EFFECTS OF SALINITY ON ANTIOXIDANT ENZYMES AND PROLINE IN LEAVES OF BARLEY SEEDLINGS IN DIFFERENT GROWTH STAGES

B. TURKYILMAZ UNAL¹, L. Y. AKTAS² and A. GUVEN²
¹ Nigde University, Ulukisla Vocational School, 51900 Ulukisla-Nigde, Turkey
² Ege University, Faculty of Science, Department of Biology, 35100 Bornova-Izmir, Turkey

Abstract


The participation of antioxidant defence under mild and severe salt stress conditions (120 and 240 mM) on barley (Hordeum vulgare L.) seedlings in different growth stages, antioxidant enzyme (superoxide dismutase, peroxidase and catalase) activities and proline content were determined. Plants grown in Hoagland solution served as control. Salinity induced proline accumulation in both 2- and 4-week-old-seedlings (up to 8.7-fold and 1.8 fold, respectively, as compared with control). The activities of antioxidant enzymes in leaves increased under NaCl stress, the seedlings in the early growing stage (2-week-old) being generally more responsive than 4-week-old ones. The highest peroxidase (POX) and superoxide dismutase (SOD) activities were 165 and 152 % of control, respectively. Catalase (CAT) activity reached about 7- fold increase in NaCl treatment of 2-week-old seedlings. This dramatic increase might indicate that CAT is a major enzyme among antioxidant enzymes examined in barley under salt stress. Thus, antioxidant defence system induced by salinity plays prominent role particularly in early growth periods and its efficiency decrease with age of the plants.

Key words: barley, catalase, Hordeum vulgare, NaCl, peroxidase, proline, superoxide dismutase

Introduction

About one-third of irrigated land is considered to be affected by salinity (Flowers and Yeo, 1997) and expanding salinization is posing a greater threat in the world (Xiaoli et al., 2009; Kausar et al., 2013). In Turkey, salinity and sodicity were detected on 1 518 722 ha of the land resource, indicating saline soils constitute a large part of the barren lands (74%) (Kendirli et al., 2005). Salinity represents one of the most important environmental stresses since it limits crop production.

Salinity can be alleviated through either soil reclamation or growing tolerant crops. In plant breeding studies, the use of some physiological and biochemical markers for improving the salt tolerance in plants is crucial. Antioxidant enzymes are related to the tolerance to various abiotic stresses including salinity. In barley, the salt tolerant varieties have higher antioxidant enzyme activities than the salt sensitive varieties (Xiaoli et al., 2009). Also, proline accumulation is one of the common characteristics in many monocotyledons under saline conditions (Tani and Sasakawa, 2006; Ashraf and Foolad, 2007).

Material and Methods

Salt tolerant cultivars (Anadolu 98, Efes 3, FIGem, Sulleyman Bey and Vamik Hoca) of barley (Hordeum vulgare L.) were chosen for this study based on data from previous work (Turkyilmaz et al., 2011). Seeds were sown in pots (20 x 30 cm², 50 seeds for each) filled with perlite and grown under controlled conditions (16-h photoperiod, irradiance at leaf level 350 μmol m⁻² s⁻¹, temperature 19±1°C, relative humidity of 60-70%). After germination, seedlings were sup-
plied every week with Hoagland nutrient solution added with
120 and 240 mM NaCl and irrigated with distilled water at
two-day-intervals. Plants grown in Hoagland solution served
as control. Seedlings were harvested reaching two- or four-
week-old stage.

Proline content was determined according to the modi-
fied method of Bates et al. (1973). The concentration was
calculated from a proline standard curve and expressed as
µmol/g FW.

Harvested fresh leaf samples were frozen in liquid nitro-
gen. Leaves were homogenized in 0.05 M Na phosphate buffer
pH 7.8 including 1 mM EDTA and 0.2 g Dowex 1 x 8 (200x
400 mesh). Homogenates were centrifuged and supernatants
were used for enzyme activity and protein content assays.
Total soluble protein content was determined according to Brad-
ford (1976) using bovine serum albumin as a standard.

