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Alfalfa (*Medicago sativa* L.) clones tolerant to salt stress: *in vitro* selection

Research Article

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Abstract: In order to quickly and efficiently evaluate the salt tolerance of alfalfa, salinity tests were conducted on *Medicago sativa* L. var. australis, var. icon, var. loi, and var. gea, under *in vitro* conditions. Pregerminated seeds of four varieties were subjected to five different NaCl concentrations (0, 50, 100, 150, 200 mM). The influence of saline stress was estimated on the basis of survival percentage, growth parameters, and electrolyte leakage. The seedlings surviving on the medium enriched with salt at the highest concentration were presumed to be tolerant and represented the mother plants for the production of *in vitro* clones. In the following step, the clones were evaluated *in vitro* to confirm the salt tolerance. The influence of mild salt stress (75 mM NaCl) on the growth parameters of selected clones was examined. At the end of this trial, the proline accumulation and sodium content in alfalfa shoots were also quantified. The results suggest an increased level of proline promotes salt tolerance. *Medicago sativa* L. var. icon is highly tolerant in comparison with the other varieties tested. *In vitro* selection of *M. sativa* L. varieties on salt-containing media allowed us to obtain clones with increased salinity tolerance.

Keywords: *Medicago sativa L. varieties • Salinity tests • In vitro culture • Salt tolerance • Growth • Electrolyte leakage • Proline*

1. Introduction

Medicago sativa L. is one the most common forage species in the world thanks to the quantity and quality of production, its longevity, and ability to improve the characteristics of the land where it is cultivated [1]. This important forage crop is widespread in arid and semi-arid areas, where increased salinity is one of the major constraints on crop productivity. The need to produce plants with increased salt tolerance has been extensively emphasized by increased crop research [2,3]. Alfalfa is moderately sensitive to salt levels in irrigation water and in soil [4], but its great genetic variability [5,6] may be useful to select the most salt tolerant genotypes among and within the varieties. The success of appropriate selection techniques depends on the ability to exploit this variability. Salt tolerance is achieved due to the control of salt movement into and through the plant. Salt-specific effects on growth are seen only after long periods of time [2].

Salt tolerant germplasm selection by investigation in the field needs to complete the productive cycle to measure biomass or yield. Therefore a promising, efficient and simple alternative could be to *in vitro* plants, favoring practical selection techniques based on morphological and physiological traits under controlled stress conditions [7]. *In vitro* culture is a valid and rapid tool for studying the behavior of plants in response to salt stress, while other factors (nutrients, lighting, temperature) are held constant and controlled in an optimal manner. This approach has been successfully used for the evaluation of salt stress in *brassica* [8], tomato [9-16], mulberry [17,18], potato [19], rice [20-22], poplar [23,24], strawberry [25], *eucalyptus* [26-28], grapevine [29,30], *citrus* [31,32], and *pistacia* [33].

In this study the *in vitro* methodology was recommended for the selection of alfalfa varieties *via* the use of indexes [25,30] which facilitated comparisons in salt tolerance between the four varieties tested. Using morphological measurements of plantlets grown on a salt-containing medium as the only indicator of salinity resistance can be misleading [25]. In fact, some tolerant varieties may not be affected by the salinity of the substrate as increases in morphological parameters were found not only on the saline medium, but also on the control medium. Differences between varieties growing

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