

## Proline accumulation and nitrate reductase activity in contrasting sorghum lines during mid-season drought stress

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Six lines of sorghum (*Sorghum bicolor* L. Moench) with differing drought resistance (IS 22380, ICSV 213, IS 13441 and SPH 263, resistant and IS 12739 and IS 12744, susceptible) were grown under field conditions in the semi-arid tropics and analysed for proline and nitrate reductase activity (NRA; EC 1.6.6.1) during a mid-season drought. The resistant lines accumulated high levels of proline, while the susceptible lines showed no significant proline accumulation. Most of the proline was accumulated after growth of the plants had ceased. In a separate greenhouse experiment, most of the proline was found in the green rather than the fired portions of leaves. The levels returned to that of irrigated controls within 5 days of rewatering. Proline levels increased as leaf water potential and relative water content fell, and there was no apparent difference among the different sorghum lines with change in plant water status. Susceptible lines accumulated less proline than resistant lines as leaf death occurred at higher water potentials. Proline accumulation may, however, contribute to the immediate recovery of plants from drought. Leaf NRA reached high levels at about 35 days after sowing in both the stressed and irrigated plants, after which it declined. The decline in NRA was more pronounced in the stressed than in the irrigated plants and closely followed changes in the growth rate. Upon rewatering, NRA increased several-fold in all the lines and, in contrast to proline accumulation, genotypic differences in NRA were small, both during stress and upon rewatering. The high sensitivity of NRA to mild drought stress was reflected in the rapid decline of activity with small changes in leaf water potential and relative water content. The results are discussed in the light of a possible role for proline during recovery from drought, and the maintenance of NRA during stress and its recovery upon rewatering.

*Key words* – Drought, growth rate, leaf water potential, nitrate reductase, osmotic adjustment, proline, recovery rate, relative water content, *Sorghum bicolor*.

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### Introduction

The increase in proline levels during drought stress is unique compared to other free amino acids in the same tissue (Aspinall and Paleg 1981, Handa et al. 1983), but similar to other low molecular weight solutes, such as sugars and organic acids (Ford 1984, Newton et al. 1986). The increase in proline is related to a decrease in leaf water potential and other measures of water status, e.g. relative water content (Blum and Ebercon 1976,

Patil et al. 1984). While genotypic differences in proline accumulation were shown for barley (Singh et al. 1973, Hanson et al. 1979) and sorghum (Blum and Ebercon 1976), the reason for these differences remains unclear. High proline accumulation was observed in drought tolerant varieties by Singh et al. (1973), Mali and Mehta (1977) and Karamanos et al. (1983), while others found the opposite (Hanson et al. 1979, Ilahi and Dörffling 1982). Hanson (1980) concluded that proline accumulation was not an adaptive trait but only a symptom of

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stress as proline accumulation highly correlated with leaf firing.

Nitrate reductase (EC 1.6.6.1; NADH; nitrate oxidoreductase) is the rate-limiting enzyme in nitrogen assimilation and is a key point of metabolic regulation. Nitrate reductase activity is associated with protein synthesis and plant growth, both of which are affected by drought stress (Sinha and Nicholas 1981). Soil nitrogen levels, light intensity and temperature, which affect growth of plants, also influence NRA activity (Sinha and Nicholas 1981).

Sorghum (*Sorghum bicolor* L. Moench) lines collected from different parts of the semi-arid tropics have been classified as drought tolerant and drought susceptible, based on their performance to mid-season heat and drought stress (ICRISAT 1986). The present study forms part of a series of experiments conducted in the field to identify physiological, phenological and morphological traits that are useful for plant survival and growth. The study was undertaken to find out whether or not any genotypic differences exist among the sorghum lines in proline accumulation and NRA, and also to examine their role in crop performance under drought conditions. In this paper we describe the results obtained by the measurement of proline and NRA in 4 resistant and 2 susceptible lines of sorghum during mid-season heat and drought stress.

*Abbreviations* – DAS, days after sowing; LWP, leaf water potential; NRA, nitrate reductase activity; OA, osmotic adjustment; RWC, relative water content.

## Materials and methods

### Field experiments

The experiment was conducted during the 1986 summer (March–June) and monsoon (June–Sept) season at ICRISAT, Patancheru, India, on a sandy clay with a loamy surface, defined according to USDA taxonomy (USDA 1975) as a fine-mixed hypothermic Udic Rhodustalf. Six sorghum lines chosen for their different responses to heat and drought stress (ICRISAT 1986) were sown in split block design with 3 replicate  $9 \times 12$  m plots. They were IS 12744, IS 12739 (drought susceptible), IS 22380, IS 13441 (drought resistant), and two drought resistant promising breeding lines, SPH 263 and ICSV 213. Days to physiological maturity of these lines were 84, 78, 115, 88, 100 and 120 days, respectively. Resistant lines had a low incidence of leaf firing and exhibited yield stability relative to susceptible lines when subjected to mid-season drought (Peacock et al. 1988). Seeds were sown on 12 March in 60 cm rows to give a plant population of 12 plants  $m^{-2}$ . A basal dressing of ammonium phosphate was applied ( $20 g m^{-2}$ ) at sowing and 22 days later a top dressing of urea was applied ( $10 g m^{-2}$ ).

