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Physiological analysis of salinity resistance in *Triticum turgidum* var. *durum* Desf.: Callus versus whole plant responses

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SUMMARY – The behaviour of plants from three durum wheat cultivars (Omrabi 5, drought-resistant; Belikh, salt-resistant and Cando, drought- and salt-sensitive) was studied in the presence of various NaCl doses (from 0 to 150 mM) and compared to the behaviour of mature embryo-derived calli exposed to NaCl (from 0 to 300 mM) or to iso-osmotic concentrations of PEG and mannitol. The cultivar Belikh behaved as the more salt-resistant in terms of growth at both levels of organisation. However, strategies adopted by whole plant on the one hand, and corresponding calli on the other hand may differ in respect to mineral nutrition and osmotic regulation. While salt stress induced a strong decrease in shoot K concentration, it induced a slight increase in this parameter at the callus level. Sugars appeared as the major compound involved in osmotic adjustment of stressed plants but did not clearly contribute to the decrease of internal osmotic potential at the cell level. The fact that PEG had a distinct specific effect on most physiological parameter recorded suggests that the ionic component of salt stress is a crucial point to consider. Moreover, since the internal strain appearing in response to a given dose of NaCl and quantified in terms of sodium accumulation and water content was of the same order of magnitude in Belikh and in the other cultivars, the better behaviour of the former cultivar comparatively to the latter should be linked to a strategy of tolerance.

Key words: Durum wheat, NaCl, osmotic adjustment, salinity.

RESUME *– "Analyse physiologique de la résistance à la salinité chez Triticum durum Desf. : Réponses des cals par rapport à la plante entière". Le comportement de plantes appartenant à trois cultivars de blé dur (Omrabi 5, résistant à la sécheresse ; Belikh, résistant au sel et Cando, sensible au sel et à la sécheresse) exposées à des doses de NaCl (de 0 à 150 mM) a été comparé à celui de cals obtenus à partir d'embryons matures de ces mêmes cultivars maintenus en présence de NaCl (de 0 à 300 mM) ou à des concentrations iso-osmotiques de PEG ou de mannitol. Le cultivar Belikh se comporte clairement comme le plus résistant en terme de croissance, tant au niveau des plantes entières que des cals. Les stratégies adoptées par ces deux niveaux d'organisation sont cependant distinctes. Alors que le stress salin induit une réduction marquée des teneurs foliaires en K, ces teneurs augmentent légèrement au niveau des cals. Si l'accumulation de sucres solubles contribue de façon déterminante à l'ajustement osmotique des plantes entières, cette contribution n'est que très limitée au niveau des cals. Le fait que le PEG exerce des effets spécifiques sur la plupart des paramètres physiologiques analysés suggère que la composante ionique du stress salin est un point déterminant à prendre en considération. De plus, le fait que la contrainte interne s'établissant au sein des tissus stressés du cultivar Belikh, et quantifiée en terme d'accumulation de sodium ou de teneurs en eau, soit du même ordre de grandeur que pour les autres cultivars suggère que ce génotype met en place une stratégie de tolérance au NaCl.*

Mots-clés : *Blé dur, NaCl, ajustement osmotique, salinité.*

Introduction

The presence of high concentrations of salt (NaCl) in the soil solution induces a wide range of physiological and biochemical perturbations at the whole plant level. It has been demonstrated in several glycophyte species such as rice (Lutts *et al.,* 1996a,b), potato (Sabbah and Tal, 1990) and alfalfa (Winicov and Krishman, 1996) but also in halophyte species like *Mesembryanthemum crystallinum* (Vera-Estrella *et al*., 1999), that the responses exhibited by salt stressed plants is, at least partly, determined by cellular properties. This suggests that, although salinity resistance is a complex trait resulting from the interaction of several morphological and physiological properties, it should be possible to select salt-resistant cell lines with the hope to regenerate valuable plant material which could be successfully integrated in a breeding scheme. To our knowledge, however, the relationship between whole plant and cellular response to salt stress was never exhaustively studied in durum wheat.

Salinity induces both an ionic and an osmotic strain in plant tissues. Hence, a better understanding of salt stress effect at the cell level implies the discrimination between these two components of salt stress. The aim of our work was: (i) to analyse the relationship between whole plant and cellular response to salt stress in durum wheat; and (ii) to determine the relative importance of ionic and osmotic stresses in NaCl-treated calli of three durum wheat cultivars differing in their mean level of drought and salt resistance.

Materials and methods

Seeds of durum wheat cvs. Omrabi 5 (drought-resistant), Belikh (salt-resistant) and Cando (drought- and salt-sensitive) were obtained from ICARDA (International Centre For Agriculture Research in Dry Areas, Alepo, Syria).

