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Photosynthesis capacity and enzymatic defense system as bioindicators of salt tolerance in triticale genotypes

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ABSTRACT

The present study aims at clarifying the differences in photosynthesis parameters, oxidative status and antioxidant enzyme activity among ten triticale genotypes in response to salinity stress; and utilizing the traits as biomarkers for identification of salt-tolerant triticale genotypes. The plants were cultivated in a hydroponic system with or without 220 mM salt concentration. The plants were analyzed for salt tolerance (in term of relative biomass) as well as for the traits at the vegetative (VG) and reproductive (RP) stages. Salinity resulted in significant decline in biomass (55–83%), net photosynthesis rate (12–65%), stomatal conductance (38–83%), transpiration rate (20–56%) and intercellular CO_2 concentrations (7–37%) among genotypes. In contrast, H_2O_2 and lipid peroxidation (LP) increased markedly in leaves of salt-stressed plants. Activities of total superoxide dismutase (TSOD), catalase (CAT), guaiacol peroxidase and ascorbate peroxidase due to salinity were 0.97–1.84, 0.88–1.96, 0.78–2.23 and 0.61–1.81 times over the control plant, respectively. The photosynthesis attributes, LP and TSOD at the VG stage and LP and CAT at the RP stage showed correlations with scores of salt tolerance (ST) indicating contribution of these traits to ST at least at some part of the plant growth stages, while no connection was found between ST with POD and CAT. Collectively, membrane integrity was a suitable indicator for discrimination of genotypes for ST, while photosynthetic capacity and enzymatic defense system cannot be utilized as general selection criteria for ST during screening of relatively large populations of triticale.

1. Introduction

In many arid and semi-arid areas of the world, salinity is one of the major threats to food production and security. Introduction of salt-tolerant crop plants is one of the most efficient ways to cope with salt stress in these regions (Munns et al., 2006). Triticale (×*Triticosecale* Wittmack) is a cereal crop cultivar obtained by cross-fertilization of wheat (*Triticum* spp.) and rye (*Secale* spp.) (Chapman et al., 2005). Triticale has higher tolerance to biotic and abiotic stresses than wheat and higher grain yield than rye (Tohver et al., 2005).

Earlier studies have reported that triticale tolerates high salinity levels in the root media and may be categorized as a salt-tolerant crop plant (Koebner and Martin, 1996). Kutlu et al. (2009) evaluated 13 triticale lines in varying salinity levels and found that most of them were able to survive at high salinity level (250 mM). However, few studies have focused on various biochemical and physiological aspects of triticale responses to salt stress.

Salinity causes many detrimental effects on growth, physiological responses, biochemical reactions and metabolic processes due to osmotic and ionic effects (Tester and Davenport, 2003). Salinity also causes oxidative damage as a consequence of producing large amounts of reactive oxygen species (ROS), in various forms such as superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($^{\cdot}OH$) in different cell organelles (Foyer and Shigeoka, 2011). When Calvin cycle activity is limited due to salinity, light utilization is reduced, and the absorbed light is more than the required quantity for photosynthetic capacity. Salinity suppresses enzyme activities of the Calvin cycle, but has little impact on the biophysical process of light absorption. A portion of excess energy is delivered to molecular oxygen forming free radicals, which provoke degradation of organic macromolecules and eventually cell death (Mano, 2002).

Abbreviations: P_n, net photosynthetic rate; g_s, stomatal conductance; E, transpiration rate; C, $_i$internal CO_2 concentration; TSOD, total superoxide dismutase; POD, guaiacol peroxidase; APX, ascorbate peroxidase; CAT, catalase; MDA, malondialdehyde; LP, lipid peroxidation; VG, vegetative; RP, reproductive; ROS, reactive oxygen species; ST, salt tolerance; STI, salt tolerance index.