This paper is concerned with two treatments: a drought-stressed treatment and an irrigated control

treatment. Both treatments were sprinkler-irrigated for 2 weeks after sowing to prevent soil crusting and ensure plant establishment. Thereafter, both treatments were furrow irrigated at weekly intervals. Twenty days after sowing (DAS), irrigation was withheld from the stressed treatment for the next 49 days, while the control was irrigated for the duration of the crops. From 20 to 43 DAS maximum air temperatures and pan evaporation rates were stable at  $38^{\circ}C$  and  $11 mm day^{-1}$ , respectively. At 43 DAS, air temperature progressively increased until 65 DAS, when maximum air temperatures reached  $42^{\circ}C$ . Likewise, pan evaporation rates increased to  $20 mm day^{-1}$  during this period. Rainfall was negligible throughout the experiment apart from 26 mm received on day 43. Stress was released on 69 DAS by irrigating all plots to field capacity.

### Growth analysis

At weekly intervals from 9 DAS to physiological maturity (black layer formation on seeds), 6 plants were harvested per plot. For each plant the total dry weight was measured after oven drying at  $70^{\circ}C$  for 24–36 h. Growth and recovery rates were calculated from the second and third order polynomials fitted to dry weight data.

### Leaf water relations

Measurements of leaf water relations were made at midday on the midportion of the youngest fully expanded leaf. Two leaves were sampled per replicate (6 per treatment) at weekly intervals from 25 to 88 DAS. Leaves were excised, and placed between moistened sheets of filter paper lined with muslin cloth. At the field laboratory, one side of the leaf was stripped and placed in a humidified pressure chamber (PMS Instruments, Corvallis, OR, USA) for determination of leaf water potential (LWP). The other side of the leaf was cut into 3 pieces, two of which were used for the measurement of RWC (Flower and Ludlow 1986). This leaf tissue was floated on demineralized water for 4 h at a temperature of  $25^{\circ}C$  and a photon flux of  $20 \mu mol m^{-2} s^{-1}$  before the measurement of turgid weight. The remaining tissue was placed in a micro-centrifuge vial and stored in liquid nitrogen. The tissue was then thawed in its vial and centrifuged for 5 min at 18 000 g, and the osmotic potential (OP) of the expressed sap was measured with a calibrated Roebbling osmometer (Camlab, Cambridge, UK).

Osmotic adjustment was calculated according to the formula of Wilson et al. (1979).

$$OP_{100} = OP (RWC-AWC)/(100-AWC)$$

Osmotic adjustment is the difference between the osmotic potential at full turgor ( $OP_{100}$ ) of unstressed and stressed leaves. Apoplastic water content (AWC) was unaffected by stress and taken as 12.8% (D. J. Flower, unpublished data).

## Greenhouse experiment

Two sorghum lines were selected based on their proline accumulation in the field experiment: IS 22380 representing the 4 resistant lines and IS 12744 representing the 2 susceptible lines. These lines were sown on 12 March 1987 in 30 cm diameter plastic pots containing about 20 kg sterilised sandy clay loam as mentioned previously. Ammonium phosphate was applied at amounts equivalent to 20 g m<sup>-2</sup>. Carbofuran was applied as granules for plant protection. After sowing, pots were watered to field capacity and the plants were thinned down to one per pot 15 DAS. The pots were randomized and kept in 4 replicates of 24 pots each. After germination the plants were watered to field capacity every second day and supplemented with Broughton's nutrient solution (Broughton and Dilworth 1971). The mean temperature ranged from 25 to 35°C during the day, to 20–25°C at night and the relative humidity was 60–65%. The pots were watered to field capacity 22 DAS and a set of 8 pots in each replicate was kept as control by watering every other day, while the remaining pots were subjected to stress by withholding water.

From 33 DAS the youngest fully-expanded leaf was removed from 2 plants per replicate (total of 8 plants per genotype) on alternate days and LWP was measured in the green leaf portion. On removal from the pressure chamber all tissue was separated into green and fired portions, placed in polythene bags, immersed in liquid nitrogen and stored at -70°C until analysis. For proline analysis two 0.5 g samples from the green and a 0.2 g sample from the fired portions were used.

## Proline estimation

Two of the youngest fully-expanded leaves were excised from each line, placed in a polythene bag, immersed in liquid nitrogen and stored on ice while they were brought from the field to the laboratory. This tissue was then stored at -70°C in a freezer till analysis. Four plants were collected for each line, two from each replicate.

The mid-ribs were removed from the leaves and two 0.5 g samples were taken from each of these leaves for each genotype. The leaf was placed in a precooled (5°C) mortar and ground with a pestle after the addition of liquid nitrogen. Sulphosalicylic acid (10 ml, 3%) was added, and the extract was filtered through Whatman no. 2 filter paper. Two ml aliquots were taken for proline estimation by the acid-ninhydrin method of Bates et al. (1973). A proline standard was run with each batch of assay.

## Nitrate reductase assay

Four whole plants of each sorghum line (two per replicate) were cut at the stem base above the ground, kept in polythene bags on ice and brought to the laboratory.

