Differences in the Vegetative Growth between Common and Tartary Buckwheat in Saline Hydroponic Culture

Asana Matsuura, Shinobu Inanaga and Kouhei Murata

Department of Plant Science of Kyushu Tokai University, Kumamoto 869-1404, Japan; Japan International Research Center for Agricultural Sciences; Ibaraki, 305-8686, Japan

Abstract: Common buckwheat (Fagopyrum esculentum Moench cv. Tsushima) and Tartary buckwheat (F. tataricum (L.) Gaertn. cv. Pontivy) were grown in a nutrient solution with or without added NaCl to investigate interspecific differences in their responses to salinity, based on their dry-matter production. The mechanism of salt tolerance was also studied. Addition of 100 mM NaCl to the culture solution (salt treatment) lowered the plant growth rate to 48% and 16% of the control in Tsushima and Pontivy, respectively, and decreased the net assimilation rate and mean leaf area of Pontivy more severely than in Tsushima. The salt treatment decreased the leaf growth rate and leaf area per leaf to 30% and 72% of the control, respectively, in Tsushima, and to 12% and 52%, respectively, in Pontivy. It decreased the photosynthetic rate to 67% and 35% of the control, and stomatal conductance to 25% and 15% of the control in Tsushima and Pontivy, respectively. It also decreased the transpiration rate to 41% and 50% of the control in Tsushima and Pontivy, respectively, and increased the wateruse efficiency 1.6 times in Tsushima, but did not influence the wateruse efficiency in Pontivy. In the saline solution, the accumulation of Na⁺ in leaves and stem was greater in Pontivy than in Tsushima, but that in the roots, was greater in Tsushima than in Pontivy. In both species, Na⁺ accumulated rapidly in the leaves after removal of the roots in the saline solution. We conclude that the difference in salt tolerance between common and Tartary buckwheat may result from the difference in accumulation of Na⁺ in leaves and absorption of Na⁺ by the roots.

Key words: Buckwheat, Interspecific difference, Salt tolerance, Sodium, Vegetative growth.

High salinity decreases crop growth and yield, but its severity can be mitigated by a large amount of precipitation, and good drainage to streams, rivers, the sea and deep water table (BOSTID and NRC, 1990). There is a short-term reduction in salinity after precipitation in saline desert sites and arid regions (Orcutt and Nilsen, 2000). Buckwheat may be suitable for cultivation in saline conditions because its growing period is very short. Interspecific differences in the reduction of growth due to salinity have been reported for several plants (Maas and Hoffman, 1977; Yamanouchi et al., 1990; Katerji et al., 2000). However, there is little information on the salt tolerance of buckwheat. Kobayashi (1954) cultivated many plant species in a polder land in Okayama Prefecture, Japan and reported that buckwheat grew as well as Brassica and Solanum species under saline conditions of around 1% salt concentration and that its salt tolerance was higher than that of several gramineous crops and legumes, but lower than that of cotton, rape, wheat and barley. Some crops can utilize sodium instead of potassium, and some crops need sodium for their growth (Subbrarao et al., 2003). Common buckwheat cannot utilize sodium even in a potassium-deficient condition (Harmer and Benne, 1945). Our objective was to investigate the differences between common and Tartary buckwheat in the response to salinity based on dry-matter production, and to determine the mechanism of salt tolerance.

Materials and Methods

1. Plant materials and culture

Common buckwheat (Fagopyrum esculentum Moench cv. Tsushima) and Tartary buckwheat (F. tataricum (L.) Gaertn. cv. Pontivy) were used. Seeds of both species were sown in vermiculite, and the seedlings were transplanted into a culture solution 7 days after sowing. Transplanted plants were grown in a greenhouse at Kyushu Tokai University, Kumamoto, Japan during the summer. The culture solution was half-strength Hoagland and Arnon's nutrient solution (KNO₃: 1057 mg l⁻¹, NH₄H₂PO₄: 115 mg l⁻¹, MgSO₄·7H₂O: 493 mg l⁻¹, Ca(NO₃)₂·4H₂O: 945 mg l⁻¹, EDTA-Fe: 22.6 mg l⁻¹, MnCl₂·4H₂O: 1.801 mg l⁻¹, H₃BO₃: 2.860 mg l⁻¹, H₂BO₃: 945 mg l⁻¹, MnCl₂·4H₂O: 1.801 mg l⁻¹, H₃BO₃: 2.860 mg l⁻¹, H₂BO₃: 945 mg l⁻¹).

