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Influence of GA_3 Pre-soaking of Seeds on Biochemical Changes in Seedling Parts of *Pennisetum typhoides* Rich.

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A comparative biochemical study was carried out to assess the changes in soluble protein, free amino acids, organic acids and keto acids in the seedlings from control and GA_3-presoaked seeds of *P. typhoides*. Most seedling parts from treated seeds have recorded higher levels of soluble protein and free amino acid pool at various growth stages. GA_3 caused a retention of free nitrogen pool in coleoptile and root than translocated to the primary leaf. Succinic and malic acids were the dominating organic acids followed by citric acid in control as well as treated ones. The distribution pattern of organic acids in seedling parts from GA_3-treated one may indicate their rapid utilization in the synthesis of various amino acids and amides.

Phosphoenolpyruvic acid and pyruvic acid were present in large concentrations in various seedling parts followed by oxaloacetic acid while α-ketoglutaric acid was traced only at few growth stages. *P. typhoides* leaves, known to possess a C_4 photosynthetic pathway was exhibiting the efficient use of PEP in the synthesis of oxaloacetate.

Key Words: Protein, Free amino acids, Keto acids, Organic acids, GA_3 pre-soaking, Seeds, *Pennisetum typhoides*

Introduction

Various metabolic changes in proteins, amino acids, organic acids and keto acids accompanying seedling growth have been studied by many workers including Oota et al. (1953), Fowden and Webb (1955), Towers and Mortimer (1956), Rai and Laloraya (1965), Pant et al. (1974), and Mukherjee and Laloraya (1974, 1979 & 1980). Recently, correlative studies were carried out in *Saccharum officinarum* leaves of different maturity levels (Afria & Mukherjee 1980a), and seedling parts of *Lathyrus odoratus* (Afria & Mukherjee 1980b), *Sorghum vulgare* and *Zea mays* (Afria & Mukherjee 1981) to throw light on the changes in aforesaid metabolites during their growth. Although, gibberellins are known to stimulate the breakdown of storage products in seeds and seedlings and some studies have also been carried out on the translocation of various biochemical components (Varner et al. 1965, Rai & Laloraya 1965, Mayer & Mayber 1975, Garcia-Luis & Guardiola 1978, Mullick 1978); information is lacking about the distribution of organic and keto-acids in different seedling parts with the advancement of growth. The present work was undertaken to reveal the influence of GA_3, after pre-soaking the seeds with this growth regulator, on the distribution pattern of soluble proteins, free amino acids, keto acids and organic acids in endosperm, root, coleoptile and primary leaf of *Pennisetum typhoides* during early stages of seedling growth.

Materials and Methods

Seeds of *P. typhoides* were surface sterilized with 0.1 percent mercuric chloride for 1 min. followed by thorough washing. One set of seeds was raised in distilled water and another kept in 2.88×10^{-4}M GA_3 in a B.O.D. incubator maintained at $25 \pm 1°C$. After 48 hr (initial stage), germinated seeds with radicles were taken out and desired amount of seedling parts was collected for biochemical analysis of free amino acids, keto acids, organic acids and soluble proteins. Rest of the seeds from GA_3-treated lot were washed thoroughly and transferred to sterilized Petri-dishes moistened with equal volume of glass distilled water. Different seedling parts, collected upto 168 hr at an interval of 24 hr were transferred immediately to a refrigerator for further analysis.

Soluble proteins from fresh plant material were determined by the method of Lowry et al. (1951) using Folin Phenol reagent. The method of Steward and Thompson (1954) was followed for free amino acid and organic acid extraction. Two-directional paper chromatography (Pal & Laloraya 1967) was used for amino acid separation while single directional chromatography was employed for the separation of organic acids as mentioned elsewhere (Hais & Macek 1963). Keto acids were extracted as their 2, 4-dinitrophenyl hydrazones (2, 4-DNP's) according to Kaushik (1966). Amino acids, keto acids and organic acids were quantified in terms of glycine, 2, 4-DNP of α-ketoglutaric acid and citric acid respectively using a Spectronic-20-colorimeter.