Sampling was done at weekly intervals from 19 DAS. Nitrate reductase activity (NRA) was measured by the method of Jaworski (1971). From each plant the two youngest fully-expanded leaves were removed, cleaned, and 50 discs (8 mm diameter) were cut from both. Ten discs were taken for fresh and dry weight determination. Twenty discs in duplicate were incubated in 0.1 M sodium phosphate buffer, pH 7.5, 5% *n*-propanol and 0.02 M potassium nitrate, ca 1 ml buffer per leaf disc in 100 ml glass beakers. The discs were then subjected to vacuum infiltration for 2 min and incubated at room temperature for 30 min in the dark. The incubation mixture was immediately filtered through a nitrate free Whatman no. 1 filter paper, and nitrite was estimated colorimetrically in a 5–10 ml aliquot. A nitrite standard was run with each batch of NRA estimation. Both measurements of NRA and proline were expressed on a dry weight basis.

## Results

The 4 resistant lines, IS 22380, ICSV 213, SPH 263 and IS 13441 accumulated very high proline levels during the drought (Fig. 1a) compared to their respective irrigated controls (Fig. 1b). Proline started accumulating in resistant lines 20 days after withholding water. Both IS 22380 and ICSV 213 accumulated ca 50 µmol g<sup>-1</sup> at about 54 DAS and proline remained high throughout the rest of the stress period. SPH 263 accumulated about 34 µmol g<sup>-1</sup> at 66 DAS, and IS 13441, 21 µmol g<sup>-1</sup> in the same period. Thus, both IS 22380 and ICSV 213 had a faster and larger accumulation of proline during stress than SPH 263 and IS 13441. In all the resistant lines proline returned to unstressed levels within 5 days after irrigation (69 DAS). In contrast, the two susceptible lines, IS 12739 and IS 12744 did not exhibit any significant increase in proline during stress compared to the respective irrigated controls. The resistant lines accumulated up to 20-fold more proline than the susceptible lines under stress (Fig. 1a and b).

In the control treatment (Fig. 2a) NRA reached a peak value by 35–40 DAS and returned to low levels by 50 DAS for all the lines. In the stressed plots, NRA decreased after ca 35 DAS in the 3 lines (Fig. 2b), and the decline in NRA was very sharp as compared to the controls. The enzyme activity stayed below that of the control in all lines after 35 DAS, and the lowest values were seen at the end of the drought period. Immediately after irrigation (69 DAS) NRA increased 20 to 30-fold compared to the level measured a few days before. NRA remained high for a period of 25 days after rewatering, and returned to control levels by ca 100 DAS. Genotypic differences in NRA were small both in stressed and irrigated treatments, although the resistant lines IS 22380, ICSV 213 and SPH 263 tended to have higher NRA than the others during the recovery period. The level of NRA in IS 13441 did not decrease to the extent recorded in other lines on DAS 60. Fur-

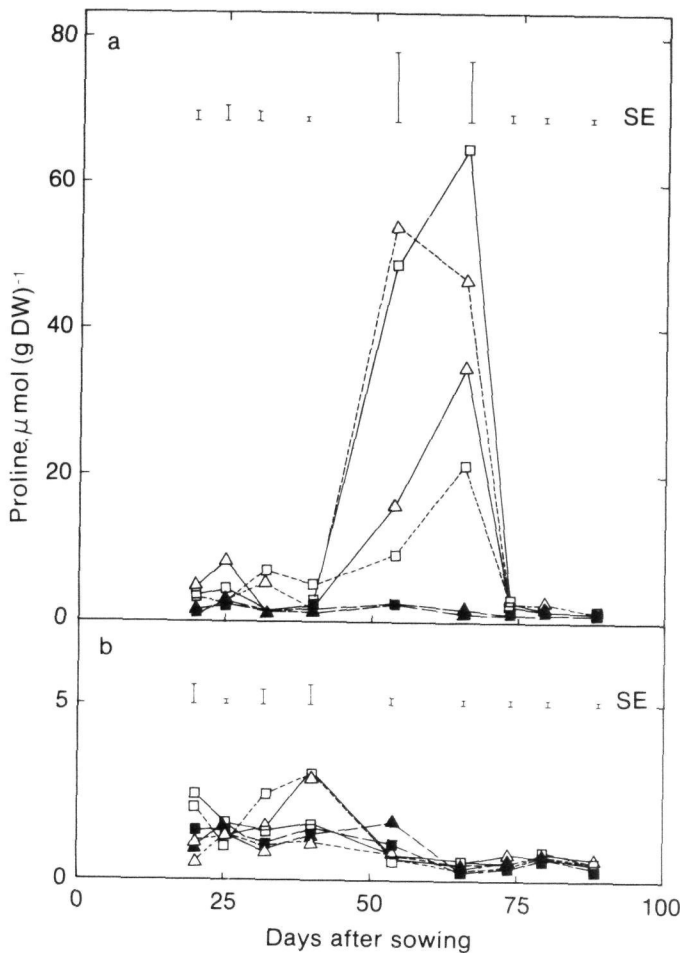


Fig. 1. Proline accumulation in leaves of 6 sorghum lines from (a) stressed plot and (b) control irrigated plot. Each data point is the mean of 8 independent determinations from 2 replicates.  $\pm$  SE is indicated by the bars. The symbols of each of the sorghum lines are as follows:

□—□, ICSV 213; △—△, SPH 263; △-----△, IS 22380; □-----□, IS 13441; ▲-----▲, IS 12739; ■-----■, IS 12744.

thermore, NRA in IS 13441 exhibited relatively little increase on rewatering, similar to the behavior of the susceptible lines. Thus IS 13441 had a pattern of NRA and proline accumulation intermediate between the other resistant and the susceptible lines.

