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in

Royo C. (ed.), Nachit M. (ed.), Di Fonzo N. (ed.), Araus J.L. (ed.).
Durum wheat improvement in the Mediterranean region: New challenges

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 40

2000
pages 319-322

A durum wheat mutant with resistance to salt stress

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SUMMARY – A mutant of durum wheat (422), obtained from Sodium Azide-treated seeds, was identified for potassium accumulation capability in leaves when grown in field. In hydroponic culture the mutant showed less growth inhibition with respect to the "wild type" and in presence of increasing concentrations of saline solutions of KNO_3, KCl and NaCl. 422 mutant seeds were also able to germinate at salt concentrations higher than 50 mM. Therefore, this mutant will be studied further to investigate the genetic basis and physiological mechanisms of salt tolerance.

Key words: Durum wheat mutant, salts stress, growth analysis, germination, osmo-tolerance.

RESUME – *"Un mutant du blé dur avec résistance au stress salin". Un mutant du blé dur (422), obtenu par les semences traitées avec Sodium-Azide, avait été identifié grâce à la capacité des feuilles d'accumuler le potassium lorsqu'elles ont poussé au champ. Dans la culture hydroponique le mutant montrait une inhibition inférieure de la croissance en comparaison avec le sauvage, en présence de concentrations croissantes de KNO_3, KCl et NaCl. Les semences du mutant 422 avaient été capables de germer à une concentration saline de plus de 50 mM par rapport au sauvage. Par conséquent, ce mutant sera encore étudié ultérieurement pour rechercher les bases génétiques et les mécanismes physiologiques de la tolérance au sel.*

Mots-clés : *Mutant du blé dur, stress salin, analyse de la croissance, germination, tolérance osmotique.*

Introduction

Salinity in soils and ground water has become a major environmental issue (Shannon *et al.,* 1994). Excessive salt accumulation in soils, in fact, has been recognized as a limiting factor for crop production of one-third of the world's limited arable land (Epstein *et al.,* 1980). Hence, a detailed understanding of the basic mechanisms involved in the plant salt tolerance is an important prerequisite to improve the performance of crop plant in saline soils (Binzel and Reuveni, 1994).

In addition to its important affects on yield, salt stress affects numerous cellular activities, including cell wall composition (Jones and Turner, 1978), photosynthesis (Locy *et al.,* 1996), protein synthesis (Singh *et al.,* 1985), ions (Rains, 1972; Greenway and Munns, 1980) and organic solutes (proline, glycinebetaine, and polyols) content.

The role of potassium has been investigated because it is involved in many physiological processes such as turgor potential regulation, cell elongation, growth of shoot and roots, stomatal movement, transpiration. In fact, under drought conditions, potassium application has shown effects on growth, water use efficiency, dry matter production and yield (Van deer Paauw, 1958; Andersen *et al.,* 1991).

In wheat a relationship between K^+ ion accumulation and changes in thermodynamic properties of tightly bound water has been found (Rascio *et al.,* 1994). Moreover the ability to accumulate K^+ ions in organs is positive for drought and saline tolerance (Hsiao and Lauchli, 1986; Fitter and Hay, 1987).

In durum wheat, genotypic differences for the potassium accumulation capability in leaves tissues have been observed and in a drought tolerant genotype, K^+ plus Cl^- ions accounted for as much as 35% of the recorded osmotic adjustment (Rascio *et al.,* 1994).

Salt tolerant mutants have been isolated in soybean, barley, tobacco and fern (Abel, 1969; Kueh and Bright, 1982; Sumaryati *et al.,* 1992). We have identified mutants of durum wheat with altered accumulation of K^+ ions. They may contribute to identify mechanisms of K^+ uptake, accumulation and partitioning, as well as to define useful genes for transfer in traditional breeding programs.

In this paper preliminary results of physiological characterization for a durum wheat mutant (422) that over accumulate ions in the leaves are reported.

Materials and methods

Plant materials and mutagenesis. Seeds of *Triticum turgidum* L. var. *durum* Desf. (cv. Trinakria, *wild type*) were mutagenized for 2 h in Sodium Azide 0.01 mM, pH 3, at 25ºC, washed in distilled water and air dried. Mutagenized seeds were sown in the field of the Experimental Institute for Cereal Research of Foggia and grown to maturity.

