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Leaf proteinase and nitrate reductase activities in relation to grain protein levels and grain yield in four species of grain amaranth

K RAMAMURTHY NAIDU, Y SEETHAMBARAM and
V S RAMA DAS*

School of Life Sciences, University of Hyderabad, Hyderabad 500 134, India
* Department of Botany, Sri Venkateswara University, Tirupati 517 502, India

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Abstract. The relationship of leaf nitrate reductase (NR) and proteinase activities to the grain protein level and grain yield was investigated in four species of grain amaranth (*Amaranthus hypochondriacus*, *A. caudatus*, *A. cruentus* and *A. edulis*). A strikingly positive correlation between the leaf proteinase activity and the grain protein content was found. *A. edulis* with higher grain protein level possessed high leaf proteinase activity, while *A. hypochondriacus*, with relatively lower grain protein content had lower leaf proteinase levels. Although there was no definite correlation between the leaf proteinase levels and the grain yield, the integrated leaf NR activity was positively correlated with the grain yield. The total nitrogen content per plant seems to be dependent on the extent of root growth and the levels of NR activity in leaves.

Keywords. Grian amaranth ; leaf proteinase activity ; leaf nitrate reductase activity ; grain protein ; grain yield.

1. Introduction

Grain amaranth is presently one of the under-exploited crop plants with a considerable economic potential. The grain is regarded to be unique for its high protein content. It is also known to be rich in lysine and sulfur containing amino acids and can therefore be considered superior to the proteins of wheat, corn and rice (Senfit 1980).

Significant correlations were found in the past between the integrated leaf nitrate reductase (NR) activity and grain yield, reduced nitrogen levels of grain and of whole plant in the case of wheat and maize (Abrol and Nair 1978 ; Brunetti and Hageman 1976 ; Hageman 1979). On the other hand Dalling *et al* (1975) found that wheat cultivars with similar levels of NR activity could accumulate variable amounts of reduced nitrogen. Deckard *et al* (1973) identified one maize genotype with relatively low NR activity but a high capacity to accumulate reduced nitrogen. However, several studies have indicated that NR assays could be a useful predictive selection criterion for grain yield and grain protein levels (Croy and Hageman 1970 ; Fakorede and Mock 1978). Not much work was done on the role of proteinases of leaf in relation to the protein content on grain. That leaf proteinase activities were correlated with grain nitrogen was evident in wheat, rice and maize (Dalling *et al* 1976 ; Perez *et al* 1973 ; Reed *et al* 1980).

In view of meagre work on grain amaranths in general and its nitrogen metabolism in particular, the present study was carried out, leading to an understanding of the relationship between the leaf proteinase activity, grain protein, grain yield and secondly between the leaf NR activity, root growth and the accumulation of reduced nitrogen in four species of grain amaranth.

2. Materials and methods

Seeds of *Amaranthus hypochondriacus*, L., *Amaranthus caudatus* L., *Amaranthus cruentus* and *Amaranthus edulis* L. were obtained from National Botanical Research Institute, Lucknow. Plants were grown in 30 cm diameter earthenware pots on soil supplemented with manure (3 parts of red soil + 1 part of farm yard manure) under natural (approximately 12 hr) photoperiod (temperature about 35° C day and 22° C night). Three plants were retained in each pot.

The plants were harvested at three different stages, *viz.*, vegetative stage (30 days after sowing), flowering stage (45 days after sowing) and grain filling stage (60 days after sowing). At each stage, plants from 3 pots were collected and were subdivided into leaf, stem, root and panicle. Fresh and dry weights of these plant parts were determined and the shoot/root ratio was calculated.

Reduced nitrogen content in the dried samples was determined by kjeldahl method using Tecator digestion and distilling systems (Tecator Manual). After anthesis (90 days after sowing) plants were finally harvested to the ground level and the dry weights of the panicle and stover were determined. Grain protein content was calculated by multiplying the grain nitrogen by a factor of 6·25.

2.1. *Nitrate reductase* (NR) *assay*

Leaf NR activity was measured at the vegetative, flowering and grain filling stages. Fully expanded young leaves from each pot were collected (at 10 a.m.) into a polyethylene bag placed on an ice bath and were carried into the laboratory. The leaves were deribbed, weighed and were then chopped into pieces. The *in vitro* NR assay was as described by Hageman and Hucklesby (1971).