Leaf water potential and RWC are expressions of plant water status. In Fig. 3a the mid-day leaf water potential for the 6 lines of sorghum is plotted against the proline levels and growth rates observed during drought. Although there was some scatter, proline started accumulating in leaf tissue when the leaf water potential decreased below  $-2.5$  MPa in the resistant lines. In contrast, susceptible lines maintained LWP above  $-2.5$  MPa throughout the drought period and, consequently, exhibited no increase in proline. The maximum levels of proline were at the lowest LWPs measured, which occurred at the end of the drought period. As there was no significant difference between the growth rate of the different sorghum lines during the

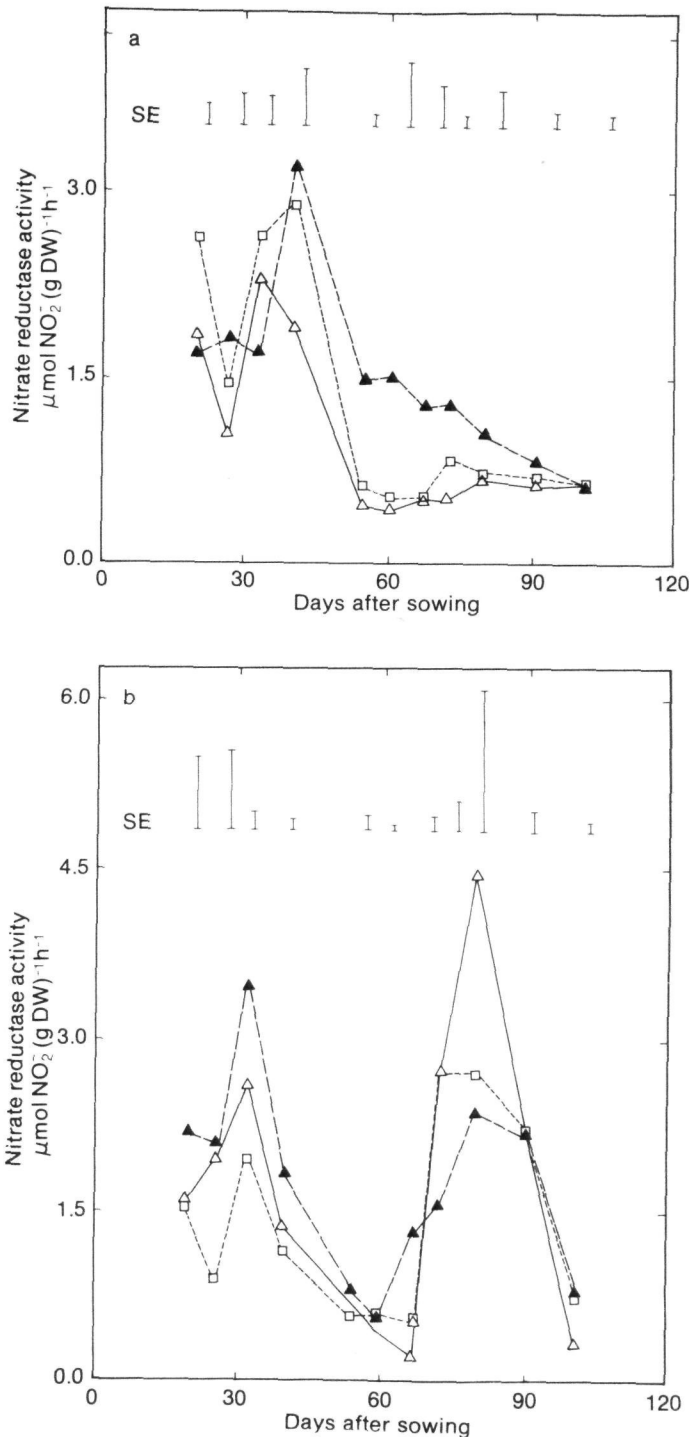


Fig. 2. Nitrate reductase activity in leaves of 3 sorghum lines from (a) control irrigated plot, (b) stressed plot. Each data point is the mean of eight independent determinations from two replicates.  $\pm$  SE is indicated by the bars. The symbols for each of the sorghum lines are as for Fig. 1

stress period, the line drawn in Fig. 3a is an average response of all the lines. Growth rate steadily decreased with the onset of stress in all the lines, and zero growth rate was reached at a mid-day LWP of ca  $-2.4$  MPa. From Fig. 3a it can also be seen that most of the proline accumulated after growth had ceased. Death and loss of lower leaves at LWP  $< -2.8$  MPa led to negative