270 M4 plants were selected from 4000 genotypes and the K^+ concentration in leaf tissues was measured by atomic absorption. Several M5 plants with abnormal K^+ concentration in the leaves were identified and the accumulation kinetic of potassium of 17 putative mutant lines was analyzed. In particular, the mutant 422 was selected for further studies as it showed greater K^+ concentration in the leaves at different growth stages.

Hydroponic culture for growth and ions accumulation analysis. 422 mutant and wild type (*wt*) seeds were germinated between wet paper towels in Petri dishes at 25ºC in the dark. After 5 d, 10 seedlings for each genotype were transferred in containers containing an aerated Hoagland's solution.

The plants were grown in a growth chamber under 14 h light (250-W high pressure sodium lamps and 400-W high pressure metal halide lamps) at 25ºC and 10 h dark at 22ºC. Containers for hydroponic culture (2 L) were black painted and covered with aluminum foil to prevent growing of algae in the nutrient solution.

Effects of salt stress were determined comparing growth in nutrient control solution and in selective media containing 100, 150 and 200 mM NaCl, KCl or KNO_3 respectively. A completely randomized design with four repetitions was adopted.

The plants were harvested on the 22th day of growth. Roots and leaves were collected separately and rinsed with distilled water for 10 min. Dry matter was determined and related to number plants. Concentration of K^+ ion was determined by drying plant tissues in a vacuum oven at 80ºC for 18 h, weighed and ashed at 550ºC for 3 h. K^+ ion content was measured by using a Perkin Elmer 370 spectrophotometer.

Salt treatments and seeds germination. Surfaces of vernalized seeds were sterilized in 3% (v/v) sodium hypochloride and rinsed with distilled water. Twenty five seeds, replicated 4-fold, were placed in Petri dishes on filter paper supplemented with salt solutions with concentrations varying from 0 to 250 mM. In order to distinguish between the effects of Na^+ and K^+, seeds were sown on solution containing KCl, KNO_3 or NaCl. Plates were sealed with parafilm and placed in a growth chamber at 20ºC. Additional germination tests were conducted on Petri dishes supplemented with mannitol concentration from 0 to 500 mM.

The mean (n = 4) germination percentage was calculated from number of seedlings, at 9 d of growth, having an axes at least 5 mm long derived from each Petri dish.

Analysis of variance was carried out on data to separate the effect due to salt, concentration and genotype.

Results

When grown in the field or in hydroponic culture, 422 mutant showed a higher leaf K^+ concentration than *wt*. Growth of 422 mutant, expressed as a percentage of dry matter accumulated in the absence of added salts, was less negatively affected than *wt* (Fig. 1). Although both genotypes showed the highest reduction in dry matter accumulation with 200 mM NaCl and the lowest with 200 mM KNO_3, 422 mutant systematically displayed a better tolerance with respect to *wt* when salt concentration was 100 mM.

The salt effect on root growth and K^+ concentration in the seeds did not differ between 422 mutant and *wt* (data not shown).