Abbreviations: Ci, intercellular CO₂ concentration; DPL, dry-matter partitioning ratio to leaf; DW, dry weight; FW, fresh weight; GR, leaf growth rate; GR₀, root growth rate; GR₁, stem growth rate; gₛ, stomatal conductance; LA₁, leaf area per leaf; LN, number of leaves; MAL, mean leaf area; NAR, net assimilation rate; PGR, plant growth rate; PT, photosynthetic rate; RH, relative humidity; RWG, relative water content; SLA, specific leaf area; Tr, transpiration rate; TW, turgid weight; WL, the dry weight of leaf per plant; WUE, water use efficiency.

Received 12 October 2004. Accepted 2 June 2005. Corresponding author: A. Matsuura (asana@ktmail.ktokai-u.ac.jp, fax +81-967-67-2659).
ZnSO₄ · 7H₂O: 0.220 mg l⁻¹, CuSO₄ · 5H₂O: 0.079 mg l⁻¹ and (NH₄)₂M₀₇O₂₄ · 4H₂O: 0.037 mg l⁻¹). Salt treatment was initiated by adding NaCl at 50 mM and increasing the concentration to 100 mM two days later. As a control, some seedlings were cultured in half-strength Hoagland and Arnon’s nutrient solution without NaCl. To investigate the role of roots in salt tolerance, we cut off the roots including the basal stem below the cotyledonary node of several plants on 8 days after the start of treatment, and cultured the rootless plants in 100 mM NaCl solution for 2 days.

2. Growth measurements

Leaf area and dry weights of whole plant were measured one day before and 8 days after the start of treatment. The shoots were dried at 65°C for 48 hours after exposure to 110°C for 30 minutes. Plant growth rate (PGR), net assimilation rate (NAR) and mean leaf area (MLA) were estimated by the following equations:

\[
PGR(\text{g day}^{-1}) = \frac{W_2 - W_1}{T_2 - T_1} \quad \text{………………(1)}
\]

\[
\text{NAR(g m}^{-2}\text{ day}^{-1}) = \text{PGR} \times \frac{\log L_2 - \log L_1}{L_2 - L_1} \times 10^4 \quad \text{………………(2)}
\]

\[
\text{MLA(cm}^2) = \frac{L_2 - L_1}{\log L_2 - \log L_1} \quad \text{………………(3)}
\]

Where, \(W_1\) and \(W_2\) were the dry weight of whole plant one day before \((T_1)\) and 8 days after \((T_2)\) the start of treatment, respectively; \(L_1\) and \(L_2\) were the total leaf area per plant one day before \((T_1)\) and 8 days after \((T_2)\) treatment, respectively.

The growth rates of leaf (GRL), stem (GRS) and root (GRR) were calculated by the following equations:

\[
\text{GRL(g day}^{-1}) = \frac{WL_2 - WL_1}{T_2 - T_1} \quad \text{………………(4)}
\]

\[
\text{GRS(g day}^{-1}) = \frac{WS_2 - WS_1}{T_2 - T_1} \quad \text{………………(5)}
\]

\[
\text{GRR(g day}^{-1}) = \frac{WR_2 - WR_1}{T_2 - T_1} \quad \text{………………(6)}
\]

where \(WL_1\) and \(WL_2\) were the dry weight of leaf per plant one day \((T_1)\) before and 8 days \((T_2)\) after the start of treatment, respectively; \(WS_1\) and \(WS_2\) were the dry weight of stems per plant one day \((T_1)\) and 8 days \((T_2)\) after the start of treatment, respectively; \(WR_1\) and \(WR_2\) were the dry weight of roots per plant one day \((T_1)\) and 8 days \((T_2)\) after the start of treatment, respectively.