Results and Discussion

Results have been summarized in figures 1–4. The endosperm of *P. typhoides* seedlings raised from GA_3 pre-soaked or control seeds showed a rapid decline in the amount of protein but the rate of degradation was faster in the former (figure 1). Treated endosperm samples also exhibited a simultaneous increase in the free amino acid pool as compared with control upto 48 hr stage followed by a lower concentration at 72 hr stage but again at 96 hr and 120 hr stages, they

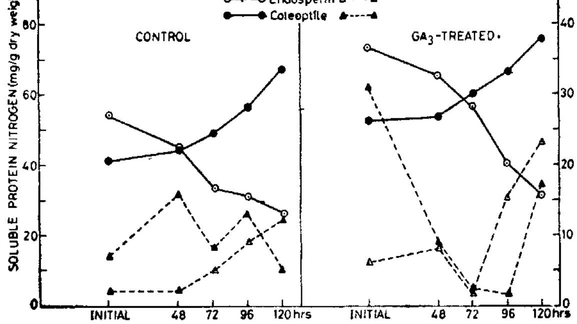

Figure 1

exhibited higher concentrations. Leucine, phenylalanine, valine, γ-aminobutyric acid, tyrosine, proline, methionine, α-alanine and glutamine in the endosperm of treated seeds dominated quantitatively upto 48 hr stage, recorded a decline at 72 hr followed by a gradual increase at later periods while in control, valine, γ-aminobutyric acid, serine, glycine and threonine recorded increasing levels with seedling growth (figure 3). The coleoptile of GA_3-soaked seeds had a higher value of free amino acid pool (figure 1) and also protein content in the beginning but 72 and 96 hr stages recorded a sharp drop in the free amino acid pool and at the corresponding stage root samples had a sharp rise which may be accounted for internal disturbances in the redistribution by GA_3 in comparison to control (figure 2). Leucine, phenylalanine, valine, γ-aminobutyric acid and α-alanine recorded a marked decline at 72 hr and 96 hr stages followed by an increase in the coleoptile of treated seeds while these amino acids declined from 48 to 72 hr stage and increased at 96 hr in the control (figure 3). It was of interest to note that leaves of treated samples recorded a low level of free amino acid pool indicating that more is retained in

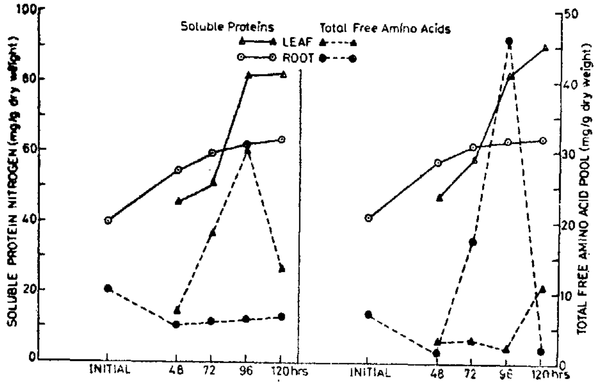

Figure 2

coleoptile and root than translocated to the primary leaf at this stage. In leaf samples free amino acid pool had recorded a gradual increase in control upto 96 hr stage followed by a decline but on the contrary treated ones exhibited a decrease from 48 to 96 hr stage and 120 hr stage recorded a considerable increase.

Paleg et al. (1964) also showed increased mobilization of protein nitrogen and reducing sugars released from the barley endosperm treated with GA_3. Gibberellic Acid, known as the enzyme mobilizing hormone, increases the level of the enzymes, amylase and protease (Mullick 1978). Bradbeer and Pinfield (1967) suggested that action of GA on *Corylus avellana* seeds may occur in the cotyledons indicating increased levels of the enzyme activity especially those concerned with the mobilization of cotyledonary reserves.

Proline has been traced only at few growth stages of different seedling parts in *Pennisetum typhoides* (figure 3). It is known to be a major component of the storage proteins of cereal seeds and may be important in subsequent nutrition of germinating seedlings (Miflin & Lea 1977). γ-Aminobutyric acid was noticed in higher concentrations in both control and GA_3-treated seedlings. It is quite widespread as a non-protein constituent of plants (Hasse & Schumacher 1950, Steward & Thompson 1954, Kulkarni & Sohonie 1956).

Among organic acids, succinic and malic acids dominated quantitatively followed by citric acid and were reported less frequently at different growth stages maintained after GA_3-treatments (figure 4A). Earlier also Pinfield (1968) while studying the effect of exogenous gibberellin on *Corylus avellana* seeds noticed higher activity of the enzyme

