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Stress studies in lentil (*Lens esculenta* Moench)

II. Sodicity-induced changes in chlorophyll, nitrate and nitrite reductase, nucleic acids, proline, yield and yield components in lentil

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Abstract

The response of soil exchangeable sodium percentage levels to nitrate reductase activity, nitrite reductase activity, free proline, DNA, RNA, chlorophyll a and b contents and yield components in lentil (*Lens esculenta* Moench) *cv.* PL 406 was studied in a replicated pot experiment. All the biochemical observations were recorded at four growth stages i.e. 30, 60, 90 and 120 days after sowing (DAS). Germination occurred up to exchangeable sodium percentage of 30, but plants survived only up to 25. With increasing exchangeable sodium percentage, there was a continuous decrease in chlorophyll a and b content, nitrate and nitrite reductase enzyme activities and DNA and RNA content. Increasing level of sodicity enhanced the free proline content up to 60 DAS, after which values fell.

Number of pods per plant, 1000 grain weight and grain yield were significantly reduced with increasing level of sodicity, but the number of grains per pod was not affected.

Introduction

Salinity and sodicity are very important constraints in crop productivity in India. There are about 7 million hectares of salt affected soil in different parts of the country (Abrol and Bhumbla, 1971) of which 2.8 million hectares are alkaline. In the past, efforts to reclaim salt-affected soil by soil scientists and agronomists have had little success, mainly because of expensive management practices. Salt generally alters a wide array of metabolic processes culminating in stunted growth, reduced enzyme activities and photosynthetic carbon metabolism (Bajawa and Bhumbla, 1971; Gill, 1987; Hanson, 1987; Rausser and Hanson, 1966; Singh andAbrol, 1985; Singh et al., 1990).

Although much work has been done on the response of crops to salinity (Ashraf and McNeilly, 1990; Plaut et al., 1990; Singh et al., 1990; Walker et al., 1981), little information is available on sodicity (Byrne, 1988; Singh and Abrol, 1986). Therefore, the present investigation was conducted to understand the nature of impairment of physiological and biochemical processes in salt-stressed plants. The information will be of great value for breeding salt tolerant lentil cultivars.

Materials and methods

A pot experiment was conducted during 1988–89 with lentil *cv.* PL 406 with four replications in a randomized design. The soil used was sandy loam with initial pH and ESP of 8.3 and 10 respectively. To create different levels of ESP, desired quantities of sodium bicarbonate ($NaHCO_3$) were thoroughly mixed into the soil prior to filling the pots. Ten seeds were sown in each pot and after germination, seedlings were thinned to five plants per pot. The ESP levels were checked every 15 days and adjusted if required. All the biochemical parameters were recorded at four growth stages i.e. 30, 60, 90 and 120 days after sowing (DAS). The chlorophyll a and b content was estimated following the method of Arnon (1949) and expressed as mg L^{-1}.

Enzyme assays

Nitrate reductase activity of leaves was assayed by the procedure of Jaworski (1971) and standard curve was made using $NaNO_2$. The amount of NO_3^- reduced/g fresh weight/hour was calculated. Activity of nitrite reductase (NIR) was measured according to the procedure given by Ferari and Varner (1971).

Accumulation of free proline amino acid was estimated using toluene by the standard procedure of Bates et al. (1973). The contents of DNA and RNA were estimated by the method of Neiman and Poulsen (1963) using diphenylamine (Burton, 1956) and oricinol reagent (Peach and Tracey, 1955) respectively.

Results

Biochemical parameters

Chlorophyll a and b contents decreased with increasing levels of sodicity (Table 1). The reduction in chlorophyll a and b contents with increasing soil sodicity were found at all stages of crop growth. Chlorophyll a and b contents were relatively high at 60 DAS. Increasing ESP reduced chlorophyll a and b contents most at 60 and 90 DAS, respectively. The activity of nitrate

Table 1. Influence of soil sodicity on chlorophyll content in lentil (*cv.* PL 406)

Treatment (ESP level)	Chlorophyll a content (mg L^{-1}) DAS				Chlorophyll b content (mg L^{-1}) DAS			
	30	60	90	120	30	60	90	120
10 (Control)	0.776	1.01	0.568	0.00	0.900	1.259	0.965	0.00
15	0.839	0.806	0.523	0.00	0.882	1.141	0.826	0.00
20	0.677	0.809	0.477	0.00	0.842	0.917	0.581	0.00
25	0.637	0.749	0.427	0.00	0.820	0.863	0.506	0.00
Se (m±)	0.070	0.073	0.005	0.00	0.003	0.008	0.004	0.00
*L.S.D. 5%	0.03	0.04	0.01	0.00	0.07	0.02	0.01	0.00

*L.S.D. 5%: Two means (in a column) are significantly different at the 5% level if their difference (in absolute value) exceeds the L.S.D. (least significant difference).

