

## CHANGES IN RESPIRATION, ITS ALLIED ENZYMES, PIGMENT COMPOSITION, CHLOROPHYLLASE AND HILL REACTION ACTIVITY OF HORSEGRAM SEEDLINGS UNDER SALT STRESS

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### SUMMARY

The effect of NaCl or Na<sub>2</sub>SO<sub>4</sub> (100 meq/l) on respiration and its allied enzymes, the levels of free titrable acids, chlorophylls, chlorophyllase and the Hill reaction activity in horsegram seedlings was studied. Aldolase, isocitrate dehydrogenase (ICDH), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) were found to increase considerably in shoots and roots of stressed seedlings. Except aldolase, the ICDH, SDH and MDH activities were higher in roots than in shoots. The higher activities of these enzymes under salt stress were associated with increased respiration rate in roots than in shoots during 7th day. The free titrable acids were significantly increased by both salt treatments. Further, the pigment composition and Hill reaction activity in leaves was more in NaCl stressed seedlings. Chlorophyllase activity did not show much variation between control and stressed seedlings.

### INTRODUCTION

Salinity is a major constraint in agriculture and induces a series of metabolic, physiological and morphological changes in crop plants. Salt stress inhibits growth throughout the plant life cycle but seed germination is generally the most sensitive stage (Bernstein and Hayward, 1958). Morphological changes have been found to correlate with different abnormalities in salt affected seedlings (Weimberg, 1970). Salinity has also been found to affect the rate of respiration (Poljakoff-Mayber and Gale, 1975). Relatively less information is available on respiratory metabolism of plants under salt stress (Levitt, 1980). A few reports indicate a rapid increase in respiration (Divate and Pandey, 1981 ; Younis, *et al.*, 1987). Among the number of plant metabolites (sugars, amino acids, free organic acids, betaines etc.), the total

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free organic acids play a protective role in saline habitats besides their metabolic functions (Martinez *et al.*, 1987). Preliminary observations indicate that horsegram possesses a better tolerant nature to NaCl or Na<sub>2</sub>SO<sub>4</sub> during germination and early seedling growth (Sudhakar *et al.*, 1987). Since different species behave differently with regard to the qualitative and quantitative nature of enzymes under salt stress, an attempt was made in the present study to find out the salt induced changes in respiration, its allied enzymes, total titrable acids, photosynthetic pigments, chlorophyllase and Hill reaction activity of horsegram under NaCl or Na<sub>2</sub>SO<sub>4</sub> salinity during early seedling growth.

### MATERIALS AND METHODS

A local variety (VZM 1) of horsegram (*Macrotyloma uniflorum* Lam. (Verdc.)) seeds were procured from Agricultural Depot., Anantapur, India. The seeds were surface sterilized with 0.1% HgCl<sub>2</sub> solution, washed repeatedly with distilled water, germinated on petridishes lined with filter paper. Salt stress was imposed by adding NaCl or Na<sub>2</sub>SO<sub>4</sub> at 100 meq/l concentration in Arnon's half-strength nutrient medium. Medium without salts served as control. The salt stress was imposed from the beginning of germination and continued till the end of the experiment. The petridishes were maintained under a light of approximately 150 Wm<sup>-2</sup> at 27°±2°C for 10 days. The solutions were replaced everyday with fresh solutions. The shoots and roots were separated, rinsed in distilled water prior to biochemical analysis.

The rate of respiration was measured by the rate of O<sub>2</sub> uptake in a Gilson 5/6 Oxygraph. The enzyme aldolase (E.C. 4.1.2.13) was assayed according to Stribling and Perham (1973) and isocitrate dehydrogenase (E.C. 1.1.1.42) activity was determined by U.V. method according to Bernt and Bergmeyer (1974). The enzyme succinate dehydrogenase (E.C. 1.3.99.1) and malate dehydrogenase (E.C.1.1.1.37) were extracted following the method of Nachlas *et al.*, (1966). The two enzymes were extracted in 0.3 M sucrose each at 0–4°C and the homogenate was centrifuged in a refrigerated centrifuge and the supernatant was taken as the enzyme source.

Succinate dehydrogenase was assayed in a reaction mixture of 2.0 ml containing 0.4 M phosphate buffer (pH 7.7), 0.2 M Sodium succinate, 1.0 ml of 0.004 M INT (2-(p-indophenol)-3-nitrophenyl-5-phenyltetrazolium chloride), 0.1 ml of enzyme. Similarly malate dehydrogenase was assayed in a reaction mixture of 2.0 ml containing 30 μ moles of sodium malate, 100 μ moles of phosphate buffer (pH 7.4), 0.1 μ moles of NAD and 4 μ moles of INT and the enzyme. The reaction mixture was incubated at 37°C for 30 min. After incubation 5.0 ml of glacial acetic acid was added to stop the reaction and the colour was extracted into 5.0 ml of toluene. The

tubes were kept over night in a refrigerator at 4°C. The colour intensity was read at 495 nm in a Bausch and Lomb spectronic 88 spectrophotometer. Acidity was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator.