The dry-matter partitioning ratio to leaf (DPL), leaf area per leaf \((LAL)\) and specific leaf area (SLA) were calculated by the following equations:

\[
\text{DPL(\%)=} \frac{WL_2 - WL_1}{W_2 - W_1} \times 100 \quad \text{………………(7)}
\]

\[
\text{LAL(cm}^2) = \frac{L_2}{\text{LN}} \quad \text{………………(8)}
\]

\[
\text{SLA(cm}^2\text{g}^{-1}) = \frac{L_2}{\text{WL}_2} \quad \text{………………(9)}
\]

\(\text{LN}\) was the number of leaves per plant 8 days after \((T_2)\) the start of treatment.

3. Gas exchange rate and relative water content

The photosynthetic (Pr) and transpiration rates (Tr), stomatal conductance (gs) and intercellular CO₂ concentration (Ci) in the fully expanded leaves of three plants each from the control and treatment were measured 7 days after the start of salt treatment using a portable photosynthesis system (Li-Cor Inc., LI-6400). Relative water content (RWC) was also measured in the fully expanded leaves. During the measurement, photosynthetic photon flux density was set at 1000 \(\mu\text{mol m}^{-2}\text{s}^{-1}\), CO₂ concentration 350 ppm and air temperature 30°C during the measurement. The fresh weight (FW) of fully expanded leaves was determined immediately after detachment from the stem at midday. After weighing, leaves were kept at 20°C in sealed plastic bags containing small amounts of distilled water. The turgid weight (TW) was measured five hours later. The dry weight (DW) of the leaves was measured after oven-drying as described previously and the RWC was calculated by the following equation:

\[
\text{RWC(\%)=} \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100 \quad \text{………………(10)}
\]

4. Na⁺ and K⁺ ion content

About 0.5 g each of the oven-dried leaves, stems (including petiole) and roots of control and salt-treated plants harvested 8 days after the start of treatment were digested with sulfuric acid and hydrogen peroxide (Mizuno and Minami, 1980). Leaves and stems of the rootless plants harvested 10 days after the start of treatment were also digested in the same way. The contents of Na⁺ and K⁺ were determined by atomic absorption spectrophotometry.

5. Statistical analysis

All measurements were conducted in triplicate and data were analyzed by the t-test. A regression analysis was also carried out to explore the correlation between PGR with NAR and MLA.

Results and Discussion

1. Plant growth

The treatment with 100 mM NaCl significantly
lowered the plant growth rate (PGR) in both buckwheat species (Table 1). Both buckwheat species died when the NaCl concentration was increased to 200 or 300 mM at increments of 50 mM per day (data not shown). These results showed that common and T artary buckwheat were as sensitive as rice and soybean to salinity when the salinity of the culture solution was increased rapidly. The PGR decreased to 48% and 16% of the control in Tsushima and Pontivy, respectively. The net assimilation rate (NAR) and mean leaf area (MLA) also decreased to 81 and 60%, respectively, in Tsushima, and 34 and 48%, respectively, in Pontivy (Table 1). These results showed that Tsushima was significantly more tolerant to salinity than Pontivy. The dry-matter accumulation of salt-stressed maize was influenced by the degree of leaf expansion (Cramer et al., 1994). In the present experiments, PGR of both buckwheat species significantly correlated with NAR (Tsushima; r = 0.983**, Pontivy; r=0.986**) and MLA (Tsushima; r = 0.982**, Pontivy; r=0.988**) as shown in Fig. 1, indicating that the reduction of PGR was influenced by NAR and MLA in both species. Salt stress lowered the growth rate of leaf (GR_L), stem (GR_S) and root (GR_R) to 30, 48 and 25% of the control, respectively, in Tsushima, and to 11, 18 and 11% of the control, respectively, in Pontivy (Table 2). Thus, salt stress lowered the growth rate more severely in leaves and roots than in stems in both species. Our results showed that the interspecific differences in salt tolerance were caused by the difference in the response of both MLA and NAR to salt stress.

