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SHORT COMMUNICATION

PROLINE METABOLISM IN SORGHUM AND CHICKPEA CULTIVARS DURING WATER STRESS

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Drought tolerant and susceptible cultivars of both sorghum and chickpea were evaluated for proline accumulation, Pyrroline 5-carboxylate synthetase (P5CS) activity and soluble proteins in relation to imposed water stress. The result in the present study revealed that an increase of proline content from 14.58 to 70.75 µmol g^{-1} fr.wt in sorghum and 11.74 to 131.30 µmol g^{-1} fr.wt in chickpea with concomitant increase in P5CS activity and soluble proteins. The significant differences were recorded in free proline content of drought tolerant and susceptible genotypes of both sorghum and chickpea that could be attributed to increased P5CS activity and the loss of feedback inhibition of P5CS by proline during moisture stress.

Key words: **Chickpea, PEG-6000, proline, P5CS, sorghum**

A well recognized cellular response to water deficit is accumulation of osmotically active compounds that allow cell to regulate turgor and extract additional water from the soil (Morgan 1980). Proline is one such compatible osmolyte and high accumulation of cellular proline upto 80% of the total amino acid pool under stress as against 5% under control has been documented in many plant species (Delauney and Verma 1993). Chickpea and sorghum are the major crops of the semiarid tropics and are grown on the residual moisture. An understanding of mechanism for higher accumulation of compatible osmolites such as proline in these crops will certainly provide insight for planning the breeding programme for development of drought tolerant genotypes.

The significant increase in the proline content under stress could be attributed to increased rate of synthesis, reduced rate of catabolism and loss of feedback regulation of the key regulatory enzyme in proline biosynthesis, viz. pyrolline-5-carboxylate synthetase (P5CS). However, plant P5CS has been reported to be less sensitive to end product inhibition as compared to bacterial enzyme. A correlation between transcript level of pyrolline-5-carboxylate synthetase,a rate limiting enzyme in proline biosynthesis and free proline content appears to be a key factor and overexpression of P5CS has been reported to confer stress tolerance in tobacco and rice (Kavi Kishore *et al*. 1995, Zhu *et al.* 1998). The present investigation was therefore undertaken to study the differential response of stress tolerant and susceptible genotypes both under osmotic stress induced by PEG-6000 and withholding of water on proline metabolism.

An experiment was conducted with sorghum genotypes, viz. ICSV-272 (drought tolerant) and SPRU-94008B (drought susceptible) and chickpea genotypes, viz. PG-96006 (drought tolerant) and Jakki (drought susceptible). The seeds were surface sterilized with

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0.1% (w/v) $HgCl_2$ followed by 70% ethanol for 5 min. and washed with sterile water and were placed separately in sterilized petriplates containing 0.8% agar (control) and 0.8% agar containing PEG-6000 (stress) corresponding to osmotic stress of -0.5 Mpa. The seeds were allowed to germinate for 7 days in the dark (Fig. 1). Free proline was extracted from the root and shoots with 3% (w/v) sulphosalysilic acid and estimated colorimetricaly (Bates *et al*. 1973). The enzyme P5CS was extracted in 50 mM Tris-HCl (pH 7.2) containing 1mM dithiothretol and the activity in the supernatant was assayed by estimating amount of gamma glutamyl hydroximate produced (Hayzer and Leisinger 1980). In a separate experiment, sorghum plants were raised in earthen pots filled with medium black soil in net house under natural conditions and water stress was imposed by withholding irrigation for 7 days, while the control plants were watered daily. Leaf samples were collected and analysed for RWC, free proline content and activity of P5CS.

