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Metabolic studies in *Sorghum vulgare* Pers. and *Zea mays* L. during seedling growth

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Abstract. Protein content declined in the endosperm while an increase was noticed in root and shoot of young seedlings of *S. vulgare* and *Z. mays*. Seedlings of both plants in light had a larger pool of α-alanine, leucine-phenylalanine, glutamic acid, aspartic acid, valine, asparagine, serine-glycine and γ-aminobutyric acid than other amino acids. Tyrosine, proline, threonine and tryptophan which were recorded in light could not be detected in dark. Asparagine and glutamine increased with the seedling growth in most of the samples studied. Phosphoenolpyruvate and pyruvic acid constituted the bulk of keto acid pool while succinate, malate and citrate of organic acid pool. Protein content and citric acid level were found higher in light than in dark grown seedlings of both plants. The higher level of PEP and pyruvic acid in young shoots in light and their sharp decline at advanced stages may be due to a shift from C_3 to C_4 metabolism.

Keywords. Metabolic studies; seedling; *Sorghum vulgare*; *Zea mays*.

1. Introduction

With the development of chromatography, the role of amino acids, keto acids and organic acids in plant metabolism has received greater attention. Changes in the amino acids and organic acids (keto acids) during seed germination, seedling growth and under different photoperiods have been studied by various workers (Fowden and Webb 1955; Webb and Fowden 1955; Towers and Mortimer 1956; Krupka and Towers 1958; Mukherjee 1972, 1978; Mukherjee and Laloraya 1974, 1977). During germination, soluble protein changes have also been recorded by various workers including Koundal *et al* (1977), Balasimha *et al* (1977), Srivastava *et al* (1978) and Mukherjee and Laloraya (1979).

However, little attempt has been made to undertake the comparative biochemical studies of different seedling parts, viz., root, shoot and endosperm both in light and dark. The present work was aimed at studying the changes in soluble proteins, amino acids, keto acids and organic acids during early stages of seed germination in light and dark.

2. Materials and methods

Seeds of *S. vulgare* var. local and *Z. mays* var. Vijay composite were surface sterilized with 0·1% mercuric chloride for 1 min. After washing with sterilized distilled water, seeds were soaked in distilled water for 16 hr. Thereafter seeds were grown on paper moistened with distilled water in petri plates in dark or light (970 lux provided by fluorescent tube). Watering was done once in a day. Protein, organic acids and free amino acids were determined quantitatively in different parts of seedlings, viz., endosperm, root and shoot, after 48 hr (stage I) and 96 hr (stage II) of sowing of both light and dark grown seedlings in addition to initial soaked seeds.

Soluble protein quantity was determined with the help of Folin's reagent as described by Lowry *et al* (1951). The method described by Steward *et al* (1954) has been followed for free amino acid and organic acid extraction. Two-dimensional paper chromatography has been used for amino acid separation as described by Pal and Laloraya (1967) while single-dimensional chromatography was employed for separation of organic acids as mentioned elsewhere (Hais and Macek 1963). Keto acids have been extracted as 2,4-dinitrophenyl hydrazones (2,4-DNP's) as explained by Kaushik (1966) and Mukherjee (1972, 1974). Milligrams of different amino acids, keto acids and organic acids were quantified

Table 1. Changes in total protein and free amino acids (mg/g dry wt) in different parts of seedlings of *S. vulgare* at stages I and II in light.

Proteins/Amino acids	Soaked seeds (Endo-sperm)	Endosperm stages		Shoot axis stages		Root axis stages	
		I	II	I	II	I	II
1. Proteins	120·6	92·1	86·8	102·8	127·0	72·9	108·7
2. Amino acids							
Leucine-Phenyl-alanine	0·086	0·13	1·52	2·37	2·60	0·49	3·60
Valine	0·054	0·25	0·64	0·87	3·10	0·08	1·20
γ-Aminobutyric acid	0·064	0·78	0·60	..	1·80	0·12	1·60
Tyrosine	0·021	0·25	0·24	..	..	..	..
Proline	0·032	0·13	0·32	..	..	..	..
α-Alanine	0·25	0·94	1·52	2·12	4·40	0·49	2·20
Tryptophan	..	..	0·58	..	..	..	..
Glutamic acid	0·14	0·36	1·08	0·75	4·70	0·12	1·40
Threonine	..	0·29	0·32	0·85	1·20	..	..
Aspartic acid	0·032	0·25	0·60	1·75	4·50	0·41	1·80
Serine-Glycine	0·090	1·03	1·64	2·25	4·30	0·33	1·80
Asparagine	0·042	0·33	0·64	1·65	3·20	0·12	1·60
Glutamine	..	0·25	0·40	1·12	1·50	..	0·60
Histidine	0·054	0·28	0·40	..	1·70	..	0·60
Cysteic acid	..	..	..	..	0·38	..	..
Unidentified	0·070	..	1·60	..	0·35	..	3·20