Plants (105 per cultivar) were cultivated in greenhouses according to Almansouri *et al.* (1999) on nutritive solution renewed once a week. When the seedlings were 21 day-old, the solution was adjusted to the desired salinity (0, 50, 100 and 150 mM NaCl) either in 25 mM daily increments (progressive exposure) or in a single step (sudden exposure). For callus induction, mature embryos were aseptically separated from the endosperm of pregerminated sterilized seeds and placed with the scutellum upwards on a LS medium pH 5.6 supplemented with 2 mg/l 2,4-D, 100 mg/l *myo*-inositol, 0.1 mg/l thiamine-HCl, 30 g/l saccharose and 3 g/l Gelrite (Phytagel; Sigma). After one month in the dark, calli of uniform size (ranking from 7 to 10 mm) were individually weighed and placed on LS medium supplemented with stressing agents: NaCl (100, 200 or 300 mM), mannitol (180, 350 and 505 mM) or PEG 6000 [18, 24 or 29% (w/v)]. These iso-osmotic various concentrations correspond to osmotic potential of -0.78, -1.24 and -1.69 MPa. For each treatment (cv x osmotic agent x osmotic potential), 30 calli were used.

Plants were physiologically characterized after 14 days of stress and calli after 30 days of treatment. Fresh and dry weights of all samples were recorded and dry weight percentages were estimated after drying the tissues at 70ºC for 48 h in an oven. Relative growth rates were estimated for roots, shoots and calli. Estimation of K and Na concentrations was conducted using an inductively coupled argon plasma emission spectrophotometer (Jobin-Yvon JY 48) after digestion in a 3:1 nitric:hydrochloric acid mixture. Cell sap was extracted according to Lutts *et al.* (1996b). Osmotic potential (Ψs) of the collected sap was assessed with a Wescor vapour pressure osmometer Wescor 5500. Free proline was quantified using the ninhydrin reagent (Bates *et al.,* 1973) and total soluble sugars were estimated by the anthrone reagent method using glucose as the standard (Yemm and Willis, 1954). For callus culture, cell viability was determined using the TTC test according to Lutts *et al.* (1996c).

Results and discussion

A sudden NaCl application affected whole plant growth further more than a progressive salinisation. Heckenberger *et al*. (1998) recently pointed out that the relationship between the rate of cell division and the rate of cell elongation may be a direct function of the kinetics of stress development. Belikh clearly behaved as the more salt-resistant cultivar in terms of growth at both levels of organisation, thus suggesting that the resistance of this cultivar probably involved a cellular component as previously demonstrated for salt resistant rice cultivars (Lutts *et al*., 1996a). The growth of calli obtained from Belikh was however strongly inhibited by PEG and even presented a negative relative growth rate expressed on a fresh basis in relation to a drastic loss of water. Mannitol was the least detrimental osmoticum for all cultivars. It was notheworthy, however, that differences between the effects of the various stressing agent on callus growth were not directly correlated to cell viability, thus reinforcing the view of Munns *et al.* (1995) suggesting that growth *per se* is not an exhaustive criteria of salinity resistance.

In both stressed shoots and NaCl-treated calli, sodium concentration was of the same order of magnitude in all cultivars, suggesting that the cultivar Belikh developed a physiological strategy of tolerance. At the whole plant level, K concentration decreased in response to salt stress, but, surprisingly, slightly increased in response to the same treatment at the callus level. Although a slight increase of K concentration was already reported in salt-treated callus of the halophyte species *Atriplex halimus* (Bajji *et al*., 1998), such a discrepency between whole plants and callus behaviour in durum suggest that K/Na discrimination is not controlled in the same way in both systems. On the other hand, the fact that PEG had a drastic effect on K nutrition of calli may contribute to its strong negative effect on growth, as previously suggested in other species by Zhao and Harris (1992).

Shoot osmotic potential was strongly affected in response to 100 and 150 mM NaCl. Belikh exhibited the highest Ψs values, corroborating once again the hypothesis of a tolerance mechanism in this cv. Osmotic potential of NaCl-treated calli in the salt-resistant cv. Belikh were not significantly different from those of the salt-sensitive cv. Cando and were higher than those of the drought-resistant cv. Omrabi 5, which exhibit a high level of osmotic adjustment. PEG induces a strong decrease in osmotic potential in all genotypes. At the whole plant level, an important part of osmotic adjustment may be due to soluble sugar accumulation while proline accumulated only at the highest concentration of NaCl in suddenly treated plants but not in progressively exposed ones. Although sucrose is available at a high concentration in the external medium, soluble sugars did not accumulate in stressed calli. Stress-induced accumulation of proline at the callus level was highest in response to NaCl and lowest in PEG-treated calli, although these later calli exhibited the highest level of osmotic adjustment. This confirms that proline accumulation alone does not explain the whole osmotic adjustment in durum wheat cell lines, as recently noted by Bajji *et al.* (1999). The precise nature of other compounds involved in such osmotic adjustment remains unknown.

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