Chlorophyll and carotenoid content of the leaves (10-day-old) was determined according to Arnon (1949) and Jensen and Jensen (1971) respectively. The chlorophyllase activity was determined according to Holden (1961) and the activity was expressed as chlorophyllide formed/250 mg acetone powder/24 hr. The Hill activity was measured according to Vishnaic (1957) and expressed as  $\mu$  moles DCPIP reduced/mg chlorophyll. The results are mean of five replications.

### RESULTS AND DISCUSSION

The respiration rate was measured in shoots and roots of 7-day-old seedlings under NaCl or Na<sub>2</sub>SO<sub>4</sub> at 100 meq/l concentration (Table I). The rate of respiration increased over control in shoots and roots of salt stressed seedlings. Salinity was found to affect the rate of respiration in plants (Poljakoff-Mayber and Gale, 1975). One of the dominant theories explaining the general stunted growth in response to salinity relates growth reduction to the diversion of energy from accumulative growth to maintenance. Higher concentration of NaCl reported to increase the rate of respiration in plants (Nieman, 1962; Rains, 1972; Divate and Pandey, 1981). Energy requirements are assumed to increase due to pumping of ions against electrochemical potential gradient (Strogonov, 1973; Lambers and Day, 1987). The high respiration rate in horsegram may provide required energy for the growth and development of seedlings under stress conditions. The increase in respiration in response to salts may

Table I. Changes in the rate of respiration and levels of free titrable acids in 7-day-old control and salt stressed (100 meq/l) seedlings of horsegram  $\pm$  S.D.

| Treatment                       | Respiration <sup>a</sup> |                   | Titrable acids <sup>b</sup> |                   |
|---------------------------------|--------------------------|-------------------|-----------------------------|-------------------|
|                                 | Shoot                    | Root              | Shoot                       | Root              |
| Control                         | 3.15 $\pm$ 0.25          | 1.47 $\pm$ 0.25   | 40.5 $\pm$ 4.8              | 23.46 $\pm$ 3.6   |
| NaCl                            | 3.75* $\pm$ 0.36         | 1.88* $\pm$ 0.10  | 75.2** $\pm$ 1.6            | 44.26** $\pm$ 2.4 |
| Na <sub>2</sub> SO <sub>4</sub> | 3.35 $\pm$ 0.21          | 2.00** $\pm$ 0.01 | 71.46** $\pm$ 1.4           | 38.93* $\pm$ 4.0  |

a— $\mu$  moles of O<sub>2</sub> consumed/g fresh wt/5 min.

b—meq/g fresh wt.

\*—Significant at 5% level

\*\*—Significant at 10% level.

Table II. Activities of certain respiratory enzymes in 7-day-old control and salt stressed (100 meq/l) seedlings of horsegram.  $\pm$  S.D.

| Treatment                       | Aldolase <sup>a</sup> |                    | ICDH <sup>b</sup>     |                        | SDH <sup>c</sup>      |                         | MDH <sup>d</sup>        |                         |
|---------------------------------|-----------------------|--------------------|-----------------------|------------------------|-----------------------|-------------------------|-------------------------|-------------------------|
|                                 | Shoot                 | Root               | Shoot                 | Root                   | Shoot                 | Root                    | Shoot                   | Root                    |
| Control                         | 4.15<br>$\pm 0.34$    | 1.49<br>$\pm 0.22$ | 614.8<br>$\pm 12.6$   | 560.8<br>$\pm 20.7$    | 1229.9<br>$\pm 49.9$  | 760.0<br>$\pm 87.1$     | 820.8<br>$\pm 124.3$    | 1073.7<br>$\pm 68.4$    |
| NaCl                            | 8.05**<br>$\pm 0.55$  | 1.73<br>$\pm 0.10$ | 843.2**<br>$\pm 28.2$ | 888.9**<br>$\pm 6.8$   | 964.5**<br>$\pm 10.4$ | 1744.0**<br>$\pm 16.7$  | 1127.6**<br>$\pm 70.7$  | 2114.4**<br>$\pm 126.2$ |
| Na <sub>2</sub> SO <sub>4</sub> | 7.18**<br>$\pm 0.43$  | 1.74<br>$\pm 0.15$ | 1176.2**<br>$\pm 57$  | 1188.2**<br>$\pm 25.3$ | 1285.1<br>$\pm 104$   | 1497.2**<br>$\pm 197.0$ | 1181.5**<br>$\pm 101.8$ | 2010.7**<br>$\pm 182.8$ |

a—mg pyruvate formed/g fresh wt/hr

b—n moles NADP reduced/g fresh wt/min

c and d  $\mu$ g formazone formed/g fresh wt/hr

\*\*Significant at 10% level.

be an adaptive feature as ascribed by Morozovski and Kabanov (1970) in order to survive under adverse conditions. Further, the increased respiration rate in the present study was associated with the increased activities of respiratory enzymes.