Table 2. Changes in total protein and free amino acids (mg/g dry wt) in different parts of seedlings of *S. vulgare* at stages I and II in dark.

Proteins/Amino acids	Soaked seeds	Endosperm stages I	Endosperm stages II	Shoot axis stages I	Shoot axis stages II	Root axis stages I	Root axis stages II
1. Proteins	120·6	82·5	80·5	90·6	120·6	74·8	90·4
2. Amino acids							
Leucine-Phenylalanine	0·086	0·25	1·48	1·20	0·80	0·85	2·10
Valine	0·054	0·30	0·76	..	0·13	0·49	0·14
γ-Aminobutyric acid	0·064	0·94	0·92	1·80	0·04	0·94	..
Tyrosine	0·021	..	..	..	..	..	..
Proline	0·032	..	..	..	..	..	0·16
α-Alanine	0·25	0·78	0·28	2·40	0·41	0·85	0·56
Glutamic acid	0·14	0·45	0·28	0·85	..	0·72	0·28
Threonine	..	..	..	..	..	0·28	1·40
Arginine	..	0·39	..	..	..	..	..
Aspartic acid	0·32	0·49	..	1·65	..	2·10	0·90
Serine-Glycine	0·090	0·30	..	2·40	0·04	1·60	0·82
Asparagine	0·042	..	0·14	2·10	..	3·12	0·08
Glutamine	..	1·25	0·28	1·12	0·08	2·80	..
Histidine	0·054	0·28	..	0·80	..	0·60	2·20
Cysteic acid	..	..	..	..	..	..	1·20
Methionine	..	1·20	..	..	..	2·10	0·80
Unidentified	0·070	..	..	..	..	..	..

Table 3. Changes in total protein and free amino acids (mg/g dry wt) in different parts of seedlings of *Z. mays* at stages I and II in light.

Proteins/Amino acids	Soaked seeds	Endosperm stages I	Endosperm stages II	Shoot axis stages I	Shoot axis stages II	Root axis stages I	Root axis stages II
1. Proteins	98·27	51·5	26·2	49·7	123·7	38·5	114·5
2. Amino acids							
Leucine-Phenylalanine	0·21	0·63	0·38	0·89	1·83	2·15	1·99
Valine	0·24	0·20	0·19	0·99	3·33	2·25	2·27
γ-Aminobutyric acid	0·31	0·18	0·26	0·78	1·49	2·00	3·72
Tyrosine	0·15	0·18	0·14	0·31	1·49	0·90	0·54
Proline	0·11	0·15	0·14	..	..	0·75	0·63
α-Alanine	0·44	0·45	0·28	1·52	5·33	4·80	4·81
Tryptophan	..	0·08	0·35	..	..	..	..
Glutamic acid	0·11	0·13	0·12	0·94	2·45	1·30	1·45
Threonine	0·16	..	0·13	1·49	..	..	..
Aspartic acid	0·18	0·09	0·07	0·89	3·16	1·25	1·72
Serine-Glycine	0·39	0·08	0·14	1·94	2·99	4·20	3·90
Asparagine	0·22	0·22	0·14	0·99	4·83	1·40	1·63
Glutamine	0·32	0·14	0·14	0·45	1·99	0·95	0·98
Histidine	0·28	0·20	0·15	..	..	0·60	0·81
Cysteic acid	0·08	..	..	0·21	0·23	0·15	..
Unidentified	..	..	..	..	..	1·30	1·72

Table 4. Changes in total protein and free amino acids (mg/g dry wt) in different parts of seedlings of *Z. mays* at stages I and II in dark.

