Effects of salinity stress on growth in relation to gas exchanges parameters and water status in amaranth (*Amaranthus cruentus*)

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Salinity is a major detrimental abiotic factor for plant growth. The main purpose of this study was to analyze the effects of different NaCl concentrations on growth and some physiological parameters related to gas exchanges and water relations in amaranth (*Amaranthus cruentus*) plants. Three weeks old amaranth plants from the cultivar ‘Locale’ were exposed in nutrient solution to 0, 30 or 90 mM NaCl (electrical conductivities of 1.915; 4.815 and 11.70 dS.m⁻¹ respectively) in phytotron conditions. Shoot elongation as well as fresh and dry masses of shoot and root were determined after two weeks of stress exposure. Net photosynthesis (A), intercellular CO₂ concentration (Ci), instantaneous transpiration (E), stomatal conductance (gs), osmotic potential (Ψs) as well as the efficiency of the instantaneous carboxylation (A/Ci), intrinsic (A/gs) and instantaneous (A/E) water use efficiency were estimated. Results reveal that salt stress induced a significant reduction in growth of aerial part as well as net photosynthesis, instantaneous transpiration, stomatal conductance and leaf and root osmotic potentials. In contrast, no significant reductions were recorded for root growth, shoot water content, intercellular CO₂ concentration and instantaneous carboxylation efficiency. However, a significant increase was observed for intrinsic (A/gs) and instantaneous (A/E) water use efficiency. The plant growth reduction observed hinges upon a drop in photosynthetic activity due mainly to stomatal closure. These data suggest that photosynthetic activity may be used as a reliable criterion for physiological estimation of salt-tolerance in *A. cruentus* cultivars.

Key words: Saline stress, net photosynthesis, stomatal conductance, osmotic potential, water use efficiency.
INTRODUCTION

Salinity is one of the most important environmental constraints that limits plant productivity, particularly in arid and semi-arid climates (Ashraf and Harris, 2004; Hussain et al., 2009). Indeed, more than 800 million hectares of arable lands are affected by soil salinity worldwide including about 45 million hectares of irrigated lands (Munns and Tester, 2008). The problem increases due to inadequate agricultural practices (Shannon and Grieve, 1999; Villa-Castorena et al., 2003; Munns, 2005) and sea level rise (Munns, 2005). Excess of saline ions in soils generates an elevated osmotic pressure and an accumulation of toxic ions in plant tissues, notably Na⁺, and consequently induces a decrease in growth and crop yield due to a disruption of several physiological processes (Munns, 2002).

Photosynthesis is an important metabolic pathway that is considered to be salt-sensitive (Munns et al., 2006; Chaves et al., 2009). Salinity reduces photosynthesis by inducing stomatal closure preventing CO₂ diffusion (Brugnoli and Lauteri, 1991). Salinity may also affect non-stomatal properties such as chlorophyll synthesis, photosystem structure, electron transport (Lee et al., 2004), efficiency of the ribulose-1,5-bisphosphate carboxylase/oxygenase for carbon fixation (Delfine et al., 1998; Jaleel et al., 2007; Megdiche et al., 2008), and photophosphorylation (Stoeva and Kaymakanova, 2008).

Salt stress also impacts the water supply of the plant. Plant-water relations have rather important implications on the physiological and metabolic processes conditioning plant growth (Passioura, 2010). Salinity indeed frequently induces plant dehydration in relation to a decrease in the osmotic potential of external soil solution which prevents water absorption by the root system (Álvarez et al., 2012).