2. Leaf expansion

The salt treatment decreased the total leaf area (L_T) to 41% and 28% of the control in Tsushima and Pontivy, respectively, and the LA_T to 72% and 52%, respectively (Table 3). The SLA was not significantly influenced by the salt treatment in Tsushima, but significantly increased in Pontivy. Salt treatment reduced the LN in Tsushima and Pontivy similarly. DPL was not significantly influenced by the salt treatment in either species. These results showed that the interspecific difference in L_T was mainly caused by the differences in GR_L, LA_T and SLA. In salt-stressed maize, leaf area ratio was significantly lowered due to the reduction in SLA and leaf weight ratio, indicating that the salt stress altered leaf expansion and carbon allocation (Cramer et al., 1994). The increase in SLA might be important for the salt tolerance of maize because the degree of leaf expansion was related to SLA (Cramer et al., 1994).

3. Gas exchange rate in leaves

Salinity lowered the photosynthetic rate in glycophytes (nonhalophytes) and even in halophytes...
The interspecific difference in Pr may be caused by the difference in both gs and chloroplast CO₂ fixation in this study, but further studies are needed to identify the main factor limiting Pr.

The salt treatment decreased the transpiration rate (Tr) to 41% and 30% of the control in Tsushima and Pontivy, respectively (Table 4). The transpiration rate generally declines with increasing leaf salinity (Yeo et al., 1985; De Pascale et al., 2003), probably because the water potential in the leaves decreases as a result of water stress and/or lowered water potential of roots, as indicated by the movement of abscisic acid from root to shoot. Moreover, guard cells are irreversibly damaged by accumulation of excessive amounts of sodium ions (Clint, 1984), and disruption of the normal regulation of transpiration may make it difficult to survive in salt-laden soils (Robinson et al., 1997). The water use efficiency (WUE), estimated as the ratio of Pr to Tr, was increased by salt treatment to 1.6 times the control in Tsushima but was little changed in Pontivy. The increase in WUE in Tsushima under salt stress is ascribed to the maintenance of Pr, which was probably caused mainly by the maintenance of CO₂ fixation and partly by that of gs.

(Cheseman, 1988) by reducing stomatal conductance (Down et al., 1985; Brugnoli and Lauteri, 1991) and/or CO₂ fixation rate in the chloroplast (Seemann and Sharkey, 1986; Gibberd et al., 2002). We obtained similar results in our study; the photosynthetic rate (Pr) of Tsushima and Pontivy decreased to 67% and 35% of the control, respectively (Table 4). The salt treatment reduced the gs in Tsushima and Pontivy to 25% and 15% of the control, respectively, and the internal CO₂ concentration in leaf (Ci) to 67% and 35% of the control, respectively (Table 4). These results indicated that the stomatal aperture and CO₂ fixation were smaller in Pontivy than in Tsushima under 100 mM salinity. Based on the interspecific difference in stomatal response, salinity is expected to decrease Ci greatly in Tsushima than in Pontivy. A decline in Pr without a corresponding decline in Ci usually has been interpreted as a direct effect of salt stress on photosynthetic capacity (Farquhar et al., 1987). The interspecific difference in Pr may be caused by the difference in both gs and chloroplast CO₂ fixation in this study, but further studies are needed to identify the main factor limiting Pr.

The salt treatment decreased the transpiration rate (Tr) to 41% and 30% of the control in Tsushima and Pontivy, respectively (Table 4). The transpiration rate generally declines with increasing leaf salinity (Yeo et al., 1985; De Pascale et al., 2003), probably because the water potential in the leaves decreases as a result of water stress and/or lowered water potential of roots, as indicated by the movement of abscisic acid from root to shoot. Moreover, guard cells are irreversibly damaged by accumulation of excessive amounts of sodium ions (Clint, 1984), and disruption of the normal regulation of transpiration may make it difficult to survive in salt-laden soils (Robinson et al., 1997). The water use efficiency (WUE), estimated as the ratio of Pr to Tr, was increased by salt treatment to 1.6 times the control in Tsushima but was little changed in Pontivy. The increase in WUE in Tsushima under salt stress is ascribed to the maintenance of Pr, which was probably caused mainly by the maintenance of CO₂ fixation and partly by that of gs.

