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Conserving plant genetic diversity
for use now and in the future

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Front cover: *Coffea mauritiana* Lam, Black River Gorges National Park, Mauritius
Photo: Ehsan Dulloo, Bioversity International

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Above: Nikolai Vavilov. Photo: N.I. Vavilov Research Institute of Plant Industry (VIR).

Determination of salt tolerance in wild einkorn wheat (*Triticum boeoticum* Boiss.) under *in vitro* conditions

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Salinity is a global problem and one of the major environmental stresses that largely affects plant growth and development (Greenway and Munns, 1980). Soil salinity affects a substantial portion of the Earth—as much as 25% of the total arable land available in the world can be described as being saline (Abrol *et al.*,1988). There are different types of salinity according to their causes, including irrigation, dryland and urban. Salts originate from mineral weathering, inorganic fertilizers, soil amendments (e.g., gypsum, composts and manures), and irrigation waters. Soil salinity affects many parts of Armenia's ecosystems, both rural and urban. The Ararat valley, which represents 13% of Armenia's arable lands, provides almost half of total agricultural production and about 42,000 ha in the Ararat valley have soil salinity problems, of which 9,000 ha are classified as severe (soil pH > 9) (Haykazyan and Pretty, 2006) (Fig. 1).

Figure 1. Saline soils (yellow region) of Ararat valley in Armenia.

Wheat (*Triticum* spp.) is the world's major cereal crop, with annual production of over 627 million t in 2004 (http://faostat.fao.org/). Wheat is grown under irrigated and rain-fed conditions—both types of agriculture are threatened by salinization (Ghassemi *et al.*, 1995; Mujeeb-Kazi and Diaz de Leon, 2002). If cropping is to continue on these salt-affected soils, substantial increases in the salt tolerance of crops are needed. Wild relatives, including some halophytes, might be sources of tolerance to improve wheat. There is no precise definition of a halophyte. In ecological terms, halophytes are plants that are able to complete their life cycle in salt concentrations approaching those found in sea waters (around 500 mM NaCl).

The identification of suitability of cultivars and their wild relatives to saline conditions will contribute to genetic improvement of the crops and help to increase their yield and quality. For these purposes an efficient screening method is required—one that identifies plants tolerant to saline or alkaline soils. The screening of genotypes under *ex vitro* conditions is difficult as it entails a large amount of resources and space. The determination of absolute salt tolerance under *ex vitro* is also difficult because of the complex interactions that exist between the plant and different soil components. *In vitro* culture is an ideal system for screening and evaluation of saline-tolerant plants as it can be carried out under controlled conditions with limited space and time (Cano *et al.*, 1998). Moreover, studies at cellular level provide better knowledge to deepen our understanding of the mechanism of salt tolerance.

Armenia is considered to be part of the centre of origin of cereals. Its flora has 13 wild wheat species and more than 360 cultivated varieties (Fig. 2). Some of the wild species are known to be salt- and drought-resistant, which is particularly important in Armenia due to its climate and frequent water shortages.

The availability of wheat wild relatives in Armenia offers an excellent opportunity for screening of salt tolerant species and genotypes. Therefore, the aim of this study was to screen wild einkorn wheat (*T. boeoticum*) populations for response to salinity and to develop an early diagnostic protocol that depends on reliable parameters using *in vitro* techniques.

Methodology

The effects of NaCl treatments on growth parameters were determined in wild einkorn wheat (*Triticum boeoticum* Boiss.) and the wheat cultivar Bezostaja 1.

Figure 2. *Triticum boeoticum* population in Erebuni Reserve (Armenia).

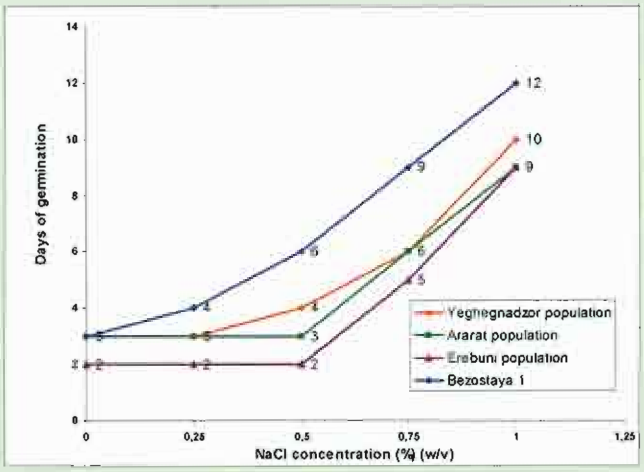

Figure 3. Effect of different concentrations of NaCl on three *T. boeoticum* populations and Bezostaja 1 germination under *in vitro* conditions.