Amaranth (Amaranthus spp.) species are tropical crops used as pseudo-cereals or leafy vegetables with a high nutritional value and large adaptability to various environments mainly marginal lands and semi-arid regions where salinity issue is sharp (Cunningham et al., 1992; Allemann et al., 1996; Bhattacharjee, 2008). In Benin, amaranth species are extensively cultivated on the arable lands from costal zones where availability of good-quality water and salinity pose serious threats (Wouyou et al., 2016; 2017). Previous studies showed that NaCl concentrations ranging from 30 to 200 mM reduce aerial and root parts growth in different genotypes of amaranth including cultivars of Amaranthus cruentus (Makus, 2003; Omami and Hammes, 2005; Ornami and Hammes, 2006; Qin et al., 2013; Amukali et al., 2015; Lavini et al., 2016; Wouyou et al., 2017). However, the physiological and biochemical mechanisms involved in such a growth reduction remain largely unknown. The main goal of the present study is therefore to analyze the effect of salinity on growth, stomatal conductance, net photosynthesis, transpiration, osmotic potential, efficiency of instantaneous carboxylation and water use efficiency in the A. cruentus in order to obtain additional information on the main factors limiting plant growth in this species.

MATERIALS AND METHODS

Plant material and salinity stress treatment

Seeds of the cultivar ‘Locale’ were germinated in jars filled with substrate (Substrate NFU 44-551) for a week. The composition of the substrate is shown in Table 1. The obtained young seedlings were individually transferred in pots containing the same substrate for one further week in a growth chamber characterized by a 25/21°C (day/night) temperature, a 16/8 h (day/night) photoperiod, a light intensity between 150-220 µmole.m⁻².s⁻¹ using white fluorescent tubes (F36W/840-T8), Daytime humidity was set to c.a. 65%. Plants were then transferred to tanks containing a modified Hoagland solution (Went, 1957) with pH 6. Stress application was carried out after one week on three weeks old plants. Treatments consisted of three NaCl concentrations: 0, 30 and 90 mM corresponding to an electrical conductivity of 1.915, 4.815 and 11.70 dS.m⁻¹, respectively. All treatments were repeated three times in a complete randomized design and each repetition consisted in a pooled sample of six plants. Eighteen plants were thus considered per treatment (Figure 1). Salinity stress was maintained over a period of two weeks.

Measurement of growth parameters and water contents

Shoot height, root length as well as fresh and dry biomasses of the shoot and roots were determined after two weeks of stress application. Shoot height was measured at the time of stress imposition (Hi) and after two weeks of treatment (Hf). The relative growth in height was calculated according to the formula: RHG = (Hf - Hi)/Hi. Root elongation was estimated according to the same procedure. Shoot and root fresh biomasses were determined after two weeks. Samples were then transferred to an oven at 80°C for 72 h for dry biomass determination. Water content was determined as [fresh mass - dry mass]/ fresh mass] x 100.

Measurement of physiological parameters

All physiological parameters were determined after two weeks treatment. Stomatal conductance (gₛ) was estimated at the mid photoperiod on the youngest fully unfolded leaf on three plants per treatment using a porometer (AP4-UM-3, Delta-T Devices, Cambridge, United Kingdom). Net photosynthesis (A, net rate of
Table 1. Composition of the Substrate NFU 44-551 used for plant growth.

<table>
<thead>
<tr>
<th>Component</th>
<th>Fertilizer</th>
<th>Dry matters/ raw product</th>
<th>Organic matters/ Dry product</th>
<th>pH (H2O)</th>
<th>Conductivity</th>
<th>Water retention capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values</td>
<td>1 kg/m³</td>
<td>50%</td>
<td>85%</td>
<td>6.5</td>
<td>40 mS/m</td>
<td>750 ml/L</td>
</tr>
<tr>
<td>NPK 14-8-14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Experimental design with plants at the beginning of stress imposition.

carbon assimilation) was measured under a constant photosynthetic flux of photons (500 µmol.m⁻².s⁻¹), instantaneous transpiration (E) and internal CO₂ content (in sub-stomatal chamber) (Ci) were measured on the youngest fully expanded leaf of three plants per treatment using a water vapor analyzer (LCA 2 8.7, ADC, Great Amwell, England) and an air supply unit (ASU 10.87, ADC) set up in series in an open system. The efficiency of the instantaneous carboxylation was calculated as A/Ci according to Zhang et al. (2001) whereas the intrinsic (A/gs) and instantaneous (A/E) water use efficiency were calculated according to de Oliveira et al. (2015). To measure the osmotic potential (Ψs), roots and leaves of three plants per treatment were rapidly rinsed in deionised water, frozen in liquid nitrogen just after harvest. They were then cut in small pieces and placed in a perforated Eppendorf tube which was encased in a second intact tube. After 3 cycles of freeze/thawing, the samples were centrifuged at 15,000 g during 15 min at 4°C. The extracted sap was used to measure the osmolarity (c) using an osmometer with steam pressure Wescor 5500 as described earlier by Lutts et al. (1999). The Ψs was then calculated with the following formula:

Ψs (MPa) = −c (mosmoles.Kg⁻¹) × 2.58 × 10⁻³ according to Van’t Hoff equation.

Statistical analysis

For all variables, the data are expressed in the form of mean ± standard error after averaging results over three replications per treatment. Stress effect on a given parameter was performed on the basis of a one-way variance analysis (ANOVA). Means were compared by the Tukey-Kramer test. All analysis was performed with the JMP software (SAS Institute, 2015).

RESULTS AND DISCUSSION

Effects of saline stress on plant growth

Salt stress effects resulted in a decrease of all estimated growth parameters (Table 2). The reduction of growth under salinity in comparison to the control was 20, 17, 22, 39, 32 and 9% with 30 mM NaCl, respectively for the relative shoot height growth (RHG), shoot fresh and dry masses (SFM and SDM), relative root elongation (RRLG), root fresh and dry masses (RFM and RDM). At
Effects of saline stress on physiological parameters

Salt stress induced a significant reduction (P < 0.05) of net photosynthesis (A) (Figure 2A). Indeed A values decreased from 2.39 μmolCO₂ m⁻² s⁻¹ (control) to 1.76 at 30 mM NaCl, and then to 1.16 μmolCO₂ m⁻² s⁻¹ under 90 mM NaCl. These values correspond to a reduction of the photosynthetic activity of 26.36 and 51.47% comparatively to control, respectively. The reduction of photosynthetic capacity under salt stress has been reported in numerous species and is considered to be, at least partly, responsible for salt-induced growth reduction (Liu et al., 2011; da Silva, 2011; Saleem et al., 2011; Shahid et al., 2011; Shaheen et al., 2013; R'him et al., 2013). However, according to Omami and Hammes (2006) and Munns and Tester (2008), the effect of salinity on the photosynthetic activity depends upon the salt concentration and the plant species. In Bruguiera parviflora, Parida et al. (2002) reported that low levels of salinity even stimulated photosynthesis while high levels clearly reduced it. In our study, a reduction of the photosynthetic activity was observed under all used NaCl concentrations, Omami and Hammes (2006) similarly showed that all NaCl concentrations up to 100 mM NaCl decreased photosynthesis in different amaranth species. We may therefore hypothesize that photosynthesis inhibition is a major component of growth inhibition in A. cruentus. The net photosynthesis reduction along with saline stress application would not only be related to the growth reduction (Cramer and Bowman, 1991; Foyer and Noctor, 2005; Passioura and Munns, 2000), but also to an increase in carbohydrate accumulation acting in a negative feed-back (Munns et al., 2000). However, Munns and Tester (2008) considered that it is always difficult to conclude whether a reduction in photosynthetic activity is the cause or the consequence of growth inhibition. Salt stress decreased the intercellular CO₂ concentration (Ci) (Figure 2B) which ranges from (control) 314 to 345 in 30 mM NaCl and 332.67 μmol.mol⁻¹ in 90 mM NaCl. The recorded decrease remained however limited from a relative point of view (9.07 and 3.67%, respectively in comparison with the control) and was not significant. In eggplant, Shaheen et al. (2013) found that saline stress did not affect the intercellular CO₂ concentration. In a study carried on two perennial Gramineae species, Liu et al. (2011) found out that in the sensitive species Eremochloa ophioides, salt stress provoked an increase of the intercellular CO₂ concentration, notably at high NaCl concentrations, whereas in the tolerant species Paspalum vaginatum, evolution of intercellular CO₂ concentration was variable depending on stress duration and intensity. In our study, NaCl had no impact on the instantaneous carboxylation efficiency (A/Cl) (Figure 2C) ranging from 0.0069 (control) to 0.0057 under 30 mM NaCl and 0.0035 mol.m⁻² s⁻¹ under 90 mM NaCl. The observed losses correspond respectively to 17.39 and 49.28% reported to the control treatment and might be explained by an inhibition of the carboxylase activity of RubisCO (da Silva et al., 2011).