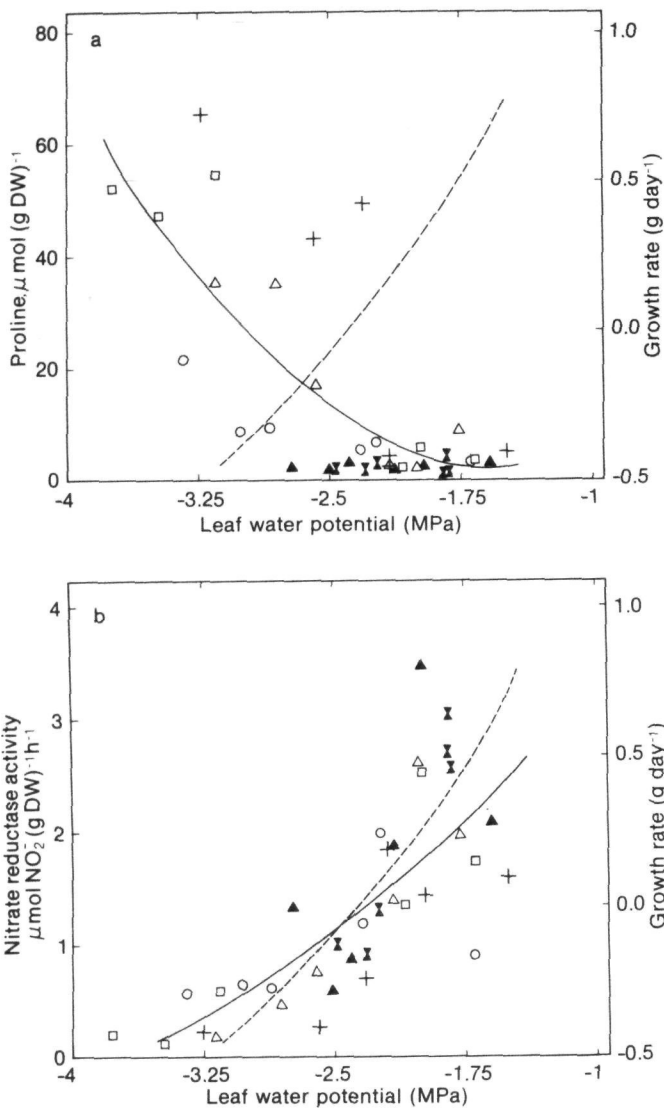


Fig. 3. (a) Proline level and growth rate as related to mid-day leaf water potential in 6 sorghum lines during mid-season stress. Proline levels are plotted as a scatter diagram with a line fitted by the equation  $Y = 27.3 + 34.4x + 11.4x^2$  ( $r^2 = 0.53$ ), while the growth rate is shown by a line (---) fitted by the equation  $Y = 2.15 + 1.14x + 0.1x^2$  ( $r^2 = 0.63$ ) with no points shown. Symbols for the various lines are (+) ICSV 213, ( $\Delta$ ) SPH 263, ( $\square$ ) IS 22380, ( $\circ$ ) IS 13441, ( $\blacktriangle$ ) IS 12739, ( $\times$ ) IS 12744.

(b) NRA and growth rate as related to mid-day leaf water potential in 6 sorghum lines during mid-season stress. NRA levels are plotted as scatter diagram with a line fitted by the equation  $Y = 5.11 + 2.02x + 0.17x^2$  ( $r^2 = 0.54$ ), while the growth rate is shown by a line fitted (---) by the same equation as in Fig. 3a. Symbols are the same as in Fig. 3a.

growth rates, at the same time as proline accumulation was observed.

A large decrease in NRA occurred at a LWP higher than  $-2.0$  MPa during drought in all the lines, and a rather slow change in NRA was seen at LWP lower than  $-2.5$  MPa (Fig. 3b). The rapid change in NRA was coincident with the growth rate becoming zero, and a marginal decline in NRA continued in the absence of any appreciable growth. The change in NRA with LWP