Seeds from different populations of T. *boeoticum* and Bezostaja 1 were germinated in test tubes on half-strength Murashige and Skoog salt solution, pH 5.5, added with 0 (control), 0.25, 0.50, 0.75, 1.00 and 1.50 % (w/v) NaCl. After three weeks seedlings were removed from their media and evaluated for growth and chlorophyll content analysis. A tolerance index was calculated to summarize the general effect of five different NaCl concentrations and to compare *T. boeoticum* populations and cultured wheat Bezostaja 1 (La Rosa *et al.*, 1989). Total chlorophyll content was calculated according to Holden (1976).

Results and discussion

The differences with respect to their response to different levels of salinity were observed among the populations of *T. boeoticum* and cultivar Bezostaja 1. An increase in salinity did not affect the germination ratio. In general, germination was delayed with increased salinity in the medium (Fig. 3).

NaCl at concentrations of 0.25 and 0.5% did not delay germination of *T. boeoticum* seeds from populations from Ararat, Yeghegnadzor and Erebuni, but for Bezostaja 1, seed germination was delayed by two days in 0.5% NaCl. The addition of extra salt to the medium delayed germination by three days in 0.75% NaCl and by more than five days in 1.0% NaCl for all of the three studied populations of *T. boeoticum*, while for Bezostaja 1, germination was delayed by four and nine days respectively. This delay in germination in higher salt media (0.75% and 1.0%) may be due to the increased osmotic potential of the saline medium—the higher osmotic potential of the medium affects water and nutrient uptake, which may in turn inhibit the metabolic activities necessary for seed initiation and growth. However, *T. boeoticum* is able to germinate well under higher saline conditions. Research of salt tolerance in plants was focused primarily on tolerance in post-germination seedlings and mature plants. Less information exists on the ability of seeds to germinate under saline conditions. It is known that mannitol-synthesizing plants have been shown to grow well under osmotic and salt stress. One of the possible mechanisms of the ability of *T. boeoticum* seeds to germinate under osmotic stress is the conversion of starches to glucose or other simple sugars during germination. It is known that the enzyme α-amylase releases monomeric sugars from starch. For example, a drought and salt tolerant cultivar of chickpea, *Cicer arietinum* L., shows higher α-amylase activity in cotyledons of germinating seed than a drought- and salt-sensitive cultivar (Gupta *et al.*, 1993). The greater concentration of monomeric sugars acts to adjust osmotic potential. The role of carbohydrates in salt tolerance of *T. boeoticum* is an important point for further investigations.

In the Yeghegnadzor population, relative growth did not decrease due to the increase in NaCl concentrations in the 0.5% NaCl treatment. However, the 0.75 and 1.00% NaCl treatments inhibited relative growth and decreased total chlorophyll content of explants. At 0.75% NaCl, the average decline in relative growth and total chlorophyll content was 43.4 % and 41.5 % respectively, and at 1.00% NaCl, the reduction was 68.8% and 62.3% (Fig. 4).

During three week salt treatments (0.25–1.00% NaCl) of *T. boeoticum* from the Ararat and Erebuni populations, significant decreases were determined in the relative growth and total chlorophyll content at 1.00 % of NaCl treatment. At 1.00 % NaCl, the average decline in relative growth and total chlorophyll content was 91.2% and 67.7% in the Ararat population, and 78% and 75% in the Erebuni population, respectively (Fig. 2). The total chlorophyll content did not change during a three week treatment with 0.25–0.75% NaCl—it remained stable at a high level. The response in extra salt treatment of these two populations of *T. boeoticum* was similar.

The results obtained suggest that the Ararat and Erebuni populations are more tolerant to extra concentrations of salt in comparison with the Yeghegnadzor population. These differences between populations may be a result of the long-term adaptation of Erebuni and Ararat populations of *T. boeoticum* to specific ecological factors (clay soil, semi-desert climatic conditions etc.) of the ecosystems where they are growing.

Differences were also observed in response to extra salt concentrations in the widely distributed cultured wheat Bezostaja 1 (Fig. 2). Although explants maintained their relative growth in 0.25%, 0.5% and 0.75% NaCl treatments over three weeks, decreases in total chlorophyll content were significant in 0.75% and 1.00 % NaCl treatments. In contrast, in wild wheat *T. boeoticum*, the total chlorophyll content did not decrease in treatments of 0.25%–0.75% NaCl.

