A SELECTION METHOD IN SUNFLOWER BASED ON PROTON EXTRUSION ACTIVITY.

G. Logozzo *, L. Stigliani *, E. Alba **, C. Soave **
* Metapontum Agrobios, 75010 Metaponto (MT) - Italy
** Faculty of Agriculture, Istituto Biologia Agraria, 85100 Potenza - Italy

SUMMARY

A method is described for a simple and rapid evaluation of the acidification capacity of roots of agricultural plants.
The method can be used to select for genotypes where the proton extrusion activity of roots is more tolerant to various environmental stresses.

INTRODUCTION

Increasing use of salt tolerant crop species is a mean of reclaiming saline agricultural land and bringing marginally productive soils under cultivation.
Examples of intraspecific variation and successful selection for NaCl tolerance have been reported in many crops (Epstein, 1985). However, the rate of genetic improvement could be much faster if the physiological traits which contribute most to salt tolerance are known and suitable selection methods for applying them are developed.
Osmotic adjustment and ion toxicity avoidance are operating in plant responses to salinity environment (Yeo, 1983). Both mechanisms rely, at least in part, on transmembrane electrical potential and on proton gradient built up by energy linked H+ extrusion as driving forces for uptake or exclusion of external ions (Marre', 1979).
In addition, there is now considerable evidence that proton extrusion activity, sustained by plasmalemma ATP-ase (the proton pump), is directly involved in several physiological functions, other than solute transport, such as control of internal pH, cell enlargement and growth, turgor maintenance and stomata opening (Marre', 1979). With these concepts in mind, we developed a simple and rapid method to evaluate the acidification capacity of plant roots (which is related to proton pump activity) and applied the method for estimating the sensitivity of different sunflower genotypes to increasing NaCl concentration.

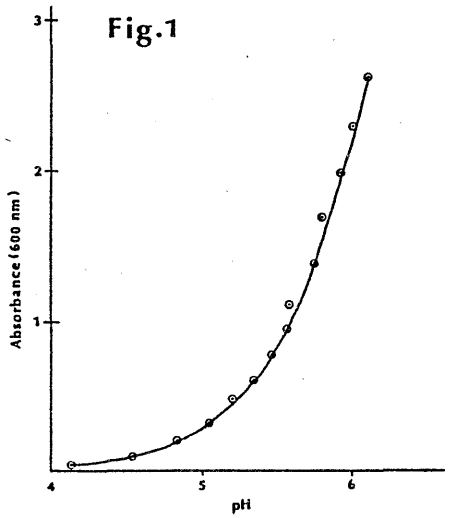
MATERIALS AND METHODS

Seeds of sunflower (Helianthus annuus L.) were germinated for 3 d on filter paper moistened with 0.5 mM $CaCl_2$ and 0.5 mM KCl at 25° C in the dark.

Seeds with primary roots 1.5-2.5 cm long were selected, briefly washed in the germinating solution and placed on the top of disposable spectrophotometric cuvettes covered with parafilm. The root passed trough a hole in the parafilm and dipped for at least 1.0 cm in the cuvette solution. Cuvettes were filled with 3 ml of a solution consisting of 0.5 mM KCl, 0.5 mM $CaCl_2$, 1 mM MES (Morpholinoethanesulfonic acid) adjusted to pH 6.3 with 0.1N NaOH and 5 mg/100 ml of the pH indicator dye bromocresol purple. The absorbance at 600 nm of the starting solution was measured at thebeginning of each experiment. When needed, NaCl was added at concentrations indicated in the text. Seeds were then incubated at 25°C in the dark and the absorbance at 600 nm recorded at different time intervals. Usually ten seeds per line per treatment were analyzed and each experiment was carried out two or three times. Data were subjected to multiple regression analysis for the evaluation of the results.

RESULTS AND DISCUSSION

The pH indicator dye method. The method used is a modification,of that proposed by Weinseel et al. (1979) allowing quantitative evaluation.
Briefly (a full description of the method will be published elsewhere), as the proton extrusion activity of roots takes place, acidification of the medium occurs producing a colour change (purple → yellow) of the pH indicator. From spectral analysis of bromocresol purple at different pHs, a distinct peak of absorbance at 600 nm and at pH around 7 is observed which progressively decreases as pH lowers. Fig. 1 shows the absorbance at 600 nm of a solution of bromocresol purple in the pH range 4 to 6.



