Differential Protein Expression in Olive Tissues Due to Salt Treatments

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ABSTRACT

In this work, differential protein expression in olive tissues after salt treatments has been investigated. Olive clones selected *in vitro* according to their salt tolerance were submitted, also *in vitro*, to high salt concentrations.

After each treatment, the leaves and stems were collected and protein extracts of them subjected to polyacrilamide gel electrophoresis to analyse their band patterns.

Some differences were observed referring to 2 bands of approximately 24 kD and 40 kD which appeared in the extracts after exposition to salt.

It is discussed the possible implication of this polypeptide in the response of olive tree to salt stress and similarities with other proteins described in other plant species.

INTRODUCTION

Salt stress is a major concern in the fruit tree industry. Although olive could be considered as relatively tolerant to soil salinity, a sustanaible oliviculture would require more tolerant varieties which could, for example, be irrigated with saline waters.

For the selection of these new varieties or to test the salt tolerance of other already known varieties, it would be of great help to find specific markers associated to salt stress which could assist the selection process. Such markers would enable the acceleration and increased reliability of selection for resistance among cultivars in breeding programmes. Furthermore, these markers could be instrumental in identifying relevant genes, thus helping in the efforts of introducing stress resistant by gene transfer.

Up to date work on markers for different physiological situations in the olive tree has been scarce. Differential protein expression was reported for juvenile and mature olive tissues (García et al., 2000; García et al., 2002). In relation to stress resistance, Bartolini et al. (1994) found different protein patterns between tolerant and non tolerant clones of cv. Leccino when submitted to cold stress.

In this work we have compared the electrophoretic protein patterns of two different clones of cv Jabaluna after cultured *in vitro* with different concentrations of NaCl in seeking differential protein expression due to the salt treatments.

MATERIAL AND METHODS

Two clones of olive cv Jabaluna were used as plant material. These 2 clones (J0 and J79) were obtained by *in vitro* germination of isolated embryos as described elsewhere (Acebedo et al., 1997). Clone J0 was obtained from an embryo germinated in normal medium without salt while clone J79 was obtained from an embryo grown on 120 mM of NaCl, then this was considered as tolerant. After germination, the plantlets selected were subcultured to attain enough plant material of each clone. To check the tolerance to salt of both clones, explants from them were cultivated for 7 weeks on medium OM + 15 $g.l^{-1}$ of mannitol and 1 $g.l^{-1}$ zeatine as base medium and 3 different treatments: control (no salt added), 50 mM, and 90 mM of NaCl. Single node explants of

each clone were placed individually in test tubes with 10 ml of media and grown in a culture chamber at 23±2 °C with a 16 h photoperiod and 30 μmol $m^{-2}s^{-1}$ of light intensity.

After culture, total nº of nodes and shoot length was measured. Leaves and stems of each treatment and clone were grounded separately in liquid nitrogen and stored at –80 °C until use. Protein extraction was performed as described previously (García et al., 2000) and protein concentration of the extracts according to Bradford (1976).

Samples of each extract with equal amounts of protein (30 μg) were subjected to SDS-polyacrilamide gel electrophoresis on 12% running and 4% stacking gels (Laemmli, 1970). Electrophoresis was carried out at 15 mA. After running, gels were stained with 0.25% Coomassie brilliant blue and then destained. The molecular mass of the protein bands was determined by comparison with reference proteins ranging from 14.4 to 116.3 kD (Bio-Rad).

RESULTS AND DISCUSSION

Table 1 shows the growth of the explants of both clones after 7 weeks of culture with the different treatments. As shown in the Table, 50 mM of NaCl provoked an inhibition of both the number of nodes and shoot length in both clones as compared to the control treatments. However, survival was lower for clone J0 and the shoot length was half of the clone J79. When 90 mM of salt was applied to the medium all the explants of J0 died while those of J79 presented a 69% of survival and some growth. This confirmed the previous supposition of J79 as more tolerant to salt.

From the examination of the electrophoretic patterns (Fig. 1) it was observed that 2 bands appeared to respond differentially to the salt treatments, since they presented more signal in the extracts corresponding to salt treated samples compared to control. One of these bands, of around 40 kD, was more strongly expressed in the stem extracts of J79 plantlets grown on 50 and 90 mM (Fig. 1b, lanes 4 and 5) and seemed to show increasing signal with the increasing concentrations of salt which may indicate a linear response to the salt dose. The other band of around 20 kD also seemed to increase its expression after salt exposure, both in leaves (Fig.1a, lanes 3-5) and stems (Fig.1b, lanes 3-5) of the J79 clone. In the extracts of clone J0 this behaviour was less marked. In this clone, the band of 40 kD showed a slight increase in expression between the control and the 50 mM stem extracts (Fig. 1b, lanes 1-2), while the 20 kD band displayed only a faint signal in the extracts of both tissues of this clone (Fig. 1). As shown from the data of culture (Table 1), clone J79 was more tolerant to salt *in vitro*, so these bands may be related to the physiological and adaptative responses to salt stress and markers for the tolerance to this kind of stress. This hypothesis would be consistent with the fact of the higher expression of the polypeptides in the tissues of the more tolerant clone J79. If so, this would be the first time, up to our knowledge, in describing polypeptides related to salt stress in olive tissues, although more work is needed to confirm these results in different olive varieties and culture conditions.

