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Assessment of salt tolerance in *Populus alba* clones using chlorophyll fluorescence

H. SIXTO, I. ARANDA, and J.M. GRAU

Forestry Research Centre (CIFOR), National Institute for Agricultural Research (INIA),
Crta. de la Coruña Km. 7, Madrid 28045, Spain

Abstract

Cuttings of five *Populus alba* clones (S18 F1-26, Al29 F8-35, J3 F1-4, GU1 F16-36, PO9 F21-88), *Populus euphratica*, and *Populus*×*euramericana* (I-214) were submitted during 45 d to regular watering with NaCl solutions of electrical conductivity of 7 and 14 dS m^{-1}. Chlorophyll *a* fluorescence in response to the salinity stress was assessed, using F_0 and F_v/F_m. Differences in reaction to the salt were found in *P. alba* clones, F_0 and F_v/F_m being the fluorescence parameters used to check out this stress. Minimal constant fluorescence of dark-adapted plants (F_0) showed a better correlation with the disease index exhibited by plants and also with salinity dose than the parameter F_v/F_m. Some of the *P. alba* clones showed the same behaviour, assessed through fluorescence parameters, as *P. euphratica*, which was previously defined as salt tolerant, while the rest exhibited the same characteristics as I-214, which was very sensitive.

Additional key words: disease index; photosystem 2; salinity.

Introduction

Large areas of irrigated land in semiarid areas have been converted into non-profitable land due to the increase of salt content in the soils (Ghassemi *et al.* 1995). Thus, selection of salt-tolerant genotypes of forest tree species may be a solution from an economic and environmental point of view. The use of fast growing species such as *Populus* sp., with a known tolerance to salinity (Marcar *et al.* 1993), would be a possible alternative in the use and management of these lands. *Populus euphratica* Oliv. is recognized as one of the most salt-tolerant species of aspen (Chen *et al.* 2002). Furthermore, clonal variability in the sensitivity to salinity was observed in different species of the genus, such as *Populus deltoides* Marshall (Singh *et al.* 1999) or *Populus alba* L. (Cuevas *et al.* 1997, Sixto *et al.* 2005). The latter is more tolerant to salinity than the species of other genera (Kuzmysky *et al.* 1999).

P. alba (white poplar) has a wide distribution range in the south, centre, and east of Europe, being also present in Central Asia and the north of Europe (FAO 1980). Its presence in riverside ecosystems throughout the Mediterranean has resulted in its being included in the European Program of Forest Genetic Resources (EUFORGEN 1999). Their importance lies in being a fast growing species as well as in having the capacity to adapt to stress conditions. This has aroused interest both in their selection for forestation and in conservation programs of the species (Peso *et al.* 1997), as an alternative to be considered in the recovery of marginal lands. A high variability in some phenotypic characters has been observed in Spanish populations (Alba 2001), which could be interpreted as a high degree of geographical structure in these populations. This could be used as background in the selection programs of salt-tolerant genotypes.

Soil salinity causes adverse effects on different physiological parameters which, in the end, affect the growth and survival of plants negatively. Different processes related to the photosynthetic pathway (Papp *et al.* 1983, Singh *et al.* 1996), the reduction of leaf area (Neuman *et al.* 1988), or to a general decrease in growth (Fung *et al.* 1998), have proved to be sensitive to soil salinity stress. In recent years, non-invasive methods such as chlorophyll (Chl) *a* fluorescence have been used to observe different types of stress and disease affecting the photosynthetic machinery (Lichtenthaler 1996). This facilitates the design of protocols for the use of Chl *a* fluorescence analysis as a tool for the screening of genotypes with different tolerance to stresses, including salinity.

Chl fluorescence has been used to assess the response by aspen trees to different stresses such as frost (Renaut *et al.* 2001), irradiance (Zhang *et al.* 1999), or ozone (Guidi *et al.* 2001). The effect of different salt treatments

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Fax: ++34913572293, e-mail: sixto@inia.es

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on *P. euphratica* and hybrids of the species has been also analysed successfully by the use of Chl *a* fluorescence (Ma *et al.* 1997). The goal of this work was to make clear differences in salt tolerance among *P. alba* genotypes using different parameters of this non-invasive technique.