An inhibition of shoot growth may also lead to...
photosynthetic accumulation in stressed tissues that would, in turn, generate a feedback signal towards the reduction of photosynthetic activity. The physiological and enzymatic mechanisms implied in these regulations have been questioned in details by Cramer and Bowman (1991), Passioura and Munns (2000), Apel and Hirt (2004), Fricke et al. (2004), Foyer and Noctor (2005), Logan (2005) and Møller et al. (2008). Sobrado (2005) reported that inefficiency of stomatal regulation in stressed plants may directly impair both leaf photosynthetic capacities and biochemical processes. Several works also reported a decrease in chlorophyll content (Koyro 2006; Geissler et al. 2009). The drop of the chlorophyll’s content could be assigned either to the reduction of its biosynthesis or to a stimulation of chlorophyllase activity (Ashraf and Bhatti 2000). In our study, saline stress induced a significant reduction (p<0.01) of the stomatal conductance (Figure 2D) that was 63.33 (in control), 40.50 (with 30 mM NaCl in the growth media) and 21.17 mmol H$_2$O m$^{-2}$s$^{-1}$ (under 90 mM NaCl). Investigating different genotypes of amaranth, Ornami and Hammes (2006) also reported a significant reduction of stomatal conductance. Results presented here matched well with those mentioned earlier in pepper (Lycoskoufis et al., 2005; Niu et al., 2010; R’him et al., 2013) and tomato (Baker and Rosenqvist, 2004).
Stomatal closure is required for maintenance of water content and could thus be regarded as an adaptive strategy of plants to uphold water whenever facing the osmotic stress under salinity (Shaheen et al., 2013; Davies et al., 2005). This stomata closure might be responsible for the low photosynthetic intensity recorded under salinity conditions. The fact that shoot water content did not change significantly under salt stress (Table 3) supports the hypothesis that stomatal closure efficiently contributes to regulate shoot water content in salt-treated *Amaranthus*.

The effect of salt stress resulted in a significant reduction (p<0.05) of instantaneous transpiration (E) (Figure 2E) ranging from 1.16 (without salt stress: control) to 0.61 (by 30 mM NaCl stress) and 0.53 mmol H$_2$O m$^{-2}$s$^{-1}$ (under 90 mM NaCl stress), consisting in a reduction of 47.41 and 54.31%, respectively in comparison with the control. In four amaranth genotypes, Oram and Hammes (2006) observed a decrease in instantaneous transpiration (transpiration rate) of salt-treated plants. A similar tendency has been reported in the genus *Brassica* (Ashraf, 2001). The reduction of instantaneous transpiration observed in our study is obviously the consequence of a decrease in stomatal conductance. Other studies showed that the salt-induced stomatal closure might contribute to avoid the build-up of the toxic ion flux through the transpiration stream (Kerstiens et al., 2002; Vysotskaya et al., 2010). Thus, Koyro (2006) suggested that stomatal conductance reductions represent an adaptive mechanism to face salt excess, reducing the amounts of toxic ions in leaves and thus contributing to avoid premature senescence of photosynthetic tissues.

