentials of −0.22, −0.35, −0.73, −0.02 MPa, respectively. The ECsoil increased with the salinity of the irrigation water. At high salinity (1%), the soil where Px107 plants were grown showed a significantly higher salinity compared to the soil of Xp102. For both genotypes, the soil water content increased at increasing salinity and during the growth season. Increasing salinity progressively reduced the leaf turgor pressure and enhanced the cellular osmotic adjustment. The latter resulted to be more pronounced in Px107 compared to Xp102 (0.36 vs. 0.20 MPa). At higher salinity (0.5% and 1%), both genotypes showed reduced leaf surface area, dry matter accumulation, water use, net assimilation rate (NAR) and crop growth rate (CGR). Px107 roots were more sensitive than shoot to salinity (3% reduction per dS m⁻¹) and compared to Xp102 roots, which showed a reduced development only at 1% salinity. Assessment of plant salt tolerance according to the Maas and Hoffman model revealed a slope of 1–2% for both genotypes, indicating that these tobaccos are relatively more salt tolerant compared to other species. [Beitr. Tabakforsch. Int. 20 (2003) 394–401]

**RESUME**

Les effets de l’ irrigation avec de l’ eau salée sur la croissance et les relations hydriques chez deux génotypes de tabac sun-cured Xp102 et Px107, appartenant aux écotypes Xanthia et Perustitza, ont été examinés. Trois concentrations en eau de mer de l’ eau d’ irrigation commerciale (0.25%, 0.5% et 1% w/v) et un témoin alimenté avec de l’ eau non-salée, correspondant à une conductivité électrique (CEw) de 4.4, 8.5, 15.7 et 0.5 dS m⁻¹, et ayant des potentiels osmotiques de −0.22, −0.35, −0.73 et −0.02 MPa ont été comparées. La CEw augmente avec la salinité de l’ eau d’ irrigation. Dans le cas d’une salinité élevée (1%), le sol où
INTRODUCTION

In the areas where Oriental tobaccos are generally cultivated, the spring-summer rainfall is sufficient to fulfill plant water requirements. In contrast, where high evaportranspiration and scarce summer rainfall coexist, tobacco crops are exposed to severe water stress, which will eventually affect quality and quantity of harvested leaves (27). Consequently, this crop is generally irrigated. However, since the available water in those areas is scarce and often saline, the choice of the most suitable genotype may be of critical importance for optimal productions (16).

**Nicotiana tabacum** is both a cultivated plant and a model system in plant biology, therefore the effects of NaCl toxicity on plant physiology and biochemical activities have been thoroughly documented in the literature. An excess of salt in the soil solution decreases its osmotic potential and the water availability, which will subsequently cause cellular turgor loss in leaves and roots (35). In saline environments, photosynthesis and ion uptake will be impaired, also. Plants will cope with this unfavourable situation by activating several adaptive mechanisms such as osmotic adjustment, one of the most characterised physiological responses (21). During osmotic adjustment tobacco and many other species, accumulate Na+ and Cl− in the vacuole, whereas organic molecules (generally soluble sugars and free amino acids) are “de-novo” synthesised in the cytoplasm (2). Increased proline and osmotin concentrations have been positively correlated to osmotic adjustment in tobacco (1,30). The production of free amino acids in stressed tobacco plants has been observed at different developmental stages (29). In general, older leaves accumulate more proline than younger leaves. It has been also shown that abscisic acid (ABA) activates the synthesis of proline [via regulation of the *P5CS* gene (7)], which in turn facilitates adaptation to hypersaline environments (14,15). The salt tolerance of Oriental tobaccos cultivar Basma grown in a hydroponic system has been associated with the translocation of Na+ and Cl− into oldest leaves (19). In line with this view, ORPHANOS (24) found that the Cl− concentration in flue-cured tobacco cultivar C139, at the flowering stage, linearly increases from the top to the bottom of the plant.