Materials and methods

Plants: Rooted cuttings of 5 autochthonous clones of *P. alba* L. from Spain (S18 F1-26, Al29 F8-35, J3 F1-4, GU1 F16-36, PO9 F21-28), coming from the watersheds of the Ebro, Guadalquivir, and Almanzora rivers, were used in the study. *P. euphratica* and *P.* ×*euramericana* I-214 which are tolerant and sensitive to salt stress (Sixto *et al.* 2000), respectively, were used as references.

The plants were grown in a greenhouse under controlled temperature (25±3 ºC max. and 10±3 ºC min.), relative air humidity (≈70 %), and irradiance (photosynthetic photon flux density of 1 000 μmol m^{-2} s^{-1}, on the average). Cuttings, 25-cm long, were planted in individual pots using river sand as substrate. Plants were regularly well-watered and a nutrient solution was applied weekly (Hewitt 1963). After flushing, plants were fertilized and well-watered for two months without salt.

Salinity treatments were established by watering with two different solutions of NaCl (68 and 137 mM with an electrical conductivity of 7 and 14 dS m^{-1}, respectively). A control treatment was added without salt containing only the nutrient solution (Hewitt 1963). Salt solutions were applied twice weekly during six weeks. A dripping system enabled the application of the same amount of solution to each pot. During the first week, the conductivity of the solution was increased to a rate of 2 dS m^{-1} each two days until reaching the target salinity. Eight rooted cuttings were used in each plot (treatment/clone combination) in a randomised layout of the factors in the greenhouse.

Chl fluorescence: Minimum (F_0) and maximum fluorescence (F_m) were recorded using a portable device (*PSM Mark A*-Plant Stress Metre). Both fluorescence parameters were recorded after full acclimation of leaves to darkness, with all photosystem 2 (PS2) reaction centres open. Variable fluorescence was calculated as the difference between F_0 and F_m. F_v/F_m was used as a surrogate of the maximum quantum efficiency of PS2, as a tool to detect genotypic differences in the sensitivity of white poplar clones to salinity.

Measurements were carried out three times during the trial, in the beginning of the watering cycles with NaCl (T0), in the middle (T1), and at the end of the experiment (T2). Two measurements were taken in each replicate for the tree treatments. Chl *a* fluorescence was recorded at 12:00 after 30 min of dark adaptation of the leaves.

The disease index is defined, after the criteria of Cuevas *et al.* (1997), as the percentage of plants with symptom-free leaves.

Data analysis: To test differences among clones in sensitivity to salinity, one-way ANOVA was applied to the analysis of the different parameters according to the model: $x_{ijk} = \mu + C_i + TR_j + T_k + CTRT_{ijk} + \varepsilon_{(ijk)l}$, where x is the analysed variable, μ is the general mean, C_i is the clone effect (I = 1…7), TR_j is the treatment effect (j = 1…3), T_k is the time effect (k = 1...3), $CTRT_{ijk}$ is the clone-treatment-time interaction, and $\varepsilon_{(ijk)l}$ is the residual (l = 1…24). Differences among treatments, times, and clones were established from a Newman-Keuls test ($p<0.05$). The relationship between F_0 and F_v/F_m with the disease index in the different salinity treatments was tested by linear regression models.

Results and discussion

The ANOVA analysis for the fluorescence parameters studied (F_0 and F_v/F_m) showed significant differences ($p\leq 0.001$) concerning the main factors (treatment – TR, clone – C, and time – T) and their interaction, except for TR×C×T interaction in F_v/F_m. The main differences in F_v/F_m between clones were observed at T2 (Fig. 1). *P.* ×*euramericana* clone I-214 showed the highest decrease in F_v/F_m as well as in F_0 with regard to the control, which is in accordance with the previously shown sensitivity of this clone to salinity (Sixto *et al.* 2000). This was observed for both treatments of salinity (7 and 14 dS m^{-1}), and at T1 and T2. On the contrary, *P. euphratica*, which has been considered tolerant to high concentrations of NaCl by different authors (Liu *et al.* 1990, Wei 1993, Ma *et al.* 1997), showed no significant changes in Chl fluorescence parameters between control and salt treated cuttings (Fig. 1).