The enzymes involved in respiration viz., aldolase, ICDH, SDH and MDH increased significantly by salt stress (Table II). The enzyme aldolase was higher in shoots than in roots. In contrast, the ICDH, SDH and MDH were higher in roots than in shoots. Higher activities of these enzymes in roots may be due to the excessive ion accumulation at higher rates in roots than in shoots (unpublished data). However, Weimberg (1970) and Greenway and Osmond (1972) have shown that the ICDH and MDH specific activities were not altered significantly in the leaves of pea and *Phaseolus aureus* seedlings under salt stress.

Organic acids represent a link between the carbohydrate and nitrogen metabolism of plants, and play a protective role in saline conditions in addition to their metabolic function. These protective functions include the binding of excess ions penetrating the plant, regulating the ionic balance of plants, maintaining electrical neutrality of cells and neutralizing the basic compound (Strogonov, 1973) and also contribute in lowering the osmotic potential of leaves under stress conditions (Hellebust, 1976). In the present study there was a substantial increase in the levels of titrable acids in shoots and roots of horsegram under stress conditions. These results confirm the observations of Martiney *et al.*, (1987) and Krishnamurthy *et al.*, (1987). The increase in respiration rate with concurrent increase in respiratory enzymes and total free amino acids could provide sufficient energy to maintain the normal metabolic functions of the seedlings under salt stress.

Table III. Pigment composition, Chlorophyllase and Hill reaction activity in the leaves of (10-day-old) control and salt stressed (100 meq/l) seedlings of horsegram. Chlorophylls=mg/g. fresh wt; Carotenoids= $\mu$ g/g fresh wt; Chlorophyllase= $\mu$ g chlorophyllide formed/250 mg acetone powder (Chlorophyll); Hill activity= $\mu$  moles DCPIP reduced/mg chlorophyll

| Treatment                       | Total Chl.          | Chl. a             | Chl. b             | Total carotenoids  | Chlorophyllase      | Hill activity       |
|---------------------------------|---------------------|--------------------|--------------------|--------------------|---------------------|---------------------|
| Control                         | 2.96<br>$\pm 0.025$ | 1.90<br>$\pm 0.01$ | 1.04<br>$\pm 0.06$ | 186.2<br>$\pm 5.3$ | 326.6<br>$\pm 8.23$ | 578.5<br>$\pm 15.1$ |
| NaCl                            | 2.02<br>$\pm 0.012$ | 1.44<br>$\pm 0.04$ | 0.58<br>$\pm 0.01$ | 165.9<br>$\pm 6.2$ | 316.8<br>$\pm 6.86$ | 459.2<br>$\pm 8.5$  |
| Na <sub>2</sub> SO <sub>4</sub> | 2.44<br>$\pm 0.08$  | 1.64<br>$\pm 0.01$ | 0.80<br>$\pm 0.04$ | 170.6<br>$\pm 4.5$ | 320.2<br>$\pm 9.46$ | 509.4<br>$\pm 9.6$  |

Further, the total chlorophylls, Chl *a* and Chl *b* decreased in the leaves of 10-day-old stressed seedlings when compared to controls and the decrease being more by NaCl than by Na<sub>2</sub>SO<sub>4</sub> treatments (Table-III). This may be due to the specific anion effect as ascribed by Bhivare and Nimbalkar (1984). The carotenoids also showed a similar trend as total chlorophylls in stressed seedlings. These results are in agreement with those of Rao and Rao (1981).

The reduction in leaf chlorophylls under salinity has been reported and attributed to the destruction of the chlorophyll pigments and the instability of the pigment-protein complex (Poljakoff-Mayber and Gale, 1975; Levitt, 1980). Salinity was found to enhance the Chlorophyllase activity which results in lowering the chlorophyll content (Rao and Rao, 1981). However, the activity of the chlorophyllase did not show much variation between control and stressed leaves of horsegram during early seedling growth. It may, however, be inferred from the results that low levels of chlorophyll caused by salt stress could be partly due to interference of salt ions with the *denovo* synthesis of proteins, the structural component of chloroplast rather than the breakdown of chlorophylls.

The Hill activity inhibited in salt stressed seedlings. The degree of inhibition was higher in NaCl than in Na<sub>2</sub>SO<sub>4</sub> treated seedlings. This may be due to the impaired chloroplasts in salt stressed seedlings. Inhibition in Hill activity suggests the inhibition of PS II activity in response to salinity.

From the present study it may be concluded that these altered metabolic activities could possibly play a protective role in salt tolerance of horsegram.

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