2.2. *Proteinase activity*

The proteinase activity was measured thrice at 15 day intervals after anthesis (60, 75 and 90 days after sowing). The proteinase activity in the middle leaf of the plant was assayed by the modified procedure of Peoples and Dalling (1978). Leaves were homogenised by grinding in a mortar and with a pestle for 90 sec with 5 ml/g extracting medium containing 23 mM sodium citrate ; 155 mM sodium phosphate ; 5 mM L-cysteine; 5 mM EDTA and 1% PVP, pH 6·8. After straining through cheese cloth the homogenate was centrifuged at 25000 g for 10 min. The supernatant was dialysed at 4° C for 48 hr against 50 mM potassium phosphate buffer, pH 7·0.

1% Bovine serum albumin solution was prepared in 0·05 M Tris–HCl (pH 7·8). 0·1 ml of extract was incubated with 0·5 ml of substrate for 2 hrs at 37° C. The reaction was terminated by adding 0·7 ml of 15% trichloroacetic acid and the soluble nitrogen in the mixture was determined by ninhydrin (Spices 1957). Leucine was used as the amino acid standard.

3. Results

3.1. *Shoot dry weight*

There was a wide variation in the dry weight of shoot at the vegetative stage between the four species of grain amaranth studied (table 1). Maximum dry matter production during the vegetative stage was in *A. hypochondriacus* followed by *A. edulis*, *A. caudatus* and *A. cruentus*. Though a similar trend was observed at flowering stage, the variation in dry matter content was not significant suggesting that the growth rate during different stages of growth period varied among four species (table 2). The higher dry matter accumulation noticed in *A. caudatus* than that in *A. hypochondriacus* during filling stage may be due to the faster growth rate in *A. caudatus* from flowering stage (table 3). The greater dry matter accumulation in shoots of *A. hypochondriacus* (table 4) than the other three species, might be due to the bigger panicles in the former species.

Table 1. Dry weights and leaf reduced nitrogen during vegetative stage (30 days after sowing) of four species of grain amaranth.

Species	Total dry wt/plant			S/R	Leaf reduced N%	Reduced N of stover g/plant
	Shoot g	Root g	Total g			
A. hypochondriacus L.	9·0 ± 0·4	0·80 ± 0·1	9·80 ± 0·45	11·25 ± 0·25	3·85 ± 0·12	0·28 ± 0·02
A. caudatus L.	3·5 ± 0·2	0·25 ± 0·05	3·75 ± 0·26	14·00 ± 0·36	4·27 ± 0·15	0·13 ± 0·02
A. cruentus L.	3·1 ± 0·3	0·40 ± 0·04	3·5 ± 0·32	7·75 ± 0·15	4·13 ± 0·08	0·11 ± 0·01
A. edulis L.	5·8 ± 0·4	0·45 ± 0·03	6·25 ± 0·42	12·89 ± 0·28	3·99 ± 0·07	0·19 ± 0·03

Table 2. Dry weights and leaf reduced nitrogen during flowering (45 days after sowing) of four species of grain amaranth.

Species	Total dry wt/plant			S/R	Leaf reduced N%	Reduced N of stover g/plant
	Shoot g	Root g	Total g			
A. hypochondriacus L.	44·0 ± 1·8	8·2 ± 0·5	52·2 ± 2·1	5·36 ± 0·25	3·92 ± 0·06	1·27 ± 0·07
A. caudatus L.	41·5 ± 2·4	9·5 ± 0·7	51·0 ± 2·0	4·37 ± 0·28	4·47 ± 0·08	1·39 ± 0·06
A. cruentus L.	35·5 ± 1·6	8·0 ± 0·3	43·5 ± 1·8	4·44 ± 0·16	3·71 ± 0·04	0·09 ± 0·05
A. edulis L.	37·5 ± 1·5	7·5 ± 0·2	45·0 ± 1·4	5·00 ± 0·32	3·64 ± 0·05	1·02 ± 0·05

Table 3. Dry weights and leaf reduced nitrogen during grain filling (60 days after sowing) of four species of grain amaranth.