High Cl− concentrations in the soil water affects N, P and K+ uptake, whereas it enhances the assimilation of Ca2+, Mg2+, Na+, Si4+, Mn2+ and Zn2+ (3). Such a nutritional imbalance may affect yield and quality of the harvested leaves. High Cl− concentrations, for example, will delay maturity and will likely yield highly hygroscopic and fragile leaves with a reduced combustibility (9,12,22,33).

Most of the growth results described for mineral nutrient treatments with Oriental tobaccos refer to cell culture and/or *in vitro* experiments, whereas the number of whole plant and/or field studies are rather limited. From an agronomic perspective, plant response to salinity is also dependent on those conditions that will affect the soil-plant-air system and will therefore affect both plant growth and salt tolerance response (4).

The specific goal of this research was to evaluate the salt tolerance of two different Oriental tobacco genotypes.

**MATERIALS AND METHODS**

The experiment was carried out in 1998 at the University of Naples Federico II (Parco Gussonne). We compared the salinity tolerance of two sun-cured tobacco genotypes: the Xp102, which belongs to the Xanthia type tobaccos and the Px107 a Perustitza type variety. Xp102 seeds were kindly donated by the Tobacco Institute of Lecce (Italy), where this cultivar was originally selected based on a polyethylene glycol (PEG)-adapted cell culture selection screening. The Px107 is a widely grown variety. Plantlets were grown at the Tobacco Institute of Scafati (SA, Italy) using an overhead watering system. Seeds were sown on April 1 (1998) and transplanted on May 19. At transplanting, three plants of the same variety were placed into each of 14-L containers filled with soil (Table. 1). Two varieties and three salinity levels (0.25, 0.5 and 1%, w/v) plus a non-salinized control were arranged in a randomized block design with three replications.

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**Table 1. Soil physical and chemical characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Values measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>75%</td>
</tr>
<tr>
<td>Loam</td>
<td>14.0%</td>
</tr>
<tr>
<td>Clay</td>
<td>10.5%</td>
</tr>
<tr>
<td>Lime</td>
<td>traces</td>
</tr>
<tr>
<td>Organic matter</td>
<td>0.76%</td>
</tr>
<tr>
<td>N total</td>
<td>0.066%</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
</tr>
<tr>
<td>F.C. a (-0.03 MPa)</td>
<td>26.3%</td>
</tr>
<tr>
<td>W.P. b (-1.5 MPa)</td>
<td>10.5%</td>
</tr>
<tr>
<td>Bulk density</td>
<td>1.35 t m⁻³</td>
</tr>
<tr>
<td>Electrical conductivity (ECw)</td>
<td>0.26 dS m⁻¹</td>
</tr>
</tbody>
</table>

a F.C. = Field capacity.  
b W.P. = Wilting point.
Destructive measurements were performed on plants from 2 containers per each treatment and each replicate. Salinity treatments were started on 10-day old after transplanting plantlets and were accomplished by distributing, at 5-day intervals, 2.5 L of saline water obtained by adding commercial sea salt (Na⁺ 12.3, K⁺ 3.8, Ca²⁺ 0.02, Mg²⁺ 0.04, Cl⁻ 14.4, SO₄²⁻ 0.03 mol·kg⁻¹) to the irrigation water. The salinity treatments (0.25%, 0.5% and 1%, w/v) corresponded to a final NaCl concentration of 43, 86 and 172 mM, respectively. Electrical conductivity (ECₙ) of and osmotic potentials for the 4 treatments were 0.5 (non-salinized control), 4.4, 8.5 and 15.7 dS m⁻¹ and −0.02, −0.22, −0.35 and −0.73 MPa, respectively. The irrigation volume (2.5 L) included an extra-volume to fulfil leaching requirements.

Twelve days after transplanting (DAT), 50 kg ha⁻¹ N, 75 kg ha⁻¹ P₂O₅ and 125 kg ha⁻¹ K₂O were supplied. Plant growth parameters were measured at 7-day intervals. During the growth season, plant water relations, plant height, number of leaves per plant, leaf area, dry weight, crop growth rate (CGR), net assimilation rate (NAR), soil EC and water content were measured. In addition, soil samples were taken for root analysis. Soil EC was measured only 4 times (June 25, July 2, 16 and 23; i.e., 37, 44, 58, and 65 DAT). Leaf area development was expressed as root density (root length per soil volume, cm cm⁻³).