A similar degree of sensitivity to salinity was observed in the *P. alba* clones S18 F1-26, Al39 F8-35, and PO9 F21-28 and with *P. euphratica*. This allows consider these clones as salt-tolerant. However, at the end of the experiment, J3 F1-4 had a significant decrease in F_v/F_m with respect to control cuttings, for both salinity treatments. GU1 F16-36 also showed a significant decrease in F_v/F_m at T1 and T2, but only in the growing media with the strongest salt concentration. Thus both clones must be considered salt sensitive. Indeed these clones showed clear visual symptoms of diseased leaves from cuttings of the salt treatments. Hence *P. alba* clones had a differential response in terms of sensitivity to salinity. The observed differences among the clones may be not related to their provenance, since Al39 F8-35 and J3 F1-4, both from the Ebro valley, showed tolerance and sensitivity, respectively. The same happened with PO9 F21-28 and GU 1 F16-36, both from the Guadalquivir valley.

The changes in F_v/F_m were paralleled by modifications in F_0. Maximum increase was thus observed for the clone I-214 at T2 and in both salinity concentrations (Fig. 1). However, leaves of *P. euphratica* maintained a similar F_0 for control and treatments during the experiment. The same was observed in *P. alba* clones S18 F1-26, Al39 F8-35, and PO9 F21-28 where F_0 was similar for all treatments. J3 F1-4, however, showed higher F_0 in plants under both salt treatments than in control, at the end of the experiment.

The F_v/F_m ratio is considered as proportional to the quantum efficiency of PS2 (Björkman and Demmig 1987), and the effect of different stressful environmental factors on PS2, including salinity (Percival *et al.* 2003), may be reflected by reduction in this ratio. Comparably, the higher F_0 values in some clones could be related to the degradation of D1 protein in PS2 (Aro *et al.* 1994) or could be associated with dissociation of the light-harvesting Chl *a/b* complexes from the reaction centre complex of PS2 (Yamane *et al.* 2000).

Changes of fluorescence parameters were followed by changes in the disease index, shown as the percentage of plants with injuries. Correlation of F_0 between both salinity treatments was higher (r = 0.94) than that of F_v/F_m (r = 0.70) (Fig. 2). Furthermore, F_0 was also better correlated with the disease index than F_v/F_m for both salinities (Fig. 3). This fairly strong relation indicates that F_0 could be a better predictor than F_v/F_m for screening salt tolerance in *Populus* clones. In a similar way, the increase in F_0 is a better index than F_v/F_m of stress tolerance to other stressors such as high or low temperatures (Ma *et al.* 1997, Froux *et al.* 2004).

