

## Biochemical Indicators of the Water Stress in Maize Seedlings

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**Abstract.** Free proline and chlorophyll contents, chlorophyllase, peroxidase and nitrate reductase activities were determined in maize seedlings grown under controlled conditions and submitted to water stress induced by 14 % PEG-4000 or NaCl (isotonic). All the parameters studied showed a significant response to the water stress, but the level of free proline and peroxidase activity together may preferentially be used for the diagnosis of osmotic stress or saline-osmotic stress.

Water stress severely modifies the metabolism to an extent depending upon the species, duration and intensity of stress (HSIAO 1973, SOLDATINI 1979). The modifications in levels of several metabolites and enzymatic activities have been used to diagnose water stress in plants (LEON *et al.* 1983). Among the most widely used characteristics there are free proline levels (HANSON *et al.* 1977, TORRECILLAS *et al.* 1984), nitrate reductase (SHAKED *et al.* 1972, PLAUT 1974) and peroxidase (STROGOV 1964, ALEKSEEVA and RAMAZANOVA 1973) activities, chlorophyll content (SIVTSEV 1973, WEIMBERG 1975) and chlorophyllase activity (SIVTSEV *et al.* 1973).

The present study analyzed the behaviour of these biochemical parameters in maize plants submitted to water stress induced by PEG-4000 addition to nutrient medium (pure osmotic stress) or with isotonic NaCl solutions (a saline-osmotic stress) (see HSIAO 1973).

### MATERIAL AND METHODS

Maize (*Zea mays* L., cv. Summer II) seedlings with roots and shoots of approximately 5 and 2 cm, respectively, were put for 5 d into a nutrient solution of HOAGLAND and ARNON (1950) under room conditions. The selected seedlings of similar size were grown under controlled conditions (temperature  $25 \pm 2$  °C, relative humidity  $90 \pm 2$  %, continuous irradiance  $29.3 \text{ W m}^{-2}$ ) for 7 d, with the following treatments : control with distilled and deio-

TABLE 1

Growth, water potential and biochemical characteristics of maize seedling submitted to different types of water stress. Values for each treatment were within any of these characteristics significantly different from values for another treatment at a 5 % probability level, according to Duncan's multiple range test

| Parameter                                                                                         | Treatment        |       |        |
|---------------------------------------------------------------------------------------------------|------------------|-------|--------|
|                                                                                                   | H <sub>2</sub> O | PEG   | NaCl   |
| Water potential, $\psi$ [MPa]                                                                     | -0.14            | -0.38 | -0.50  |
| Plant height [cm]                                                                                 | 16.06            | 12.24 | 9.57   |
| Plant fresh mass [g]                                                                              | 1.53             | 1.25  | 1.01   |
| Free proline [mol kg <sup>-1</sup> ]                                                              | 1.07             | 4.10  | 1.51   |
| Chlorophyll [g kg <sup>-1</sup> (fr.m.)]                                                          | 2.16             | 1.93  | 1.67   |
| Chlorophyllase [ $\mu$ g(chl) kg <sup>-1</sup> (chl) s <sup>-1</sup> ]                            | 37.0             | 16.4  | 22.3   |
| Peroxidase [ $\mu$ mol(ascorbic acid) kg <sup>-1</sup> (fr.m.) s <sup>-1</sup> ]                  | 756.2            | 902.5 | 1251.3 |
| Nitrate reductase [nmol(NO <sub>2</sub> <sup>-</sup> ) kg <sup>-1</sup> (fr.m.) s <sup>-1</sup> ] | 205.9            | 121.7 | 162.9  |

nized water (H<sub>2</sub>O); 14 % PEG-4000 solution (PEG); isotonic NaCl solution (NaCl). The solutions were renewed daily in order to avoid root anoxia. Six replicates were used in each treatment. Analysed in three replicates were free proline content (BATES *et al.* 1973), chlorophyll (TORRECILLAS *et al.* 1984), peroxidase (EC 1.11.1.7) activity (MARTINEZ-MOMPEAN 1983), nitrate reductase (EC 1.6.6.1) activity (SHAKED *et al.* 1972), and chlorophyllase (EC 3.11.14) activity (NAVARRO *et al.* 1979).

The water potentials were measured by the pressure chamber method of SCHOLANDER *et al.* (1965), and the mass and lengths of shoots of the seedlings were determined in each treatment.

## RESULTS AND DISCUSSION

NaCl treatment induced a lower water potential than the PEG (Table 1), in accordance with similar assays of GIANNINI (1981) with maize seedlings. This explains the reduction in height and mass of the plants as functions of the water potential (BEGG and TURNER 1976). According to these authors, growth by cellular extension depends on the turgor of the plant. However, a greater reduction in size and mass of the plants in the NaCl treatment may also be due to the phytotoxic effects of the Na<sup>+</sup> and Cl<sup>-</sup> ions.

The free proline levels significantly increased in response to the water stress favouring the osmotic adjustment (Table 1; *cf.* HANSON *et al.* 1977) and increasing the levels of the slightly toxic and easily transportable metabolites. The lesser increase in proline levels in the seedlings treated with NaCl may result from the decreased necessity of making osmotic adjustment. Whereas the PEG is not absorbed under these conditions (LAWLOR 1970), NaCl may be absorbed considerably, resulting in a decrease in the osmotic potential of the plants (YANCEY *et al.* 1982).

The chlorophyll content of leaves (Table 1) decreased more in the NaCl treatment; contrary to our results GIANNINI (1981) found an increase in this

pigment content in analogous assay situation. The greater decrease induced by NaCl than by PEG may be explained, in agreement with KENNEDY (1977), by the complementary action of the  $\text{Na}^+$  and  $\text{Cl}^-$  ions on the structure and function of chloroplasts, and also by the higher chlorophyllase activity (Table 1).

The activity of the peroxidase increased more as a result of saline-osmotic stress (similar results by WEIMBERG 1975) than owing to osmotic stress (*cf.* NIR *et al.* 1970) (Table 1). The activity of nitrate reductase was lower in the PEG treatment than in the NaCl treatment (Table 1). The reduction of this enzymatic activity by water stress has already been reported (MORILLA *et al.* 1973, PLAUT 1974). ALINA and KLYSHEV (1975) found activation of this enzyme in maize as a result of salinization. Thus in the NaCl treatment the osmotic effect has probably prevailed over the saline effect.

Although the responses of the different biochemical parameters showed similar tendencies to increase or decrease at both treatments and were statistically significant in each case, this might have been a result of careful experimentation and a high number of repetitions, which in practice may be difficult to repeat.

As the main objective of the work was to select parameters which are indicative not only of water stress, but also to show whether the cause was osmotic or saline-osmotic in nature, it was necessary to select those parameters whose variation with respect to the control treatment is the greatest. The greatest significant per cent differences were shown in activities. As proline is sensitive to a pure osmotic stress and peroxidase is sensitive to saline-osmotic stress, both of them should be considered together to differentiate between the causes of water deficit in isotonic media. As a consequence of this and due to a degree of uncertainty concerning the inductive cause of the water stress which could occur in several cases, it is necessary to determine the range of osmotic conditions induced by PEG and NaCl in the root medium.

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