Leaf osmotic adjustment (OA) during the growth season was determined as the difference between leaf fresh weight – leaf dry weight)/(leaf saturated weight – leaf dry weight) (34, 21).

Root development was expressed as root density (root length per soil volume, cm cm⁻³). Measurements were performed the day before irrigation occurred. Data were analysed by analysis of variance (ANOVA). Levels of significance of differences between treatments were determined using Duncan’s multiple range test ($P < 0.05$).

**RESULTS**

**Weather conditions**

Rainfall during the experiment was 37.2 mm and it was unevenly distributed during the season. The greater rainfall (22 mm) was on May 21, 2 DAT, therefore it did not influence soil EC and plant water status. The air temperature was within the optimal range for tobacco. Air temperature and rainfall were slightly below the 1991–99 average.

**Soil EC and pH**

The ECₙ at 25 °C increased with the salinity of the irrigation water (Figure 1). Maximum EC values (1% treatment) were reached on July 2 (44 DAT) and were relatively stable until the end of the season. The ECₙ was relatively low in the non-salinized control treatment and it did not significantly change during the season. At high salinity (1%), the soil where Px107 plants were grown showed a significantly higher salinity compared to soil of the Xp102 genotype (3.1 vs. 2.5 dS m⁻¹).

The pH was stable, around 7.4, during the growth season and among different treatments.

**Soil water content**

For both genotypes, the soil water content increased at increasing salinity and during the growth season (Figure 2). However, by the end of the season, the soil water content in Xp102 plots was significantly higher compared to Px107, especially at 0.5 and 1% salinity levels.

**Plant water status**

Total leaf water potential ($Ψ_p$) was significantly affected by increasing salinity (Table 2). However, at the end of the growing season, leaf $Ψ_p$ of 0.5 and 1% treated plants did not significantly differ. The osmotic potential ($Ψ_o$) showed an analogous trend for all treatments (Table 2), nevertheless

Figure 1. Electrical conductivity of soil extracts (ECₙ) during 4 sampling days of the growth cycle. Values are mean of 6 replications ± S.E.
no differences between 0.25% and non-salinized control plants were detected during the vegetative stage (data not shown). Similarly, cellular leaf turgor decreased at increasing salinity (Figure 3). The lowest turgor pressure values (0.2 MPa) were measured for 0.5% and 1% treated plants. Px107 leaves manifested a higher turgor pressure compared to the other genotype tested, particularly at 1% salinity (0.23 vs. 0.18 MPa).

A significant osmotic adjustment was always measured in salinized plants. Specifically, osmotic adjustment increased until the week before the appearance of floral buds and it decreased subsequently to reach a low value by the end of the season (Figure 4a). It was generally higher at high salinity levels. Interestingly, Px107 plants showed a significantly higher osmotic adjustment compared to Xp102 (0.36 vs. 0.20 MPa), at each salt concentration tested. The diurnal osmotic adjustment (Figure 4b) increased early in the season and decreased between days 37 and 44: to reach stable values when flower buds appeared.

**Plant growth**

Px107 plants height was already inhibited at low salinity (Table 2). In contrast, Xp102 plants grown at 0.25% NaCl did not differ from non-salinized control plants. The maximum leaf number per plant in Px107 was smaller than non-salinized control plants only at 0.5% and 1% salinity levels, whereas no differences were detected at 0.25% NaCl.

Leaf area was larger in Px107 plants compared to Xp102 (Figures 5a and 5b). However, both genotypes were significantly affected by salinity. The reduced leaf area at increasing salinity was associated to a smaller mean leaf area per plant (Table 2). Leaf area and turgor pressure were positively correlated ($r^2 = 0.74, P = 0.01$).

Leaf and plant dry weights accumulation in response to salinity and time were similar to the leaf area development, with a higher salt sensitivity in Xp102 plants (Table 2, Figures 6a and 6b).
Table 2. Mean values of some morphological and physiological parameters. Values with the same letters within a column are not statistically different at $P = 0.05$ level. Plant height and leaf number values are from plants at the end of the experimental period (65 DAT). $\psi_i$, $\psi_w$: dry weight, mean leaf area, root density, NAR and CGR values are average over all the growing period.