Table 2. Influence of soil alkalinity on nitrate and nitrite reductase activity in lentil

Treatment (ESP level)	Nitrate reductase activity (n mol h^{-1} g^{-1} F W) DAS				Nitrite reductase activity (n mol h^{-1} g^{-1} F W) DAS			
	30	60	90	120	30	60	90	120
10 (Control)	555.60	666.70	91.11	0.00	2722.22	2736.11	999.99	0.000
15	242.22	499.99	88.90	0.00	2395.83	2687.50	604.10	0.00
20	88.00	455.60	84.44	0.00	2184.50	2465.28	555.60	0.00
25	84.44	346.70	78.90	0.00	1666.70	2236.70	416.70	0.00
SE (m±)	6.56	15.92	3.13	Nil	62.42	82.36	84.74	Nil
L.S.D. 5%	20.23	49.07	9.63	Nil	192.45	253.90	261.25	Nil

*See footnote to Table 1.

reductase and nitrite reductase decreased significantly with increasing levels of soil sodicity (Table 2) at almost all stages of plant growth. The maximum reduction in nitrate reductase (NR) activity occurred at 30 DAS as compared to control. Maximum reduction in nitrite reductase (NIR) activity was observed at 90 DAS. In general, nitrate and nitrite reductase activities were maximum at 60 DAS and then decreased gradually with plant age.

Free proline accumulation (Table 3) rose with increasing level of soil ESP upto 60 DAS after which proline contents showed no clear trend. At 60 DAS, proline was accumulated in relatively high amounts with increasing level of soil sodicity. The DNA content clearly increased with the advancement of plant age till maturity, whereas RNA content increased only up to 90 DAS and afterward decreased (Table 4). The reduction in DNA and RNA content with increasing level of soil sodicity was observed at all the stages of plant growth.

Table 3. Effect of soil sodicity on free proline content in lentil

Treatment (ESP level)	Free proline content ($\mu g\, g^{-1}$ F W) DAS			
	30	60	90	120
10 (Control)	86.38	133.12	203.12	170.00
15	104.50	543.75	280.00	190.94
20	183.12	827.25	188.44	451.87
25	238.75	928.28	148.12	109.50
SE (mn±)	4.90	10.00	6.60	5.75
*L.S.D. 5%	15.08	30.86	20.32	17.72

*See footnote to Table 1.

Table 4. Effect of soil sodicity on DNA and RNA content in lentil

Treatment (ESP level)	DNA content ($\mu g\, L^{-1}$) DAS				RNA content ($\mu g\, L^{-1}$) DAS			
	30	60	90	120	30	60	90	120
10 (Control)	52.00	75.50	85.00	112.50	190.00	258.75	297.50	118.75
15	10.50	31.00	43.00	82.50	188.75	255.00	291.25	105.00
20	7.50	26.50	37.00	76.00	177.50	246.25	282.25	98.75
25	2.50	25.50	32.00	62.00	146.25	215.00	243.75	5.62
Se (m±)	0.60	3.98	0.68	2.22	1.84	1.70	2.80	3.00
*L.S.D. 5%	1.83	12.27	2.08	6.86	5.63	5.23	8.61	9.35

*See footnote to Table 1.

Table 5 Effect of soil sodicity on some yield components in lentil

Treatment (ESP level)	At harvesting			
	Number of pods per plant	Number of grains per pod	1000 grain weight (g)	Grain yield per plant (g)
10 (Control)	63.75	2.00	20.17	2.57
15	30.25	1.75	16.93	0.896
20	28.50	1.50	15.77	0.674
25	8.50	1.25	15.73	0.167
SE (m±)	0.70	0.10	0.27	0.03
L.S.D. 5%	2.158	0.313	0.822	0.098

*See footnote to Table 1.