4. Sodium and potassium accumulation in the plant

The ratio of Na⁺ content of the leaves in the salt-treated plants to that in the control plants was 20.5 and 12.5 in Pontivy and Tsushima, respectively (Fig 2). Pontivy accumulated a larger amount of Na⁺ in the leaves than Tsushima. Tsushima accumulated more Na⁺ in the roots than Pontivy. Many studies have shown that the low Na⁺ accumulation in the leaves correlates with the high salt tolerance in glycophytes (Schachtman and Munns, 1992; Tsuchiya et al., 1992). The difference in Na⁺ accumulation in the leaves thus seemed to be related to the difference in salt tolerance between the two species. Pr and leaf expansion were less affected by salt stress in Tsushima, which accumulated a smaller amount of Na⁺ in the leaves under salt stress. Negative linear correlations were found between gₛ and leaf Na⁺ content and between Pr and leaf Na⁺ content in carrot (Gibberd

Table 3. Influence of salt stress on leaf area (Lₒ), dry-matter partitioning rate to leaf (DPL), number of leaves (LN), leaf area per leaf (LAₒ) and specific leaf area (SLA).  

<table>
<thead>
<tr>
<th></th>
<th>Lₒ  (cm²)</th>
<th>DPL (%)</th>
<th>LN</th>
<th>LAₒ (cm²)</th>
<th>SLA (cm² g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsushima</td>
<td>C 128 (100)</td>
<td>51</td>
<td>8 (100)</td>
<td>16.4 (100)</td>
<td>391 (100)</td>
</tr>
<tr>
<td>S 53** (41)</td>
<td>42 NS</td>
<td>5* (62)</td>
<td>11.5* (72)</td>
<td>444 NS (115)</td>
<td></td>
</tr>
<tr>
<td>Pontivy</td>
<td>C 109 (100)</td>
<td>54</td>
<td>8 (100)</td>
<td>14.1 (100)</td>
<td>411 (100)</td>
</tr>
<tr>
<td>S 31** (28)</td>
<td>45 NS</td>
<td>4* (50)</td>
<td>7.3** (52)</td>
<td>632** (155)</td>
<td></td>
</tr>
</tbody>
</table>

C ; Control,S;Salt treatment with 100 mM NaCl. ** and * indicate significance at p = 1% and 5%, and NS not significant. Figures in parentheses show percentage of control.

Table 4. Influence of salt stress on photosynthetic rate (Pr), stomatal conductance (gₛ), intercellular CO₂ concentration (Ci), transpiration rate (Tr), water use efficiency (WUE) and relative water content of leaf (RWC) in common and Tartary buckwheat.  

<table>
<thead>
<tr>
<th></th>
<th>Pr (µmol m⁻² s⁻¹)</th>
<th>gₛ (µmol m⁻² s⁻¹)</th>
<th>Ci (ppm)</th>
<th>Tr (m mol m⁻² s⁻¹)</th>
<th>WUE</th>
<th>RWC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsushima</td>
<td>C 20.7 (100)</td>
<td>0.591 (100)</td>
<td>275 (100)</td>
<td>8.0 (100)</td>
<td>2.6</td>
<td>81</td>
</tr>
<tr>
<td>S 13.7** (67)</td>
<td>0.146** (25)</td>
<td>185** (67)</td>
<td>3.3** (41)</td>
<td>4.2*</td>
<td>84 NS</td>
<td></td>
</tr>
<tr>
<td>Pontivy</td>
<td>C 21.1 (100)</td>
<td>0.820 (100)</td>
<td>290 (100)</td>
<td>10.4 (100)</td>
<td>2.0</td>
<td>80</td>
</tr>
<tr>
<td>S 7.3** (35)</td>
<td>0.119** (15)</td>
<td>229* (79)</td>
<td>3.1** (30)</td>
<td>2.4 NS</td>
<td>66*</td>
<td></td>
</tr>
</tbody>
</table>

C ; Control,S;Salt treatment with 100 mM NaCl. ** and * indicate significance at p = 1% and 5%, and NS not significant. Figures in parentheses show percentage of control.
accumulation in leaves reduced Pr, g s, Tr and WUE in leaf expansion, Pr, gs and Tr were smaller in Pontivy, pepper (De Pascale et al., 2003). In our experiment, (K Tsushima. The salt treatment decreased the potassium + content of the leaves about 0.6 and 0.5 times in salt-treated plants to that in the control. Figures in parentheses indicate ratios of ion concentration in the plants with thee roots removed to that in the plants with roots remaining.