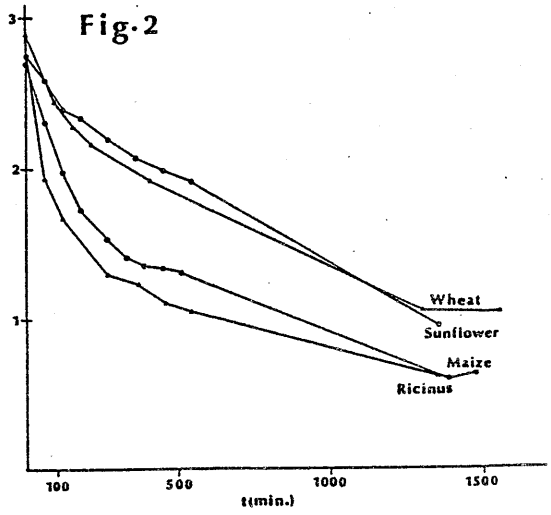


Fig. 1 pH dependence of bromocresol purple absorbance at 600 nm. A bromocresol purple solution (5mg/100ml) in MES 1mM, $CaCl_2$ 0.5mM,

KCl 0.5mM was adjusted to different pHs by addition of 0.1N NaOH and the absorbance at 600 nm recorded with an Hitachi 100-20 spectrophotometer.

Clearly, small changes in the pH of the solution induce large variations in the absorbance allowing a sensitive appreciation of the pH changes produced by protons extruded by roots. Furthermore, the analysis can be done on individual intact seeds since the acidification capacity of single roots (1.5-2.5 cm long) of several crop species is sufficient to induce appreciable changes in the pH (and then in the absorbance) of the small solution volumes used (3 ml/root).
Fig.2 shows the time course of medium acidification induced byroots of intact seeds of several species, evaluated by the decrease in absorbance of bromocresol purple.

Fig.2. Time course of medium acidification (evaluated by the decrease in absorbance of bromocresol purple) induced by roots of various species. Roots (1.5-2.5 cm long) of germinating seeds were incubated in the standard solution (see Methods) and the absorbance at 600 nm was recorded at different times. sunflower (c.v. $\pm$ 11%), wheat (c.v $\pm$ 8%), maize (c.v.$\pm$ 6%); ricinus (c.v. $\pm$ 18%). n = 10 seeds per species.
In all cases, the absorbance decreases rapidly in the first hours and lowers constantly, even at a slower rate, until the end of the experiment. The slopes of the absorbance over the logarithms of time were linear, with r values higher than 0.9 in all cases. Starting from a pH around 6.3, the initial acidification reaches a pH around 5.5 and continues until pH 4.8-5.0.
When the decrease in absorbance of the external solution at different times is plotted against length increments of roots occurred in the same interval times, linear relationships are observed with high r values and slopes differing according to the species considered.

Table I Correlation between medium acidification and root growth(1)

	r	s.e.	b	n
Maize: Lo 904 x H108	0.819	0.070	0.372	10
B 73 x B14 A	0.817	0.056	0.360	17
B 73	0.821	0.058	0.486	20
Wheat: Appulo	0.758	0.068	0.816	10

(1) Roots were incubated in cuvettes filled with 3 ml of standard solution at 25°C in the dark. After 20 hrs, the absorbance at 600 nm was measured together with the length of the roots. The absorbance decrease was then plotted against root length increment. Initial root length 15 mm.

Salt sensibility of proton extrusion activity in sunflower.