The effect of salt stress resulted in a significant increase in plant intrinsic (A/gs) (p<0.001) and instantaneous (A/E) (p<0.05) (Table 4) water use efficiency (WUE). The intrinsic WUE ranged from 0.038 (without salt stress: control) to 0.068 (by 30 mM NaCl stress) and 0.115 μmolCO$_2$/mmolH$_2$O (under 90 mM NaCl stress), consisting in an increase of 78.95 and 202.63%, respectively in comparison with the control; whereas instantaneous WUE ranged from 2.077 (without salt stress: control) to 3.252 (by 30 mM NaCl stress) and 4.336 μmolCO$_2$/mmolH$_2$O (under 90 mM NaCl stress), consisting in an increase of 56.25 and 108.65%, respectively in comparison with the control. Similar results were obtained in *Eugenia myrtifolia* and *Callistemon citrinus* plants submitted to salt stress which were able to increase their intrinsic WUE throughout most of the growing season indicating that the plants maintain higher net photosynthesis rate (A) levels despite reduced stomatal opening (Álvarez and Sánchez-Blanco, 2014; Acosta-Motos et al., 2017).

Salinity induced significant reductions in the leaf and root osmotic potentials (Ψs) (Table 5). In leaves, osmotic potential ranged from -1.23 MPa (under 0 mM NaCl) to -1.75 MPa (30 mM NaCl) and -3.42 MPa (under 90 mM NaCl stress). In roots, osmotic potential dropped from -0.87 MPa (without salt stress) to -1.35 MPa (30 mM NaCl) and -2.14 MPa (90 mM NaCl). It is well known that the presence of salt excess in rhizosphere leads to reductions in osmotic potential and consequently, contributes to the decrease in plant water potential (Sánchez-Blanco et al., 2004; Munns, 2005; Franco et al., 2011). Our results are in agreement with those reported earlier in sunflower (Akram et al., 2012).

### Table 3. Effect of different NaCl concentrations on shoot water content of *Amaranthus cruentus* cv. 'Locale' after two weeks stress application.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NaCl concentrations (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Shoot water content (%)</td>
<td>88.93±0.87$^a$</td>
</tr>
</tbody>
</table>

Each value is the mean of three replicates ± S.E. Means followed by the same letter are not significantly different at P < 0.05.

### Table 4. Effect of saline stress on instantaneous (A/E, μmolCO$_2$/mmolH$_2$O) and intrinsic (A/gs, μmolCO$_2$/mmolH$_2$O) water use efficiency in amaranth young plants of the cultivar 'Locale' after two weeks culture on media containing three NaCl concentrations (0, 30 and 90 mM).

<table>
<thead>
<tr>
<th>NaCl concentration (mM)</th>
<th>A/E</th>
<th>A/gs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.077±0.097$^a$</td>
<td>0.038±0.0026$^a$</td>
</tr>
<tr>
<td>30</td>
<td>3.252±0.211$^{ab}$</td>
<td>0.068±0.0049$^b$</td>
</tr>
<tr>
<td>90</td>
<td>4.336±0.556$^b$</td>
<td>0.115±0.017$^c$</td>
</tr>
</tbody>
</table>

Each value is the mean of three replicates ± S.E. Means followed by the same letter within a column are not significantly different at P < 0.05.
Table 5. Effect of saline stress on osmotic potential (Ψs) of leaves and roots measured at amaranth young plants of the cultivar ‘Locale’ after two weeks culture on media containing three NaCl concentrations (0, 30 and 90 mM).

<table>
<thead>
<tr>
<th>NaCl concentration (mM)</th>
<th>Leaves</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-1.23±0.11a</td>
<td>-0.87±0.14a</td>
</tr>
<tr>
<td>30</td>
<td>-1.75±0.10b</td>
<td>-1.35±0.04ab</td>
</tr>
<tr>
<td>90</td>
<td>-3.42±0.31b</td>
<td>-2.14±0.28b</td>
</tr>
</tbody>
</table>

Each value is the mean of three replicates ± S.E. Means followed by the same letter within a column are not significantly different at P < 0.05.

CONFLICT OF INTERESTS
The authors have not declared any conflict of interests.

REFERENCES

 Conclusion
The reduction of plant growth under salt stress is due to a loss in photosynthetic activity mostly related to stomatal closure. The maintenance of high water use efficiency appeared as an important strategy to face salt stress in A. cruentus. The results exposed here provide specific physiological cues for improvement of salt-tolerance in amaranth.

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