Species	Total dry wt/plant			S/R	Leaf reduced N%	Reduced N of stover g/plant
	Shoot g	Root g	Total g			
A. hypochondriacus L.	82·0 ± 4·2	12·0 ± 0·8	94·0 ± 4·5	6·80 ± 0·4	3·50 ± 0·06	2·86 ± 0·21
A. caudatus L.	87·0 ± 3·6	15·0 ± 1·2	102·0 ± 4·8	5·80 ± 0·26	3·57 ± 0·04	3·29 ± 0·18
A. cruentus L.	66·5 ± 3·8	12·0 ± 1·4	78·5 ± 4·2	5·54 ± 0·32	3·44 ± 0·07	2·41 ± 0·18
A. edulis L.	69·5 ± 2·3	11·5 ± 0·7	81·0 ± 3·1	6·04 ± 0·22	2·94 ± 0·08	2·65 ± 0·14

3.2. *Shoot/Root (S/R) ratio*

The S/R ratio in all the four species was maximum at vegetative stage and decreased gradually during flowering and grain filling stages (tables 1, 2 and 3). *A. caudatus* had the highest S/R ratio among the four species during vegetative phase of growth, but the ratio decreased during flowering and grain filling stages due to increases in root dry weight at later stages of growth.

Table 4. Mean NR activity (calculated from figure 1), dry matter at final harvest (90 days after sowing) grain yield, grain protein and mean proteinase activity (calculated from figure 2) of four species of grain amaranth.

Species	NR activity μmol NO_2^- g^{-1} h^{-1}	Dry matter (g/plant) Panicle	Stover	Total	Grain yield g/plant	Grain protein %	Proteinase activity μmol NH_3 g^{-1} h^{-1}	Total N g/plant
A. hypochondriacus L.	26·2 ± 1·4	75·0 ± 2·5	51·0 ± 1·0	126·0 ± 3·4	23·6 ± 1·8	12·5 ± 0·4	21·6 ± 0·8	2·42 ± 0·12
A. caudatus L.	23·43 ± 0·8	45·0 ± 2·2	60·0 ± 1·8	105·0 ± 4·0	16·8 ± 0·7	14·0 ± 0·3	31·5 ± 0·6	2·96 ± 0·18
A. cruentus L.	20·07 ± 1·2	44·0 ± 1·9	58·0 ± 2·1	102·0 ± 3·8	15·6 ± 0·6	12·6 ± 0·2	28·4 ± 0·7	2·15 ± 0·11
A. edulis L.	24·17 ± 0·9	69·0 ± 1·7	46·0 ± 1·4	115·0 ± 3·1	21·8 ± 0·7	15·0 ± 0·3	35·0 ± 1·2	2·24 ± 0·09

3.3. *Levels of leaf reduced nitrogen (%) and grain protein (%)*

Variation in the concentration of leaf reduced nitrogen was observed among the four species of grain amaranth. Maximum protein content was in grain of *A. edulis* while the maximum content of leaf reduced nitrogen was recorded in *A. edulis* (table 4).

3.4. NR *activity and grain yield*

The greatest NR activity was at the flowering stage of all the four species of grain amaranth and the activity decreased at grain filling stage (figure 1). The level of NR in *A. hypochondriacus* was more than that in the other three species during vegetative and flowering stages whereas, at grain filling stage, *A. caudatus* had the greatest NR activity. There was a positive correlation between the mean NR activity the three stages and grain yield ($r = +0·89$) in all the four species of grain amaranth.

3.5. *Proteinase activity*

The results of the mean proteinase activity calculated from figure 2 are given in table 4. The level of leaf proteinase activity was in the decreasing order of *A. edulis* followed by *A. caudatus* ; *A. cruentus* and *A. hypochondriacus*. The leaf proteinase activity was positively correlated with the percentage of protein

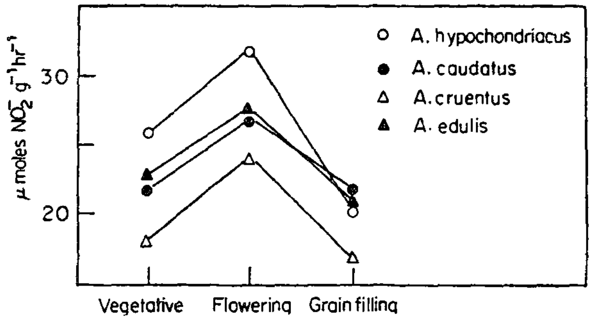

Figure 1. The leaf *in vitro* nitrate reductase activity of four species of grain amaranth during different stages of growth.