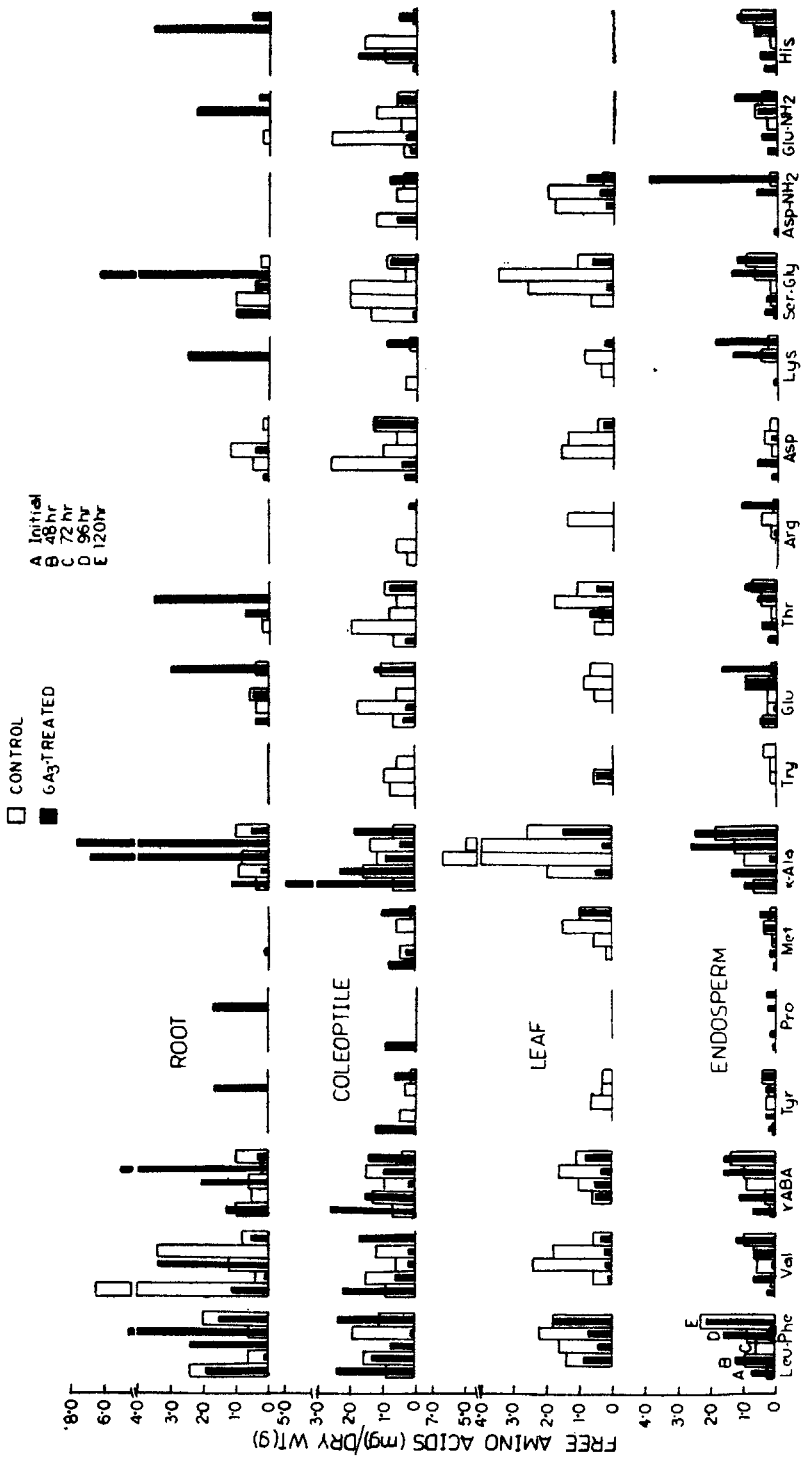
CONTROL
GA_3-TREATED
A Initial
B 48 hr
C 72 hr
D 96 hr
E 120 hr
ROOT
COLEOPTILE
LEAF
ENDOSPERM
FREE AMINO ACIDS (mg)/DRY WT(g)
Leu-Phe
Val
γABA
Tyr
Pro
Met
α-Ala
Try
Glu
Thr
Arg
Asp
Lys
Ser-Gly
Asp-NH2
Glu-NH2
His

Figure 3

isocitrate lyase and increased labelling of glutamate at the expense of TCA cycle acids. It appears, in the present study, that in the seedlings raised from GA_3-treated seeds, these acids were more rapidly utilized for the synthesis of metabolites like glutamic acid and various other amino acids and amides which further participate in transamination reactions.

Most of the seedling parts showed a similarity on comparing the levels of some of the dominant and important keto acids viz., phosphoenolpyruvate (PEP), pyruvic acid and oxaloacetic acid (OAA) (figure 4B). During growth, the leaves of treated and control seedlings exhibited a decrease in the amount of PEP while OAA-level showed an increasing trend in comparison to their respective initial values (figure 4B). However, in coleoptile, the PEP level was increased and fluctuations were noticed in the OAA-concentrations. Seedlings were unique in maintaining higher level of pyruvic acid throughout the growth stages of both coleoptiles and leaves. Indeed, it is well known that *P. typhoides* possesses Hatch and Slack pathway of CO_2 fixation in addition to Calvin cycle and that may be responsible for the efficient use of PEP in leaves in the formation of dicarboxylic acid such as OAA which was found to accumulate.