<table>
<thead>
<tr>
<th>Plant treatment</th>
<th>$\psi_i$ MPa</th>
<th>$\psi_w$ MPa</th>
<th>Plant height cm</th>
<th>No. of leaves max plant$^1$</th>
<th>NAR g d$^{-1}$ dm$^{-2}$</th>
<th>Mean leaf area dm$^2$</th>
<th>CGR g d$^{-1}$</th>
<th>Dry weight g plant$^{-1}$</th>
<th>Root density cm cm$^{-3}$</th>
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<tbody>
<tr>
<td>Xp102</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>-1.32 c</td>
<td>-1.62 c</td>
<td>80 a</td>
<td>15.8 a</td>
<td>0.0176 a</td>
<td>1.40 a</td>
<td>0.52 a</td>
<td>28.42 a</td>
<td>16.5 a</td>
</tr>
<tr>
<td>0.25 %</td>
<td>-1.46 b</td>
<td>-1.74 b</td>
<td>79 a</td>
<td>15.9 a</td>
<td>0.0172 a</td>
<td>1.32 b</td>
<td>0.49 a</td>
<td>26.91 b</td>
<td>16.0 a</td>
</tr>
<tr>
<td>0.5%</td>
<td>-1.62 a</td>
<td>-1.81 a</td>
<td>72 b</td>
<td>16.3 a</td>
<td>0.0175 a</td>
<td>1.24 c</td>
<td>0.45 b</td>
<td>24.59 c</td>
<td>16.0 a</td>
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<tr>
<td>1%</td>
<td>-1.63 a</td>
<td>-1.85 a</td>
<td>65 c</td>
<td>15.7 a</td>
<td>0.0146 b</td>
<td>1.16 d</td>
<td>0.36 c</td>
<td>18.85 d</td>
<td>11.2 b</td>
</tr>
<tr>
<td>Mean</td>
<td>-1.51</td>
<td>-1.76</td>
<td>74</td>
<td>15.9</td>
<td>0.0167</td>
<td>1.28</td>
<td>0.46</td>
<td>24.69</td>
<td>14.9</td>
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<td>Pxl07</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0%</td>
<td>-1.21 c</td>
<td>-1.53 c</td>
<td>127 a</td>
<td>24.5 a</td>
<td>0.0202 b</td>
<td>1.20 a</td>
<td>0.75 a</td>
<td>39.29 a</td>
<td>16.9 a</td>
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<tr>
<td>0.25 %</td>
<td>-1.44 b</td>
<td>-1.71 b</td>
<td>112 b</td>
<td>25.2 a</td>
<td>0.0215 a</td>
<td>1.11 b</td>
<td>0.73 a</td>
<td>37.93 b</td>
<td>15.2 b</td>
</tr>
<tr>
<td>0.5%</td>
<td>-1.57 a</td>
<td>-1.82 a</td>
<td>107 c</td>
<td>23.9 ab</td>
<td>0.0206 b</td>
<td>1.13 c</td>
<td>0.66 b</td>
<td>34.44 c</td>
<td>12.2 c</td>
</tr>
<tr>
<td>1%</td>
<td>-1.66 a</td>
<td>-1.89 a</td>
<td>100 d</td>
<td>23.3 b</td>
<td>0.0188 c</td>
<td>1.08 d</td>
<td>0.60 b</td>
<td>31.72 d</td>
<td>10.2 d</td>
</tr>
<tr>
<td>Mean</td>
<td>-1.47</td>
<td>-1.74</td>
<td>112</td>
<td>24.2</td>
<td>0.0203</td>
<td>1.13</td>
<td>0.69</td>
<td>35.85</td>
<td>13.6</td>
</tr>
</tbody>
</table>
Figure 5. Leaf area development during the growth season for a) Xp102 and b) Px107. Values are mean of 18 plants ± S.E.