Superoxide dismutase activity (SOD) assay was based
on the method of Beauchamp and Fridovich (1971), which
measures the inhibition in the photochemical reduction of
nitroblue tetrazolium chloride (NBT) spectrophotometri-
cally at 560 nm. Peroxidase activity (POX) was determined
according to Herzog and Fahimi (1973) using dianinoben-
zidine tetrahydrochloride dihydrate (DAB) as a substrate.
Catalase activity (CAT) was estimated according to the
method of Bergmeyer (1970) by the determination of the de-
stroying of H2O2, measuring the decrease of the absorbance
at 240 nm.

The mean values were calculated from two independent
experiments, each with four replicates. Experimental data
were analyzed with Tukey test at p<0.05 level. Standard er-
rors (±) are calculated.

Results and Discussion

Plant response to NaCl stress is a quite complex phe-
nomenon comprising the changes from morphology to me-
tabolism, depending on several factors such as intensity of
the stress, developmental stage of plant and tolerance poten-
tial.

Salinity exposure was very effective in proline accumu-
lation in leaves of many crop seedlings (Amirjani, 2010) includ-
ing barley (Pirasteh-Anosheh et al., 2014). NaCl treatment
caused a massive accumulation of proline in the leaves parallel
to the rise of NaCl concentrations in 2-week-old- seedlings
reaching up to an 8.7-fold increase in Efes 3 cultivar com-
pared with control (Table 1), whereas in 4-week-old-seed-
lings NaCl caused a less pronounced proline accumulation in
all cultivars. Shevyakova et al. (2009) suggest that NaCl- and
parquat-induced accumulation of proline had both osmopro-
tective and antioxidant functions. Proline ability to quench
reactive oxygen species particularly OH has convincingly
been demonstrated (Signorelli et al., 2013). Our data also
showed that induced proline accumulation co-occurred with
higher activities of antioxidant enzymes in younger seedlings
proving the proline role in activation of antioxidant defence
in plants (Rejeb et al., 2014). Proline implication in protection
of protein integrity (Szabados and Savoure, 2009) may con-
tribute to ability of seedlings to survive in early growth stage
under high saline conditions.

The activities of antioxidant enzymes of the five barley
cultivars were increased in leaves under NaCl stress (Figures
1, 2 and 3). The responses of SOD, POX and CAT were par-
ticularly significant in the leaves of two-week-old-seedlings,
indicating a high defence capability of antioxidant enzymes
to salt stress at the early growth stage of barley. The highest
rate of SOD activity was measured in 240 mM NaCl treated
2-week-old Anadolu 98 (about 52% increases as compared
with control). SOD activity was not significantly altered in
4-week-old-seedlings (Figure 1).

POX activity was almost the same in control groups of
two- and four-week-old-seedlings of Anadolu 98, Efes 3,
F1 Gem and Vamik Hoca cultivars and slightly different in

Table 1
Proline content (µmol g⁻¹ fresh weight) of the leaves of 2- and 4-week-old seedlings of five barley cultivars treated
with Hoagland nutrient medium containing 0, 120 and 240 mM NaCl

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>NaCl, mM</th>
<th>Anadolu 98</th>
<th>Efes 3</th>
<th>F1 Gem</th>
<th>Suleyman Bey</th>
<th>Vamik Hoca</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-week-old</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.36±0.01a</td>
<td>0.26±0.01a</td>
<td>0.44±0.01a</td>
<td>0.42±0.06a</td>
<td>0.45±0.05a</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>1.47±0.21b</td>
<td>1.62±0.19b</td>
<td>1.42±0.16b</td>
<td>1.73±0.09b</td>
<td>1.22±0.05b</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>2.20±0.03c</td>
<td>2.26±0.72b</td>
<td>1.81±0.12c</td>
<td>1.73±0.17b</td>
<td>1.19±0.17b</td>
<td></td>
</tr>
<tr>
<td>4-week-old</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0.99±0.05a</td>
<td>0.85±0.08a</td>
<td>0.91±0.05a</td>
<td>0.99±0.08a</td>
<td>0.94±0.07a</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>0.94±0.11a</td>
<td>1.34±0.74a</td>
<td>1.26±0.03ab</td>
<td>1.06±0.19a</td>
<td>0.95±0.08a</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td>1.78±0.25a</td>
<td>1.40±0.33a</td>
<td>1.61±0.37b</td>
<td>1.47±0.55a</td>
<td>0.99±0.04a</td>
<td></td>
</tr>
</tbody>
</table>