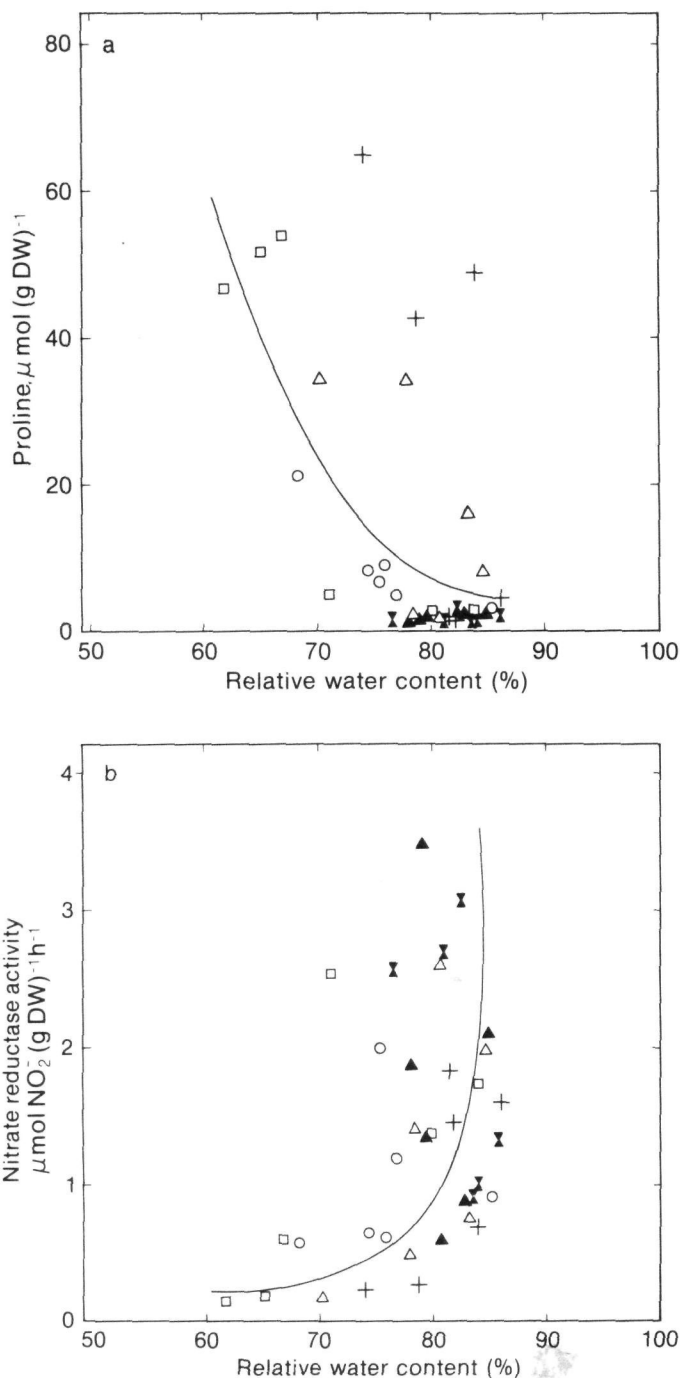


Fig. 4. (a) Proline and (b) NRA in relation to RWC of leaves in 6 sorghum lines during stress. The line showing the relation between proline accumulation during stress against RWC is fitted by the equation  $Y = 654 - 18.08x + 0.087x^2$  ( $r^2 = 0.36$ ). The line showing the relation between NRA and RWC is fitted by the equation  $Y = 15.58 - 0.177x$  ( $r^2 = 0.43$ ). Symbols are the same as given in Fig. 3.

showed only small differences between susceptible and resistant lines.

The association between proline and RWC was non-linear (Fig. 4a) and points were scattered, although there was some evidence that proline accumulated in SPH 263 and ICSV 213 at a higher water status compared to other resistant lines. In general, RWC did not fall below 80% in the susceptible lines, and this was the



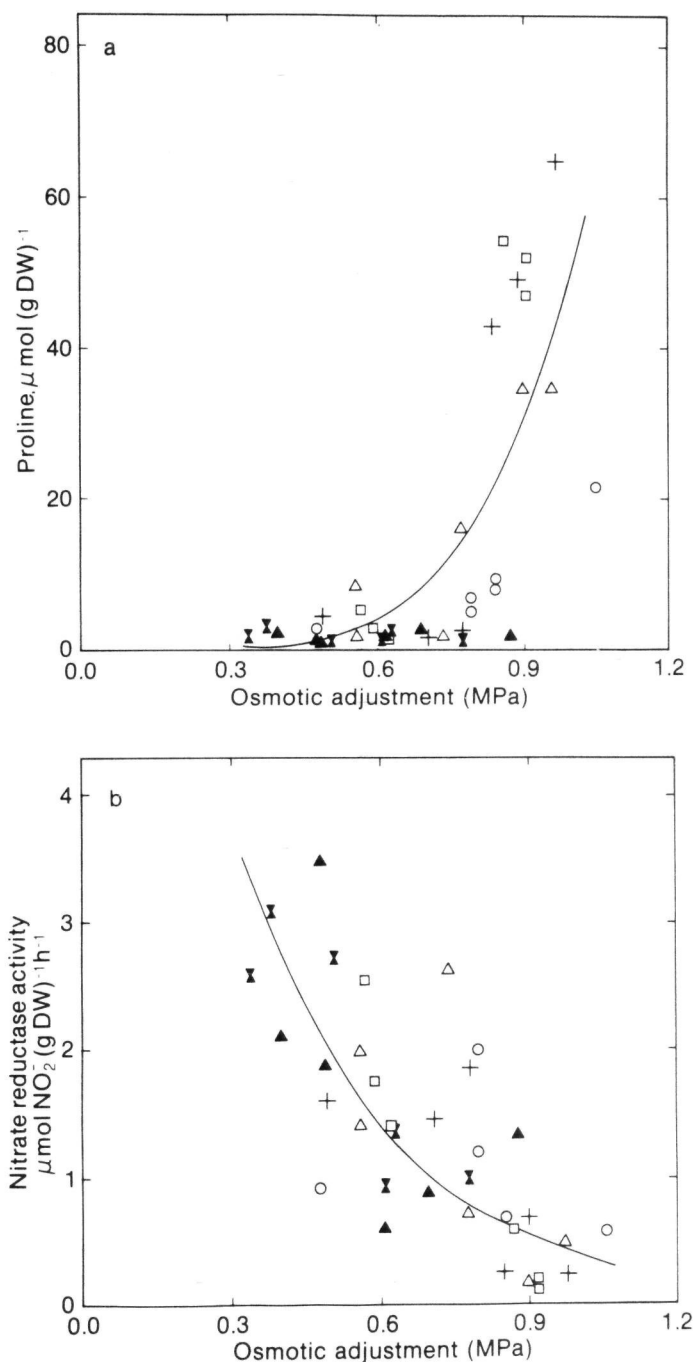


Fig. 5. Relationship between OA and (a) proline and (b) NRA during stress in six sorghum lines. The line showing the relationship between proline and OA is fitted by the equation  $Y = 42.1 - 169.7x + 171.3x^2$  ( $r^2 = 0.51$ ). The line showing NRA and OA is obtained by linear regression of log NRA on OA, and the fitted values are presented by the equation  $Y = e^{(2.336 - 3.32x)}$ , ( $r^2 = 0.51$ ). Symbols are the same as given in Fig. 3.