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All plant species naturally have various defensive networks to prevent their cells from deleterious effects of ROS which include enzymatic and non-enzymatic antioxidants (Ahmad and Prasad, 2012). Antioxidative enzymes include different enzymes such as catalase (CAT), total superoxide dismutase (TSOD), ascorbate peroxidase (APX), peroxidase (POX), glutathione reductase (GR). Non-enzymatic antioxidants include different compounds such as ascorbic acid, glutathione, tocopherols, carotenoids and phenolic compounds. Under stress conditions, activity of these enzymes is altered and the degree of alteration may be linked to stress tolerance of the plant (Ahmad and Prasad, 2012).

In general, a number of physiological and biochemical traits have been identified for screening of genotypes under stress conditions. Photosynthesis is a key physiological activity, which is mainly contributing to plant growth and development (Dubey, 2005). Increasing the rate of leaf photosynthesis appears to be a straightforward method of increasing crop yields. Several investigations have been carried out to select genotypes with higher photosynthetic ability for saline conditions such as in wheat (El-Hendawy et al., 2007) and cotton (Desingh and Kanagaraj, 2007).

At high salt concentration, ROS are able to rapidly react with polyunsaturated lipids of cell membranes, leading to an alteration of membrane permeability; with DNA and RNA molecules leading to genomic instability; with amino acids and proteins, leading to oxidative modifications (Dat et al., 2000). Such changes occur when biological systems are exposed to severe adverse environmental conditions. Thus one fast and clear indicator of oxidative stress could be excess production of ROS in living cells.

Accumulation of malondialdehyde (MDA), which is the breakdown product of the fatty acids of membranes, is also extensively used to determine the lipid peroxidation status and to distinguish between sensitive and tolerant species/genotypes to salt stress (Kim et al., 2004). A large amount of lipid peroxidation products can be noticed in cell degradation of susceptible plants after exposure to salt stress (Moradi and Ismail, 2007; Yao et al., 2010).

The measurement of antioxidant potential can also be a biochemical approach towards assessment of salt-induced oxidative stress (Jin et al., 2009). Plant growth improvement under stressful environments could be due to the significant role of the enzymatic antioxidant system in alleviation of oxidative impact and this mechanism has been generally pointed out in wheat (Mandhania et al., 2006), rice (Moradi and Ismail, 2007) and barley (Gao et al., 2013). Kravchik and Bernstein (2013) identified 72 salt-affected genes in maize leaf cells, of which 13% belonged to antioxidant defense. More recently, Gręboszet al., (2014) also reported that 32% of proteins of triticale root cells whose biosynthesis was altered by osmotic stress belonged to the antioxidative system. According to these authors, this considerable proportion is not surprising because ROS plays dual roles under salinity stress. In the first role they are necessary for natural growth of young cells but in the second role they can cause damage particularly in mature cells.

Salt stress increased POD and TSOD activity in barley genotypes differing in salt tolerance (Jin et al., 2009). Also, transcription levels for mitochondrial and chloroplastic superoxide dismutase and cytosolic ascorbate peroxidase enzymes were greatly increased in salt-tolerant pea cultivars, whereas the activities of those enzymes were not induced in the susceptible cultivars (Hernández et al., 2000). Chickpea seedlings, exposed to salinity levels from 25 to 100 mM NaCl, showed an increase in catalase (CAT) activities (Rasool et al., 2013). Moreover, some studies found a positive association between salt tolerance (ST) and ROS-scavenging system in different tissues in various plants such as maize, rice and other species of gramineae (Kim et al., 2004; Kholova et al., 2010). These results clearly indicate that up-regulation of antioxidant enzymes contributes to ST in many crop plants. In contrast, a number of recent reports showed that there were no significant correlations between ST and antioxidant enzymes (Dragišić Maksimovic et al., 2013; Fan et al., 2014). Inhibition of growth in the presence of high level of antioxidant enzymes has been also reported in leaf cells of maize (Kravchik and Bernstein, 2013). A similar finding was reported by Miller et al. (2007) in Arabidopsis (*Arabidopsis thaliana*) mutant which grows better than the salt stressed plants of the wild-type despite it is deficient in APX1.