Yield components

Number of pods per plant was significantly reduced by increasing sodicity directly affecting the grain yield (Table 5). Grain number and 1000 grain weight were also affected by ESP level but the differences were not statistically significant. Nevertheless, grain number and 1000 grain weight were positively correlated with yield. The percentage reduction in grain yield over control was 65, 74 and 94 at 15, 20 and 25 ESP level, respectively.

Discussion

An increasing level of soil sodicity reduces chlorophyll a and b contents, which is in agreement with the findings of El-Sharkawi et al. (1986) in Cotton and Millet. This reduction was related to the enhanced activity of chlorophyllase (Reddy and Vora, 1986). According to Lapina and Popov (1970) saline conditions lead to disruption of the fine structure of chlorophyll and instability of the pigment protein complex which may also be the cause of the reduced chlorophyll content.

Nitrate and nitrite reductase enzymes are sensitive to alkalinity and their activities decreased with increasing ESP levels of the soil. Similar observations have also been reported by Abdul Kadir and Paulsen (1982), Blom-Zandstra and Lampe (1983), Heimer (1973), Helal and Mengel (1979), Kozlowski (1972), Singh et al. (1990) and Smith and Middleton (1980).

The effect of salt stress on nitrate reductase activity may be attributed to inhibition of enzyme induction. It has been reported that stress causes a shift of ribosomes from the polymeric to the monomeric form in maize seedlings (Hsiao, 1970). Another reason may be the enhanced degradation of nitrate reductase by an inactivating system which may lead to a decrease in enzyme activity (Plaut, 1974). The presence of such inactivating system for the control of NRA has already been reported by Tragis et al. (1969). Although it is not yet confirmed whether nitrate reductase is an adoptive enzyme with synthesis being induced by NO_3^- via gene activation (Bresteles et al., 1978), there is general agreement that nitrate enhances NRA (Benzioni et al., 1971). In most cases NH_4^+ and amino acids have the reverse effect. Radin (1975) reported that at lower pH (5) the inhibiting effect of NH_4^+ on NRA was less than at pH 7. In the present investigation also we have observed more reduction in NRA and NIR activities with increasing sodicity levels i.e. increasing pH. It has also been reported by Lycklama (1963) that the rate of uptake of NH_4-N is much higher in the alkaline pH range than in the slightly acidic range. Therefore, it is possible that the observed effect of ESP levels on NRA activity in the present investigation could result mainly from different rates of NH_4-N uptake.

Another possibility for decreased enzymatic activities under salt stress may be because of limited substrate availability in the leaves, resulting from inhibition of NO_3^- uptake (Lacuesta et al., 1990; Trogisch et al., 1989). Although Martinez and Cuder (1989) could not find any relationship between NO_3^- content of the leaves and enzyme activity, several investigators have emphasized the importance of efflux of vacuolar NO_3^- ions and movement of NO_3^- ions from the roots to the shoots under the influence of salt stress (Aslam et al., 1984; Bloom-Zandstra and Lampe, 1983; Shaner and Boyer, 1976).

Although Na^+ and K^+ ions are essential for the synthesis and activity of nitrate reductase, their salts are strong inhibitors (Hewitt, 1975), which is one of the reasons for lower activity of NR and NIR under salt stress.

The accumulation of free proline increased with increasing level of soil ESP up to 60 DAS. After 60 DAS, the accumulated proline due to salt stress might have substituted for sugars as a respiratory substrate leading to a reduction in proline content at later stages (90 and 120 DAS) of crop growth. The increase in proline content under salt stress is mainly due to the break-down of proline-rich protein and fresh synthesis of proline amino acid.

The reduction in DNA and RNA content with increasing soil sodicity was observed at all stages of growth. This reduction might be due to increased activities of DNAase and RNAase. Similar responses in relation to soil sodicity have also been reported by Gill (1987) for Cowpea.

The number of pods per plant, grain number and 1000 grain weight were significantly reduced with increasing level of soil sodicity leading to poor crop yields. Singh and Singh (1989) have also found reductions in number of capsules per plant, number of seeds per capsule and 1000 grain weight in different varieties of linseed. Under non-saline conditions the flow of sodium into barley grain was very low and it accumulated at a very slow and uniform rate throughout the entire grain filling period (Dutt, 1988). Under saline conditions, however, sodium suddenly accumulated in the grain between 10th day an 20th day from flowering, after which there was again a negligible flow of sodium into the maturing grains (Dutt, 1988). Similar responses to sodicity have also been reported for groundnut and soybean (Singh and Abrol, 1985, 1986).

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