point at which appreciable increase in proline was noticed for the resistant lines. On the other hand, a large reduction in NRA occurred at high RWC, and at lower RWC (75%) there was little further effect of stress on NRA (Fig. 4b).

The resistant lines showed an increase in proline after an osmotic adjustment of ca 0.8 MPa was reached (Fig. 5a). Even at low LWP, susceptible lines did not have this

capacity for osmotic adjustment. It was only when low LWPs were reached ( $LWP < -2.5$  MPa) and leaves already had an adjustment of 0.8 MPa that the proline could be of benefit. With increasing osmotic adjustment NRA showed a decrease (Fig. 5b), and there was no difference between the resistant and susceptible lines.

Low LWP causes marginal firing of sorghum leaves, which expands from the edges towards the midrib with increasing intensity of heat stress. In certain cases yellowing of leaves occurs before the onset of firing as in the case of IS 12744. In the greenhouse an experiment was conducted to determine the proline levels in green and fired or yellow portions of sorghum leaves and to measure the lethal leaf water potential in the susceptible and resistant lines. Table 1 shows that proline accumulated in both the green and fired portions of the same leaf in IS 22380. Upon rewatering proline returned to control levels in the green but not in the fired portions of the leaf. Furthermore, the levels of proline were much higher in the green than in the fired portions. The same result was seen with IS 12744 (Tab. 1), although proline in the yellow portions behaved similar to green leaves upon rewatering. Table 2 gives the relative recovery rate upon rewatering of drought stressed plants. In contrast to the field experiment (Fig. 3a), the LWP decreased below  $-2.5$  MPa in IS 12744, which resulted in a considerable increase in proline levels. IS 12744 exhibited a lethal LWP of  $-2.7$  MPa compared to  $-3.4$  MPa in IS 22380.

## Discussion

Genotypic differences in proline accumulation during drought stress have been observed in a variety of crop species like barley (Singh et al. 1973, Hanson et al. 1977), sorghum (Blum and Ebercon 1976), maize (Ilahi and Dörffling 1982, Patil et al. 1984), rice (Mali and Mehta 1977) and cotton (Ferreira et al. 1979). The results presented here also demonstrate the genotypic difference in the accumulation of proline in 6 sorghum lines subjected to mid-season drought stress of the same duration (Fig. 1). Blum and Ebercon (1976) suggested that there was a threshold LWP for sorghum, below which proline started accumulating. Although a threshold LWP was not obvious in our experiments, a considerable decrease in LWP to ca  $-2.5$  MPa was reached in all the resistant lines before any substantial change in proline was observed (Fig. 3a). The data from the experiment in the greenhouse (Tab. 1) showed that LWP of the susceptible line IS 12744 did not fall below  $-2.7$  MPa (compared to  $-3.4$  MPa for the resistant line IS 22380) without the death of the youngest fully-expanded leaves. The difference in the lethal LWP between the two contrasting lines seems to have resulted in the observed difference in proline accumulation. Most of the proline accumulated after the above ground growth had ceased and, therefore, the contribution of

Tab. 1. Proline accumulation and LWP in green and fired portions of sorghum leaf during stress and on rewatering. Each value is a mean of 6 independent determinations, and values in parenthesis denote  $\pm$  SE.

Line	Portion of leaf	Control		Stress		24 h after rewatering		72 h after rewatering	
		LWP, MPa	Proline $\mu\text{mol (g DW)}^{-1}$	LWP, MPa	Proline $\mu\text{mol (g DW)}^{-1}$	LWP, MPa	Proline $\mu\text{mol (g DW)}^{-1}$	LWP, MPa	Proline $\mu\text{mol (g DW)}^{-1}$
IS 22380	Green	-1.10 (0.061)	4.6 (0.66)	-3.43 (0.028)	100.8 (4.52)	-1.96 (0.21)	44.8 (3.7)	-1.40 (0.024)	7.9 (1.37)
	Fired/ Yellow	-	-	-	48.9 (2.40)	-	56.3 (7.0)	-	57.7 (2.74)
IS 12744	Green	-0.97 (0.188)	3.2 (0.30)	-2.70 (0.055)	52.8 (4.91)	-0.99 (0.077)	9.4 (0.63)	-1.11 (0.032)	3.3 (1.19)
	Fired/ Yellow	-	-	-	8.7 (3.3)	-	5.5 (0.48)	-	3.2 (1.21)

proline to plant growth during drought is likely to be negligible.