Tolerance to saline soils is mainly achieved by plants trough adjustments of osmotic potential to maintain water uptake and turgor and trough regulation of net ion transport to mitigate the consequences of excessive internal concentration of potential toxic ions.
Both mechanism rely, at least in part, on proton gradients, built up by plasmalemma and tonoplast ATP-ase-dependent proton pumps, as driving forces for inclusion/exclusion of external ions.
Consequently, it seems reasonable to search for variability of proton extrusion capacity in crop species grown at high salt concentration in view of selecting more tolerant genotypes. For this purpose, germinated sunflower seeds were placed in our standard solution containing different NaCl concentrations and the acidification capacity was evaluated by the absorbance decrease of the pH indicator (Fig.3).
Fig.3A shows the acidification of the medium (expressed as ΔA = Absorbance at time 0 - Absorbance at the time indicated x 10^3) over the time in the presence of NaCl from 0 to 0.8%. Clearly, as NaCl increases, acidification is more and more repressed especially on relatively long times of incubation (more than 400 minutes). In 3 B, the % of inhibition of acidification at time 1300 minutes is plotted against NaCl concentration. The values observed seem indicate a linear relationship between % of inhibition and external NaCl concentration. Accordingly, a NaCl concentration of 0.6% (producing a 60% inhibition in our standard hybrid) was used to explore variability in 10 commercial hybrids (table II).

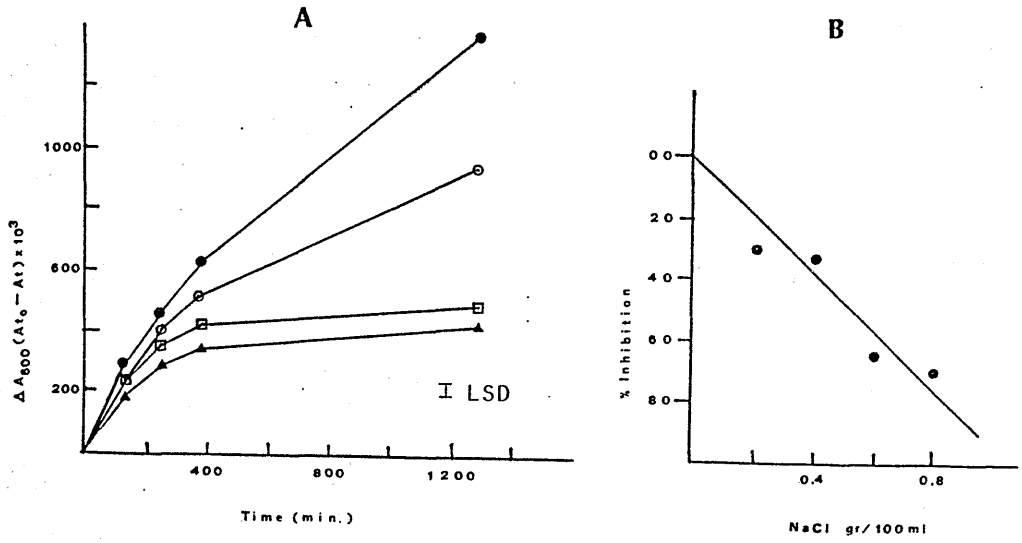


Fig.3.

Fig.3.A)-Time course of medium acidification induced by sunflower roots at different NaCl concentrations. Sunflower roots (1-2 cm.long) were incubated in cuvettes with 3ml of standard solution containing 0.0% (●—●), 0.2% (○—○), 0.4% (○—○), 0.6% (□—□), 0.8% (▲—▲) NaCl at 25oC in the dark. Absorbance at 600 nm was recorded at different time and the ΔA (A_{600} at initial time minus A_{600} at times indicated) plotted over time.
B) - % Inhibition of ΔA after 1300 min of incubation in the presence of different NaCl concentration over the control (0% NaCl). n = 10.

Table II Analysis of variance for ability of 10 commercial sunflower hybrids to induce medium acidification in the presence or absence of 0.6% NaCl.*

	DF	Mean-Square	P
Genotype	9	4436	0.003
NaCl concentration	1	227-346	0.001
Genotype x NaCl concentration interaction	9	1435	0.497
Error	368	1540	

* Experimental detail as in Fig.3 B. For each hybrid, not less than 15 seeds have been assayed.

As shown in table II, a significant difference exists among the hybrids assayed in their ability to induce medium acidification; furthermore 0.6% NaCl strongly reduces the acidification power. The variation in the extent of acidification induced by salt is not, however, genotype dependent (NaCl concentration x genotype interaction is not significative). This, likely, depends from the genotypes tested (all commercial hybrids), were a large extent of variability for the trait considered is not to be expected due to the heterozygosity of the materials.

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