High root selectivity for K^+ over Na^+ and exclusion of Na^+ and Cl^- from the shoot can be among components of ST mechanisms in triticale plants (Salim, 1988). Accumulations of proline and carotenoids have also been shown to be correlated with ST in triticale (Salehi and Arzani, 2014). However, only few studies have investigated the contribution of photosynthesis-related parameters and antioxidant enzymes activities in triticale affected by salt stress. Therefore, the aims of the present investigation were: to find out the magnitude of differences in photosynthesis parameters and antioxidant enzymes activities among 10 triticale genotypes in response to salinity stress; and to examine if any of the studied traits can be considered as a possible criterion for evaluation of different triticale genotypes for salt tolerance.

Table 1
Triticale genotypes and their pedigrees used for assessing the potential of photosynthesis parameter and enzymatic defense system as bioindicators of salt tolerance.

Genotype name	Pedigree
BL-T1	W.TCL83/KB35//FAHAD_8
BL-T2	ARDI_1/TOPO 419//ERIZO_9/3/LIRON_1-1/4/FAHAD_4/FARAS_1
BL-1	STIER_29/FARAS_1//MANATI_1
BL-2	DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9/4/FAHAD_8-1*2...
BL-3	ERIZO_6/NIMIR_4//ERIZO_15/FAHAD_3
BL-4	DAHBI_6/3/ARDI_1/TOPO 1419//ERIZO_9/4/FAHAD_8-1*2...
BL-5	02-94-012 02-93-020 3-4/4/LASKO/...
BL-6	ARDI_1/TOPO 419//ERIZO_9/3/LIRON_1-1/4/FAHAD_4/FARAS_1...
BL-S1	RONDO/BANT_5//ANOAS_2/3/VICUNA_4
BL-S2	RONDO/BANT_5//ANOAS_2/3/RHINO_3/BULL_1-1

2. Material and methods

2.1. Plant material and growth conditions

The present study was conducted from November 2012 to May 2013 under greenhouse conditions. Ten triticale (×*Triticosecale* Wittmack) genotypes with diverse pedigrees were used for this investigation (Table 1). The experiment was carried out in a completely randomized block design that had three replications.

The plant material included two salt-sensitive breeding lines (BL-S1 and BL-S2), two salt-tolerant improved breeding lines (BL-T1 and BL-T2) and six more breeding lines (BL-1 to BL-6) whose tolerance to salinity was unknown. The breeding lines were provided by the International Maize and Wheat Improvement Center (CIMMYT). Seeds from each genotype were planted in large tanks filled with washed river sand (Bulk density = 1.4 g cm^{-3}). Tanks were watered initially with tap water and after germination half strength modified Hoagland's nutrient solution was introduced. The nutrient solution was increased to full strength within 2 days after emergence (DAE). Modified Hoagland's nutrient solution contained 3 mM KNO_3, 2.5 mM $Ca(NO_3)_2.4H_2O$, 0.17 mM KH_2PO_4, 1.5 mM $MgSO_4.7H_2O$, 50 μM Fe as sodium ferric diethylenetriamine penta acetate (NaFeDTPA), 23 μM H_3BO_3, 5 μM $MnSO_4.H_2O$, 0.4 μM $ZnSO_4.7H_2O$, 0.2 μM $CuSO_4.7H_2O$, and 0.1 μM H_2MoO_4. Salinization was induced 2 DAE by adding 50 mM NaCl and $CaCl_2$ (5:1 molar ratio) to the solution twice daily over 5 days to reach the concentrations of 220 mM salt. Plants were watered three to five times daily with a nutrient solution. A closed-cycle system was used for irrigation of the plants. Each irrigation continued about 10 min until the sand in the tank was completely saturated. The drained