The NAR (Table 2) was higher in Px107. During the growth cycle, Xp102 showed NAR reductions only at high salinity (1%). In contrast, the NAR in Px107 plants decreased during the season at 0.5% and 1% levels of salinity tested. Similar trends were observed for CGR (Table 2). Root development increased until flowering and it decreased at later stages of development (Figure 7). The two genotypes showed a different response to salinity (Table 2). Xp102 roots were affected only at high salinity (1%) levels, whereas Px107 root density decreased linearly at increasing salinity.

DISCUSSION AND CONCLUSIONS

Growth rates for non-salinized control plants were similar to those obtained for other field experiments performed in similar environments (10). The increase of soil water content in Xp102 should be associated with the lower leaf area and lower transpiration of this cultivar, particularly at 0.5% and 1% salt levels (Figure 5). This has been shown also for other crops grown

Figure 6. Leaf dry matter accumulation during the growth season for a) Xp102 and b) Px107. Values are mean of 18 plants ± S.E.

Figure 7. Root density during the growth season. Values are mean of the two cultivars and 6 containers ± S.E.
in open field (5, 28). Probably, with higher soil water content, the leaching was greater and salt accumulation in the soil of the Xp102 was lower than in Px107. In general, salinity did not have an effect on plant mortality and leaf number, nevertheless the later parameter seemed to be affected in Px107 plants at high salinity. Also it has clearly been established that plants exhibit salt sensitivity during the first week after germination (6, 18), so in an agronomic context this is not critical for tobacco because during the first week following germination, plantlets are grown in the nursery and are usually irrigated with good quality water. In advanced stages of development (after transplanting), salinized plants had reduced leaf water potential and cellular turgor, which were both correlated to a reduced leaf area. Osmotic adjustment was similar to that reported for other species (21), and it significantly contributed to maintain high turgor in salinized plants. Nevertheless, for both genotypes tested, the osmotic adjustment was not sufficient to restore the turgor to the level of non-salinized control plants.

Osmotic adjustment was higher in Px107, which also had higher turgor. Variable osmotic adjustment capability among different cultivars has been reported for other species, including sorghum (37), wheat (20), and cotton (11). Based on the MAAS and HOFFMAN (17) model, it was not possible to identify a tolerance threshold with sufficient accuracy. However, leaf area, plant and leaf dry matter in Px107 and leaf area are in Xp102 decreased 1% per each dS m⁻¹ increment of the irrigation water. The tolerance slope was slightly higher, 2%, in Xp102 (as related to plant and leaf dry matter). These values are significantly lower than those reported for other species (26). At moderate salinity (0.5%), the inhibitory effect of salt on the NAR was more evident in Px107 compared to Xp102. However, these differences disappeared at high salinity (1%).

A difference in root response to salinity was observed between the two cultivars. Xp102 roots were not sensitive to salt stress until 0.5% NaCl, whereas Px107 root density decreased linearly at increasing salinity. In addition, Px107 roots were more sensitive than leaves to salt (3% decrease per each dS m⁻¹ increase vs. 2% decrease for Xp102 per each dS m⁻¹ increase). A different partitioning of photo-synthates in the two cultivars may have affected the root/shoot ratio in presence of salt, which in turn may be associated to a different level of tolerance (4). The higher tolerance of Xp102 roots may be correlated to a more efficient osmoregulation of root vs. shoot cell. It is also possible that the observed response is functionally activated to compensate for the reduced efficiency of root systems under saline conditions, as reported by other authors for other species (32).

Probably part of the differences between the two cultivars may be associated to the PEG selection of Xp102 plants, yet this cannot be conclusively established since Px107 is not the wild type of Xp102. These two ecotypes have a different genetic background. Therefore possible responses attributable to the PEG selection cannot be established.

We may conclude that both genotypes showed a significant salt tolerance compared to other crops. Specifically, Px107 leaves were able to osmoregulate more efficiently compared to Xp102. In contrast, Xp102 roots were more tolerant until 0.5% NaCl. These data suggest that, from an agronomic perspective, the choice of the most appropriate genotype may play an important role for optimal production in saline environments.

REFERENCES

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