The mean values were calculated from two independent experiments, each with four replicates. Standard errors (±) are
indicated. Different subscript letters indicate significant differences (p<0.05)
Salinity Stress Effects on Antioxidants of Barley Leaves

Suleyman Bey (Figure 2). Two-week-old-barley seedlings showed significant increases in POX activity with 240 mM salinity treatment except Suleyman Bey. Although significant increase (65%) was measured in the enzyme activity of

Fig. 1. Superoxide dismutase (SOD) activity of the leaves of 2- and 4-week-old seedlings of five barley cultivars treated with Hoagland nutrient medium containing 0, 120 and 240 mM NaCl.
The mean values were calculated from two independent experiments, each with four replicates. Vertical bars indicate ± SE.

Fig. 2. Peroxidase (POX) activity of the leaves of 2- and 4-week-old seedlings of five barley cultivars treated with Hoagland nutrient medium containing 0, 120 and 240 mM NaCl.
The mean values were calculated from two independent experiments, each with four replicates. Vertical bars indicate ± SE.
Another scavenger of $H_2O_2$, CAT activity, increased by increasing salt concentration. Especially in the early growth period changes were very dramatic reaching about 7-fold increase compared with control in Anadolu 98 cultivar in both NaCl concentrations (Figure 3). In four-week-old-barley seedlings, CAT activity was affected significantly only in 240 mM NaCl treatment. In this group, salinity caused significant changes in the activity of the cultivars Anadolu 98, Suleyman Bey and F1 Gem exposed to 240 mM NaCl concentration. The CAT activity of control groups was not changed during the growth period in Efes 3, F1 Gem and Vamik Hoca, in contrast with the big differences in Anadolu 98 in which CAT activity increased during the growth period almost 4-fold, whereas in Suleyman Bey a decrease of approximately 37% was observed.

Among the enzymes, CAT showed the highest rate of activity changes under salt stress in early growth period of barley seedlings in accordance with the experiments of Khosravinejad et al. (2008). Increases in SOD and POX activity were relatively low when compared with CAT activity in the leaves of NaCl treated barley cultivars, with this indicating the major role of CAT in the antioxidant defence of barley in salt stress conditions (Dai et al., 2009). While the highest POX activity rate measured was 165% and SOD activity was 152% of the controls, CAT activity increased about 7-fold, this pointing to the CAT contribution in maintaining steady-state levels of cellular hydrogen peroxide.

**Conclusion**

The results strongly imply a possibility that the antioxidant enzyme system is also utilized in barley to alleviate oxidative stress caused by salinity, thus protecting the cells from oxidative damage (Kim et al., 2005). The increased activities of the antioxidant enzymes upon salt stress are often related to the enhanced tolerance to salt stress (Gueta-Dahan et al., 1997; Mittova et al., 2004).

Older seedlings of the same barley cultivars were affected more by ion toxicity than by oxidative stress (Turkyilmaz et al., 2011). Also, the idea that long term NaCl exposure causes Na\(^+\) and Cl\(^-\) accumulation in older leaves and ion toxicity suggested by Munns (2002), Munns and Tester (2008) and Mane et al. (2011) supports our results. The antioxidant enzymes and proline accumulation undergo dramatic changes under NaCl stress in early growth period in comparison to four-week-old seedlings of five barley cultivars. This might suggest that oxidative stress caused by salinity is operative in early growth periods of barley plants.
Salinity Stress Effects on Antioxidants of Barley Leaves

References


Received February, 26, 2013; accepted for printing December, 2, 2013.