The content of free proline and activity of pyrroline-5-carboxylate synthetase increased in all the cultivars due to osmotic stress induced by PEG-6000. The mean proline content in sorghum root increased from 17.75 under unstressed to 62.72 µmol g^{-1} fw with an increase in P5CS activity from 0.18 to 0.35 units. Similarly in the roots of chickpea the mean proline content increased from 17.53 to 110.1 µmol g^{-1} fw with an increase in the P5CS activity from 0.30 to 0.68 units. A correlation between induction of a gene for pyrroline-5-carboxylate synthetase and accumulation of proline has been reported in *Arabidopsis thaliana* (Yoshiba *et al*. 1995). Similarly increase in the activity of P5CS in leaves and roots of *Brassica* cultivars under water stress and the trend of changes in P5CS activity compared well with differences in magnitude of proline accumulation (Phutela *et al*. 2000). The free proline content though slightly lower in the drought tolerant cultivars, viz. ICSU-272 and PG-96006 under unstressed condition was significantly increased under stress and the % increase over unstress control was more in the drought tolerant cultivars (Table 1). An increase in soluble proteins during water stress clearly suggests that the contribution of proteolysis may not be a factor for increase in the proline levels. The content of free proline can be correlated to substantial increase in the activity of pyrroline -5-carboxylate synthetase under stress over unstressed control.

Table 1. Effect of PEG induced stress (-0.5 MPa) on proline (µmol g^{-1} fw), soluble protein (mg g^{-1} fw) and P5CS activity (µmol γ glutamyl hydroximate formed g^{-1} fw min^{-1}) in sorghum and chickpea genotypes

Crop	Proline		Soluble protein		P5CS	
Genotype	Control	Stress	Control	Stress	Control	Stress
Sorghum						
ICSU-272	14.58	70.75	17.25	25.11	0.12	0.38
SPRU-94008	20.93	54.68	16.31	33.60	0.23	0.31
Mean	17.75	62.72	16.78	29.36	0.18	0.35
SE	0.03	0.02	0.03	0.035	0.02	0.02
Chickpea						
PG-96006	11.74	131.30	8.76	11.64	0.32	0.79
Jaki	23.31	88.80	7.86	10.69	0.39	0.56
Mean	17.53	110.10	8.31	11.06	0.36	0.68
SE	0.05	0.03	0.04	0.03	0.03	0.02

Leaf samples of the sorghum plant exposed to water stress by withholding irrigation for 7 days when analysed against leaf samples of unstress (control) as evident from RWC (Fig.2) exhibited significant differences in the content of free proline and P5CS activity. The free proline content in the leaves of ICSU-272 increased from 0.77 to 16.95 µmol g^{-1} fw. An increase in activity of P5CS by 208% under stress caused almost 2100% increase in the free proline (Table 2). The activity of P5CS extracted from the samples of unstress leaves was less in the presence of proline in the assay medium. However, under stress condition the activity of P5CS though higher but did not show any significant differences either in the presence or absence of proline (Table 3). Although the enzyme preparation was not purified but still it appears that proline synthesis is under feed back regulation under unstress conditions. Efforts are underway to evaluate the effect of proline on partially purified P5CS enzyme. From the results it appears that site directed mutaganised mothbean P5CS gene (Kavi Kishore *et al.* 1995) can be utilized for transformation of chickpea and sorghum plants for improving drought tolerance.

Table 2. Effect of water stress imposed by withholding irrigation on RWC (%), proline (µmol g^{-1} fw) and P5CS activity (µmol γ glutamyl hydroximate formed g^{-1} fw min^{-1}) in sorghum genotypes

Genotype	RWC		Protein		P5CS	
	Control	Stress	Control	Stress	Control	Stress
ICSU-272	89.5	67.30	0.77	16.95	0.63	1.94
SPRU-94008	94.6	41.90	1.06	11.30	0.81	2.27
M-35-1-19	98.30	63.00	0.93	13.20	0.76	2.20
Mean	94.13	57.40	0.92	18.32	0.73	2.14
SE	0.03	0.035	0.02	0.04	0.05	0.03

Table 3. Effect of water stress on activity of pyrroline-5-carboxylate synthetase (µmol γ glutamyl hydroximate formed g^{-1} tissue min^{-1}) in sorghum genotypes

Genotype	P5CS activity			
	Control		Stress	
	without proline	with proline	without proline	with proline
ICSU-272	0.63	0.50	1.95	1.92
SPRU-94008	0.81	0.69	2.27	2.20
M-35-1-19	0.78	0.67	1.84	1.77
Mean	0.74	0.62	2.02	1.96
SE	0.05	0.04	0.01	0.02

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