The content of potassium (K') and sodium (Na') in leaves, stem (including petiole) and roots of Tsushima and Pontivy under salt stress. C ; Control, S ; Salt treatment with 100 mM NaCl, E ; Removal of roots. ** and * indicate significance at p = 1% and 5%, and NS not significant. 1) Comparison of control and salt-treated plants. Figures in parentheses indicate ratios of ion concentration in the salt-treated plants to that in the control. Comparison between salt-treated plants with the roots removed and with the roots remaining. Figures in parentheses indicate ratios of ion concentration in the plants with thee roots removed to that in the plants with roots remaining.

Some crops have an ability to utilize Na' as an alternative to K' in their metabolism; however, common buckwheat is a crop that does not utilize Na' even under K' deficiency (Subbarao et al., 2003). Most plant species are “natrophobes” that are not able to readily absorb Na’ but can readily absorb K’ under a low Na’ concentration in the root zone (Shone et al., 1969). When natrophobes were subjected to a high Na’ concentration (>100 mM), they absorbed much Na’ and translocated it to the shoots (Subbarao et al., 2003). The general assumption related to salt tolerance of plants is that they compartmentalize the absorbed Na’ in vacuoles, and use it as an inorganic osmoticum in place of or along with K’. It is widely believed that cytoplasm itself does not tolerate high levels of Na’ as it interferes with normal metabolic functioning (Geenway and Osmond, 1972). “Natrophobes” that have limited or no ability to compartmentalize Na’ must spend substantial amounts of energy in preventing Na’ from entering the plant in order to survive in a saline environment (Subbarao and Johansen, 2002). In support of the above findings, our results suggest that common and Tartary buckwheat are also “natrophobes” and the interspecific difference in salt tolerance between the two species may be mainly influenced by Na’ content of leaves.

The accumulation of Na’ in the leaves depends on the uptake of Na’ by the root and its translocation to leaves and/or exclusion from leaves through the root. Phloem transport plays an important role in controlling the NaCl content of the leaves in maize cultured in the solution with 100 mM NaCl (Lohaus et al., 2000). Durand and Lacan (1994) reported that Na’ accumulation in the young leaf of soybean was prevented both by depletion of Na’ from the xylem stream, and by a high recirculation of Na’ via the phloem. The Na’ content of leaves increased when roots were removed, indicating that the roots may play an important role in the uptake, transport and/or exclusion of Na’ under salt stress. Na’ accumulated in the leaves rapidly in both species during the two days after roots were removed. The ratio (%) of Na’ content of the leaves in the plants after removal of roots to that in the plants with roots remaining was 4.0 and 2.2 in Tsushima and Pontivy, respectively (Fig.2). Thus, root excision increased Na’ accumulation in leaves more greatly in Tsushima than Pontivy, while it gave no significant effect on Na’ accumulation in the stem in both species. In Tsushima, potassium (K’) accumulation in the leaves and stems were increased by removal of roots but not in Pontivy. Yamanouchi (1989) showed that the Na’ content of shoots in rice plants increased after removal of leaf sheath including roots and concluded that both Na-holding and Na-excluding abilities of the roots affected Na’ accumulation in the shoots. We concluded that interspecific differences in salt tolerance between common and Tartary buckwheat might be influenced by the differences in the Na’ accumulation in the leaves and its absorption by the...
root.

Acknowledgments

We thank Dr. A. Egriiny Eneji, visiting professor of the Arid Land Research Center, Tottori University and Dr. S. Morita, professor of the University of Tokyo, for their critical review of the manuscript and helpful discussion. This research was supported in part by a Grant-in-Aid for Takano Life Science Research Foundation. We sincerely thank Dr. T. Tetsuka, a Grant-in-Aid for Takano Life Science Research Foundation. We also thank Messrs K. Shimogama, D. Matsui and Miss T. Iwasaki, students of the Department of Agriculture, Kyushu Tokai University, for their assistance in carrying out the experiments.

References


* In Japanese.

** In Japanese with English abstract.