In conclusion, in experimental conditions, wild wheat *T. boeoticum* is more tolerant to salinity stress than cultured wheat Bezostaja 1.

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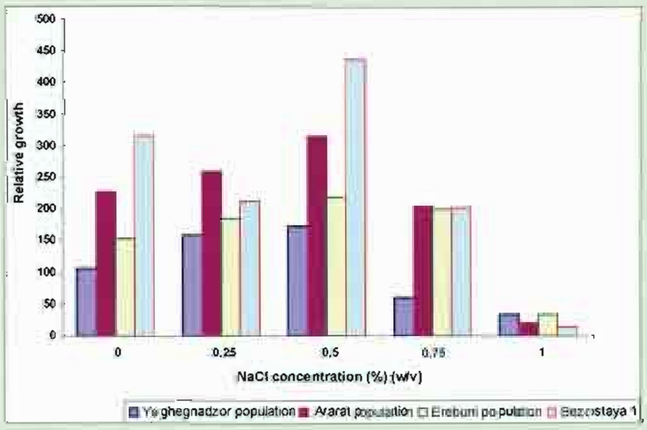

Figure 4. Relative growth of different populations of *T. boeoticum* and Bezostaja 1 under salt treatment.

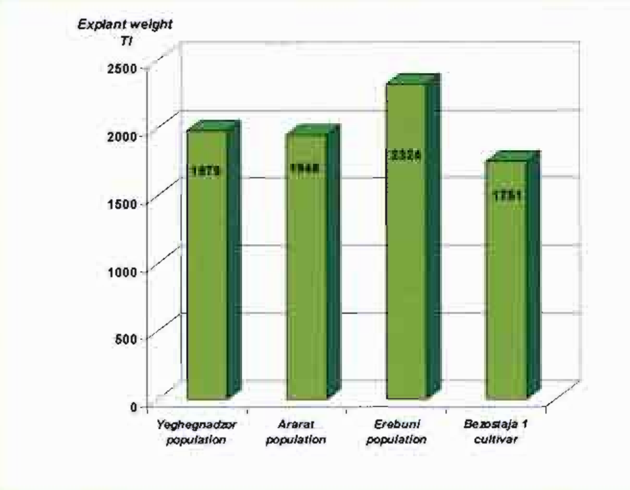

Figure 5. Tolerance indexes of *T. boeoticum* and Bezostaja 1 on the basis of explant weight.

The tolerance mechanism of *T. boeoticum* to salt stress has to be further investigated, but our results correlate with other studies in which the relative growth parameters for halophytic and potentially halophytic plants are described. The basic mechanism of a potential halophyte's salt tolerance is its ability to use the controlled uptake of Na^+ (balanced by Cl^- and other anions) into cell vacuoles for osmotic adjustment. It is known that more tolerant plants are osmoconformers, maintaining an osmotic pressure in the shoot approximately two to three times higher than the osmolality of the external solution (Farrukh, 2002). This is the result of a metabolic response to salt stress in tolerant plants. The cells of such plants synthesize compatible osmolytes (sugars, polyols, amino acids and tertiary and quaternary ammonium, and sulphonium compounds) (Hare *et al.*, 1998; Hong *et al.*, 2000).

Concerning the tolerance indexes (TIs) calculated on the basis of fresh weight of explants, differences between populations of *T. boeoticum* and cultured Bezostaja 1 were shown (Fig. 5).

At the end of the three week NaCl treatments, the highest TIs (2324) were obtained from the Erebuni population of *T. boeoticum*. The minimum TIs (1751) were determined in Bezostaja 1. So, *T. boeoticum* has a high potential of tolerance to salinity.

The results of the present study show the advantages and usefulness of salt tests conducted under *in vitro* conditions using plantlet culture. Therefore, *in vitro* salt tests could be used as an early diagnostic method to screen wild wheat and their cultivars for response to salinity or to identify salt tolerant wild wheat species, genotypes and cultivars for genetic improvement.

The utilization of tolerance indexes was found to be a good evaluation method for classifying different cultivars and their wild relatives. These parameters can be used to give a coefficient in evaluated plants species. Thus, further investigation of salt tolerance mechanisms of *T. boeoticum* will help us to understand the potential of wild einkorn wheat for genetic improvement of existing cultivars and development of more salt tolerant crops. This research is in line with other works on *in vitro* and *in vivo* studies of NaCl treatments on wild and cultured wheat and may contribute to the development of high salinity agriculture through sustainable use of the rich breeding potential of crop wild relatives (Farrukh, 2002; Colmer *et al.*, 2006; Munns *et al.*, 2006).

Acknowledgements

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