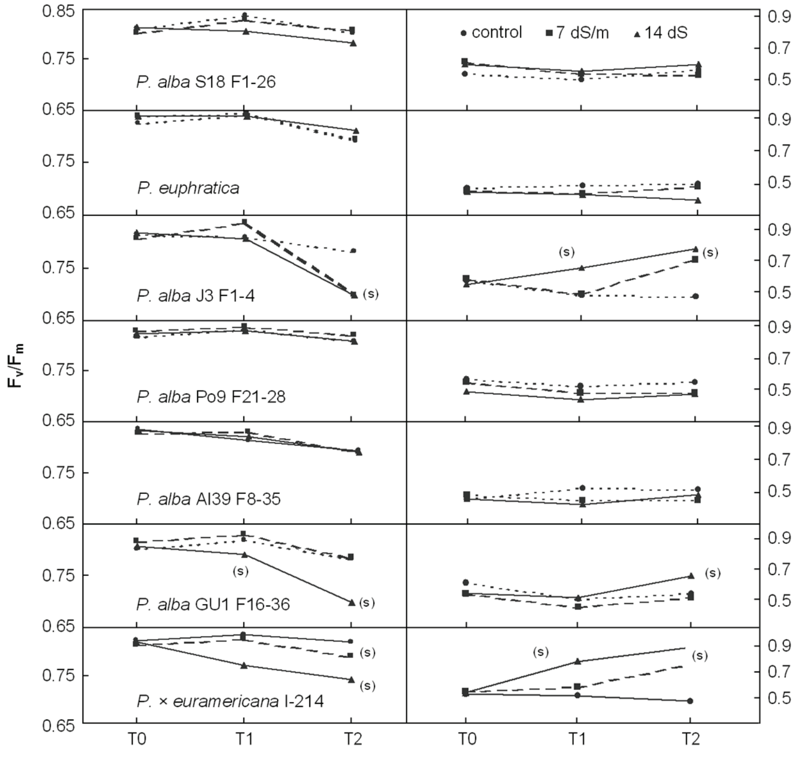

Fig. 1. Changes of F_v/F_m (*left panels*) and F_0 (*right panels*) at three times during the experiment for poplar clones submitted to different salinities. (s) indicates $p<0.05$.

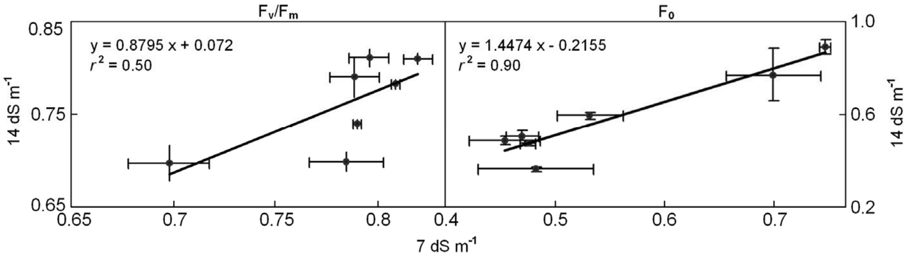

Fig. 2. Correlations between F_v/F_m (*left*) or F_0 (*right*) measured under two salinities. Means for each clone ± SE. n = 10.

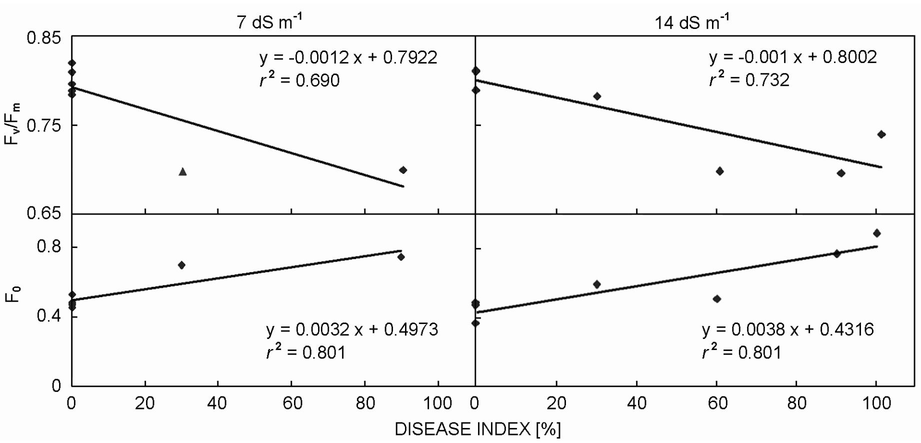

Fig. 3. Relationship between disease index and F_v/F_m or F_0 for both salinity treatments.

Chl fluorescence is an appropriate tool for earlier selection of salt-tolerant clones of *P. alba*. In this sense, there was a close agreement between the changes observed in the parameters derived from Chl fluorescence and the visual symptoms of damage in a similar way to the response to other stresses such as cold (Aranda *et al.* 2005). The methodology enables to screen the physiological state of the photosynthetic machinery (Krause and Weis 1990) and the changes observed by a differential sensitivity of genotypes to different stresses in a fast and non-invasive form (Ducruet *et al.* 1993). This methodology enables to complement the direct results obtained from trials in common garden trials under field conditions.

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