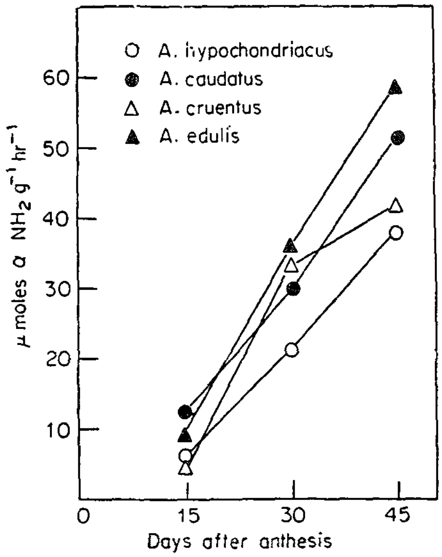

Figure 2. The leaf proteinase activity of four species of grain amaranth.

in grain ($r = +0{\cdot}85$) in all the four species. Leaf proteinase activity increased during grain development (figure 2).

4. Discussion

The total reduced N per plant was positively correlated with leaf NR activity ($r = +0{\cdot}86$) during the vegetative phase. The leaf NR activity was positively correlated with the total reduced N per plant ($r = +0{\cdot}86$) but not with leaf reduced nitrogen ($r = +0{\cdot}19$). There was also significant correlation between root growth and the total reduced N per plant ($r = +0{\cdot}87$) and NR activity ($r = +0{\cdot}65$). These data indicate that total reduced nitrogen per plant was influenced by NR activity as well as by the root growth. Therefore the higher NR activity and root growth in *A. hypochondriacus* and *A. edulis* than those in *A. caudatus* and *A. cruentus* resulted in more total reduced nitrogen per plant in the former two species.

During the flowering stage also no positive correlations could be traced between NR activity and leaf reduced nitrogen or ($r = +0{\cdot}16$) or total reduced nitrogen per plant ($r = +0{\cdot}42$) (table 2). *A. caudatus* had high N levels in leaf and in total plant during flowering stage, but its NR activity was less than that of the other three species. Reed and Hageman (1980) observed that accumulation of reduced nitrogen was dependent more on nitrate uptake than on the relative levels of NR activity per plant. Raper *et al* (1977a, b) suggested that nitrogen uptake was positively related to the rate of root growth in tobacco, cotton and soybean. The remarkable increase in root growth of *A. caudatus* during the flowering stage might have resulted in its comparatively high reduced nitrogen per plant (table 2).

A. caudatus retained more reduced nitrogen per plant during grain filling stage. Again the NR activity was not related with either leaf reduced nitrogen ($r = +0{\cdot}14$) or total reduced nitrogen per plant ($r = +0{\cdot}34$) in all the four species (table 3). The high root growth of *A. caudatus* could have facilitated the accumulation of more reduced nitrogen per plant either through mobilisation of reduced N or by augmenting the uptake of nitrate and efflux. While there was always a positive correlation between the root growth and total reduced nitrogen per plant ($r = +0{\cdot}78$), the NR activity was not correlated with the total reduced nitrogen per plant even at the harvest (table 4). Thus, our observations confirm that at any stage of growth period in the grain amaranths studied, the leaf NR activity alone is not an index of total reduced nitrogen per plant but the extent of root growth along with the NR levels would together influence the nitrogen content per plant.

The average nitrate reductase activity in the leaf was positively correlated with the grain yield ($r = +0{\cdot}89$) as well as total dry matter accumulation at harvest ($r = 0{\cdot}86$) in all the four species of grain amaranth (table 4). These findings confirm the observations of earlier workers where NR activity was correlated with grain yield (Blackwood and Hallam 1979 ; Deckard *et al* 1977 ; Dalling and Loyn 1977 ; Johnson *et al* 1976).

On the other hand, the protein levels in the grain were not related with the NR activities ($r = -0{\cdot}37$), but were strongly correlated with proteinase activity ($r = +0{\cdot}85$) in all the four species (table 4). This agrees with the results of Deckard *et al* (1977) who concluded that the NR activities were not correlated with grain protein. Differences in nitrogen translocation efficiency could reduce the correlation between NR activity and grain protein (Croy and Hageman 1970 ; Eilrich and Hageman 1973). Leaf proteinase activities were related more closely to accumulation of grain nitrogen than leaf NR activity (Reed *et al* 1980). In the present study, *A. edulis* contained greater proteolytic activity in the leaf and accumulated more protein in its grain than that in *A. hypochondriacus* in spite of high NR activity in the latter species.

Although the number of observations are limited, the present investigation nevertheless suggests that in grain amaranths the level of leaf proteinase activity is an important determinant of the grain protein content whereas the leaf NR activity and root growth modulate the total reduced nitrogen per plant.

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