While proline is one of few compounds that accumulate in drought stressed plants, its adaptive significance is far from clear. Hanson et al. (1979) showed that most of the proline accumulation during drought stress in barley leaves was irreversible as it occurred in the fired portions of the leaf. They also concluded that proline accumulation was an injury response in plants, and only a symptom of stress with no adaptive significance. In contrast, Tyankova (1967) found more proline in green than in fired (yellow) portions of wheat leaves during water deficit. Our results from both the field (Fig. 1a) and greenhouse (Tab. 1) showed that most of the proline was accumulated in the green portion of leaves. Upon rewatering, proline fell to half its stress level in 24 h and returned to normal within 72 h. In the field, little firing of sorghum was visible in all the lines until 54 DAS, and whole leaves that were taken for analysis of proline had mostly green tissue. Riazi et al. (1985) showed that proline accumulation mainly took place in growing regions of young barley seedlings subjected to moisture stress and which showed no evidence of cell death. Furthermore, Handa et al. (1986) found that proline accumulation was associated with the acclimation of tomato cells to water deficits. Therefore, proline accumulation in drought stressed tissue can not be just an injury response contrary to the proposition by Hanson (1980).

Relative water content is considered an alternative measure of plant water status, reflecting the metabolic activity in tissues and lethal leaf water status (Flower and Ludlow 1986). Proline accumulated over a narrow range of RWC (Fig. 4a), and the points for the various lines were more scattered than when they were compared with LWP. One reason for the scatter could be that RWC and LWP are instantaneous measurements, whereas proline levels are the result of an integrated response to the period of stress. Both LWP and RWC are sensitive to the balance between transpiration and soil water uptake, which are influenced by many environmental parameters.

Osmotic adjustment is an important mechanism in drought tolerance of plants. Handa et al. (1986) have suggested that proline makes a substantial contribution towards osmotic adjustment and adaptation to stress. Our results indicate that there was no genotypic difference in proline accumulation with increasing osmotic adjustment (Fig. 5a) and that an adjustment of 0.8 MPa occurred at low LWP before any substantial increase in proline was observed. Riazi et al. (1985) concluded from their findings that in growing regions of barley, proline levels did not increase until long after osmotic adjustment began.

Singh et al. (1973) and Blum and Ebercon (1976) suggested that the role of proline may be in the immediate recovery of plants after the release of stress. Our data calculated for the immediate recovery of resistant lines after rewatering (Tab. 2) compared well with the levels of proline accumulated during peak stress period. When the immediate recovery rates of these resistant lines were plotted against proline levels 67 DAS, a good correlation ( $r^2 = 0.81$ ) was obtained, suggesting that proline may have a role in the immediate recovery of plants after stress. Proline may be used to meet the immediate needs of energy and nitrogen after a recovery from drought.

The sensitivity of NRA to water deficit has been demonstrated in many crops (Sinha and Nicholas 1981).

Tab. 2. Proline accumulation and relative recovery rate on rewatering of plants after drought stress.

Line	Proline $\mu\text{mol (g DW)}^{-1}$	Relative recovery rate, g DW day <sup>-1</sup>
	67 DAS	Mean (70-72 DAS)
ICSV 213	64.4	2.02
IS 22380	46.5	1.23
IS 13441	21.1	0.78
SPH 263	34.1	0.60
IS 12744	1.45	negligible
IS 12739	0.94	negligible

Pal et al. (1976) measured NRA both in vivo and in vitro in maize seedlings and found that the enzyme from drought tolerant hybrids was less inhibited at higher temperature compared to intolerant hybrids. In contrast, Maranville and Sullivan (1976) observed that most tolerant types of sorghum genotypes lost NRA to a greater extent compared to the susceptible ones. Our results did not show any significant genotypic difference between the resistant and susceptible lines in NRA during stress (Fig. 2), in contrast to what was observed for proline accumulation (Fig. 1).

Nitrate reductase activity is more sensitive than proline, even to mild water deficit and the activity falls drastically over a narrow range of LWP (Fig. 3b) or RWC (Fig. 4b). No genotypic difference in the reduction of NRA was observed with a decrease in either LWP or RWC during stress. In spite of zero growth rate, a certain level of NRA was maintained in the leaves during the peak stress period, which may be an acclimatization to water deficit (Smirnoff et al. 1985). Many factors like enzyme level, nitrate content and NADH availability influence the decline of NRA under water deficit and the recovery upon rewatering. Although NADH could be a limiting factor under water deficit, nitrate content in leaf tissues does not always reflect the NRA status in the tissue (Sinha and Nicholas 1981). The NRA under moisture deficit expressed as a percentage of the irrigated control was positively correlated with enzyme recovery upon rewatering and grain yield in rice (Sairam and Dube 1984). The decline in NRA during drought has been attributed to a reduction in enzyme level as determined by the rate of protein synthesis and degradation (Bardzik et al. 1971) as well as inactivation of enzyme activity (Plaut 1974).

Plant growth during water stress does not seem to be influenced by proline, since most of the accumulation occurs after growth has ceased. However, the role of proline in the immediate recovery of plants from stress cannot be ignored, and a better understanding of the regulation of proline levels during this recovery phase is needed to evaluate its significance for crop performance under drought. NRA is a sensitive index of plant water status; and the stability of the enzyme during stress as reflected in the minimal value during peak stress, along with the potential for recovery upon rewatering, are two aspects that need more investigation to give a full picture of their value for crop yield.

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