The 20 kD band shows similar characteristics of the product of the gene TAS14 that has been characterised in tomato (Godoy et al., 1990). TAS14 is inducible in tomato upon salt stress and ABA treatment, and although the predicted molecular mass for this polypeptide is 14 kD the observed electrophoretic mobility is around 20 kD (Godoy et al., 1990). TAS14 is a member of a general class of genes named dehydrins (dehydration-induced). Dehydrins have been found in different plant species and are induced by ABA and different kinds of environmental stresses, including desiccation, osmotic stress and salt stress. As mentioned above, TAS14 is induced in tomato seedlings and mature plants

upon treatment with NaCl, ABA or mannitol and localized in stems and leaves of salt treated tomato plants (Godoy et al., 1994).

The 20 kD band observed in the extracts of olive tissues seems to be induced by the salt treatment and its electrophoretic mobility is very close to that described for TAS14. Then, that band would be a good candidate to be a dehydrin in the olive although further investigation is needed to check this hypothesis as well as to characterize also the 40 kD band.

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Table 1. Explant growth after 7 weeks of culture with the different salt treatments.

Clone	J0			J79		
Treatment	Surv. %	Nº of nodes	Shoot length (mm)	Surv. %	Nº of nodes	Shoot length (mm)
Control	100	7.95 ± 0.31	40.05 ± 1.91	100	7.43 ± 0.20	58.28 ± 1.54
50 mM	72.7	4.81 ± 0 .34	16.69 ± 1.30	96.7	4.73 ± 0.25	30.96 ± 1.46
90 mM	0	-	-	69.2	3.55 ± 0.33	16.09 ± 2.62

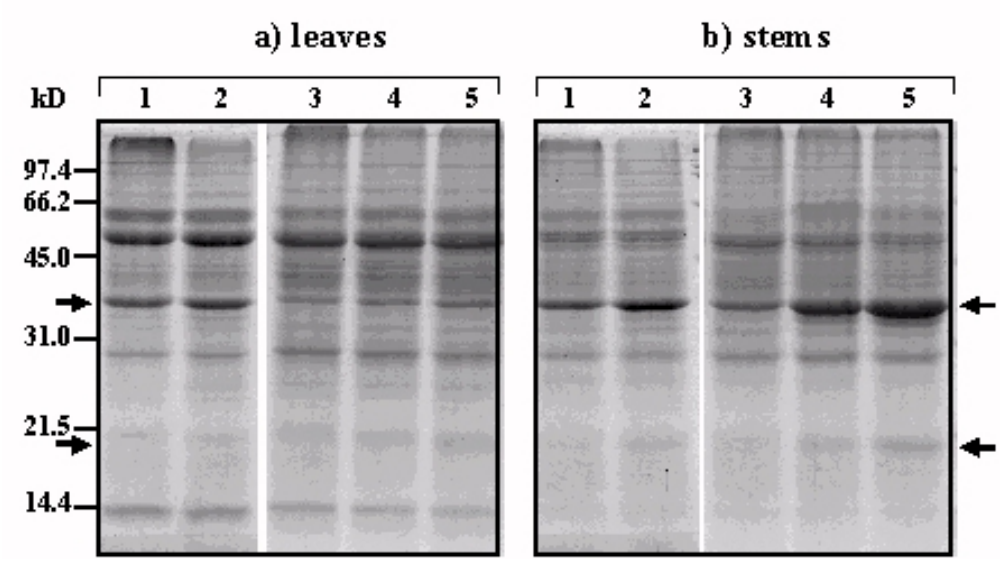

Figure 1. Electrophretic patterns of leaves and stems extracts of the explants.

a) Leaves: clone J0 control (1), clone J0 cultured in 50 mM of salt (2), clone J79 control (3), clone J79 50 mM of salt (4), and clone J79 90 mM of salt (5).

b) Stems: clone J0 control (1), clone J0 cultured in 50 mM of salt (2), clone J79 control (3), clone J79 50 mM of salt (4), and clone J79 90 mM of salt (5).

Arrows indicate the 40 and 20 kD bands that show different expression in controls and in salt treated explants.

A 12% acrylamide running gels were used and 30 μg of protein of each sample loaded onto the gels.