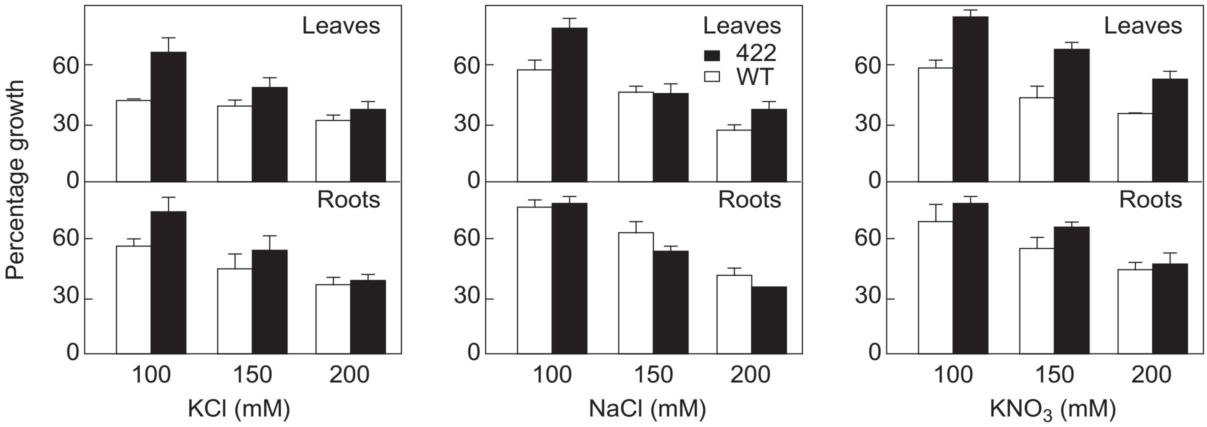

Fig. 1. Effects of salts on dry matter accumulation in leaves and roots of *wild type* (blank column) and 422 (full column) plants grown for 22 days in saline solutions. Salt effects on growth were calculated as percentage of dry matter accumulated in the absence of added salts. The values are means of four plantlets and error bars denote the SE.

The difference in germination performance between *wt* and 422 mutant seeds is shown in Fig. 2. In general, both genotypes had a similar behaviour when the seeds were grown in absence of salt or at concentration of 100 mM, with exception for KNO_3. In KCl treatment, 422 mutant showed an higher seed germinability with respect to *wt*, while this was less evident in NaCl treatment and specular to that observed in mannitol treatment.

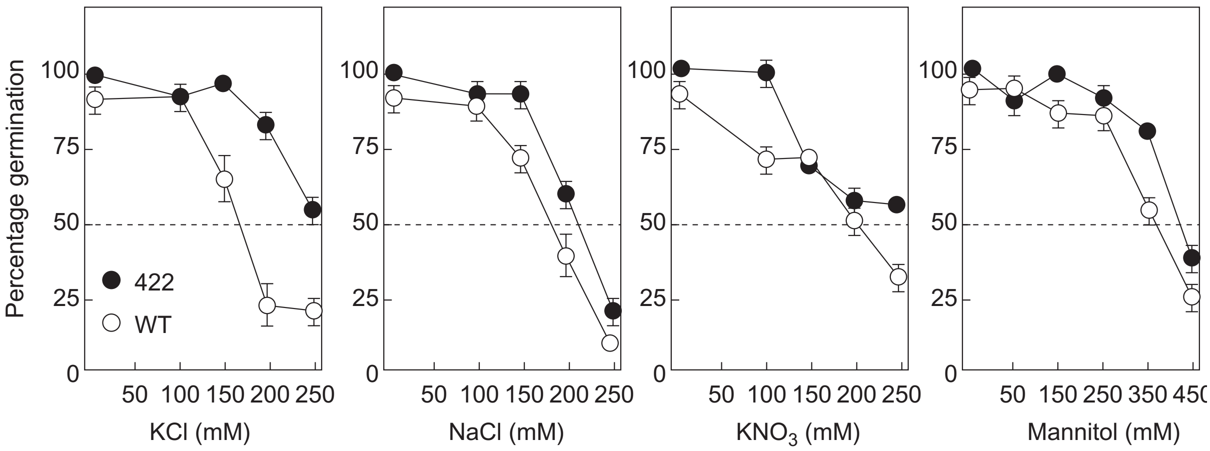

Fig. 2. Percent germination of *wild type* and 422 mutant seeds on solutions with increasing concentration of KCl, KNO_3, NaCl or mannitol. Bars indicate SE (n = 4). Intercepts between dotted lines and curves indicate salt concentrations that inhibited 50% germination.

These results idicate that 422 mutant shows a better performance under salt stress conditions. In fact, according to Stergios and Howell (1973), 422 mutant respect to *wt* reduced the seeds germination by 50% at higher salt concentrations (Table 1).

Table 1. Salt concentration that reduced the seeds germination by 50% in *wild type* and 422 mutant. The values were obtained for interpolation from curves in Fig. 2

Genotype	KCl	NaCl	KNO_3	Mannitol
Wild type	170 mM	180 mM	200 mM	400 mM
422 mutant	250 mM	200 mM	250 mM	460 mM

Discussion

The growth analysis carried out in hydroponic solution and seed germination tests have demonstrated a better tolerance to salt stress of the 422 mutant with respect to *wt*. Thus, it may be defined as a generally osmotolerant type.

Therefore, 422 mutant may represent promising material to study the genetic basis and to identify the gene(s) responsible of salt stress tolerance in tetraploid wheats.

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