The low levels of α-ketoglutarate in both control and treated seedling samples may be accounted for its rapid utilization

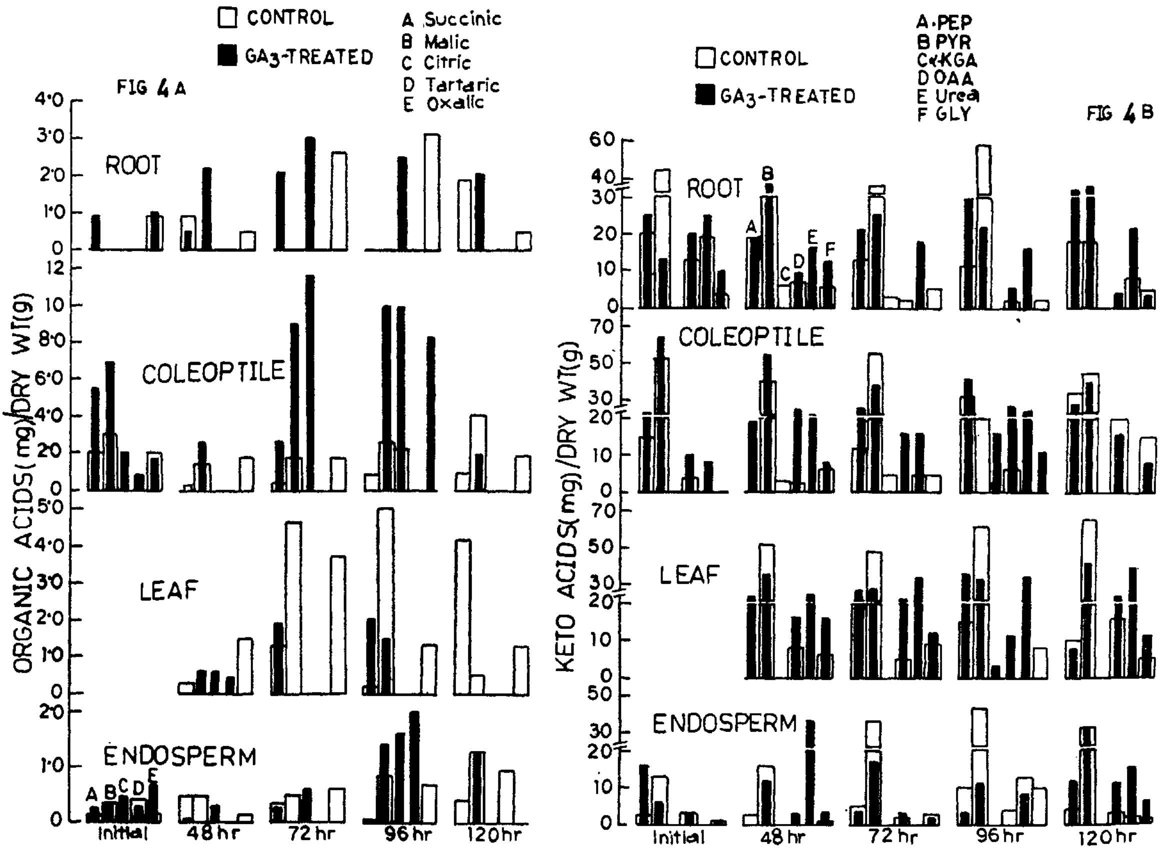

Figure 4

in transamination reactions (figure 4B). Higher amounts of keto acid hydrazones of urea in control and GA_3-treated seedling samples may account for its active role in nitrogen metabolism. Reifer and Melville (1949) confirmed urea as a widespread metabolite in higher plants and it may be formed from arginine. Seedling parts of GA_3-presoaked seeds of *P. typhoides* also exhibited comparatively higher values of the amide glutamine from respective control (figure 3). In fact, it was shown earlier that changes in various biochemical components such as protein-N, soluble-N, free amino acids and amides in light versus dark were closely parallel to those of control (light) versus GA_3-treated one (Rai & Laloraya 1965, 1967). The accumulation of amide may be due to metabolic inertness as witnessed earlier in *Bauhinia purpurea* (Mukherjee 1972, Mukherjee & Laloraya 1979).

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