Proteins/Amino acids	Soaked seeds	Endosperm stages I	Endosperm stages II	Shoot axis stages I	Shoot axis stages II	Root axis stages I	Root axis stages II
1. Proteins	98·27	47·8	24·8	42·5	78·5	40·2	85·9
2. Amino acids							
Leucine-Phenyl-alanine	0·21	0·31	0·57	2·50	0·56	7·80	..
Valine	0·24	0·27	0·85	..	0·14	9·10	0·16
γ-Aminobutyric acid	0·31	0·04	0·16	1·80	0·28	2·15	0·14
Tyrosine	0·15	..	..	0·44	..	4·12	..
Proline	0·11	..	..	..	..	..	..
α-Alanine	0·44	0·04	0·04	0·38	0·28	8·70	0·56
Glutamic acid	0·11	..	0·04	0·49	..	6·45	0·20
Threonine	0·16	..	..	..	..	0·39	0·10
Arginine	..	..	0·04	..	..	..	..
Aspartic acid	0·18	0·04	0·80	1·65	..	2·65	0·40
Serine-Glycine	0·39	..	..	2·30	0·83	4·30	0·42
Asparagine	0·22	0·04	0·04	1·80	..	2·80	..
Glutamine	0·32	..	0·80	0·85	0·57	4·99	0·50
Histidine	0·28	..	..	1·25	..	5·20	1·20
Cysteic acid	0·08	..	..	..	..	..	0·04
Methionine	..	0·15	0·04	..	0·28	1·80	..
Unidentified	..	..	..	2·40	..	..	..

in terms of glycine, 2,4-DNP of α-ketoglutaric acid (α-KGA) and citric acid respectively, using a Spectronic-20 colorimeter.

3. Results and discussion

Changes in proteins and amino acids have been summarized in tables 1 to 4. The present investigation on *Z. mays* and *S. vulgare* shows a marked decline in the endospermic protein while it has increased in root and shoot during early phase of seedling growth up to stage II. Similar observations were noted by Oota *et al* (1953) while studying the changes in protein nitrogen content of embryonic organs of *Vigna sesquipedalis* during early germination for six days. The protein content recorded in different seedling parts was higher in light than in the dark of *S. vulgare* and *Z. mays*. It has also been shown earlier that high protein content is present in light grown seedlings while low values are exhibited by the dark grown seedlings (Rai and Laloraya 1965, 1967; Banerji and Laloraya 1967).

During the present investigation, it was found that seedlings of both plants in light were having a larger pool of α-alanine, leucine-phenylalanine, glutamic acid, aspartic acid, valine, asparagine, serine-glycine and γ-aminobutyric acid than other amino acids. Among these amino acids, leucine-phenylalanine, valine, tryptophan and threonine are essential amino acids. Most of the data in the present study in dark, specially in endosperm, indicated the disappearance of tyrosine, proline, threonine and tryptophan. Proline may be converted to glutamic acid thus increasing the pool size of this amino acid as reported elsewhere (Bewley and Black 1978).

During investigations on maize and sorghum, asparagine and glutamine are found to accumulate both in root and shoot in dark at stage I of seedling growth. Endosperm, roots and shoots in light have shown a steady increase in the amounts of asparagine and glutamine during early stage of seedling growth upto stage II in both the plants except the endosperm of *Z. mays*. However, their levels declined markedly in dark from stage I to stage II. The synthesis of asparagine and glutamine in seedlings has been reported for a long time and it has been reviewed in recent years (Miflin and Lea 1977). Total free amino acids and keto acids exhibit a sharp increase in the young seedlings of both the plants which may indicate the involvement of active transamination processes. In general, in young plant parts, a high transamination activity and a direct correlation of these processes with protein synthesis could be witnessed as reported earlier (Albaum and Cohen 1943; Cook 1959).

Phosphoenolpyruvate (PEP) and pyruvic acid constitute the bulk of the keto acid pool in both the plants (figure 1). It appears that young seedling parts,

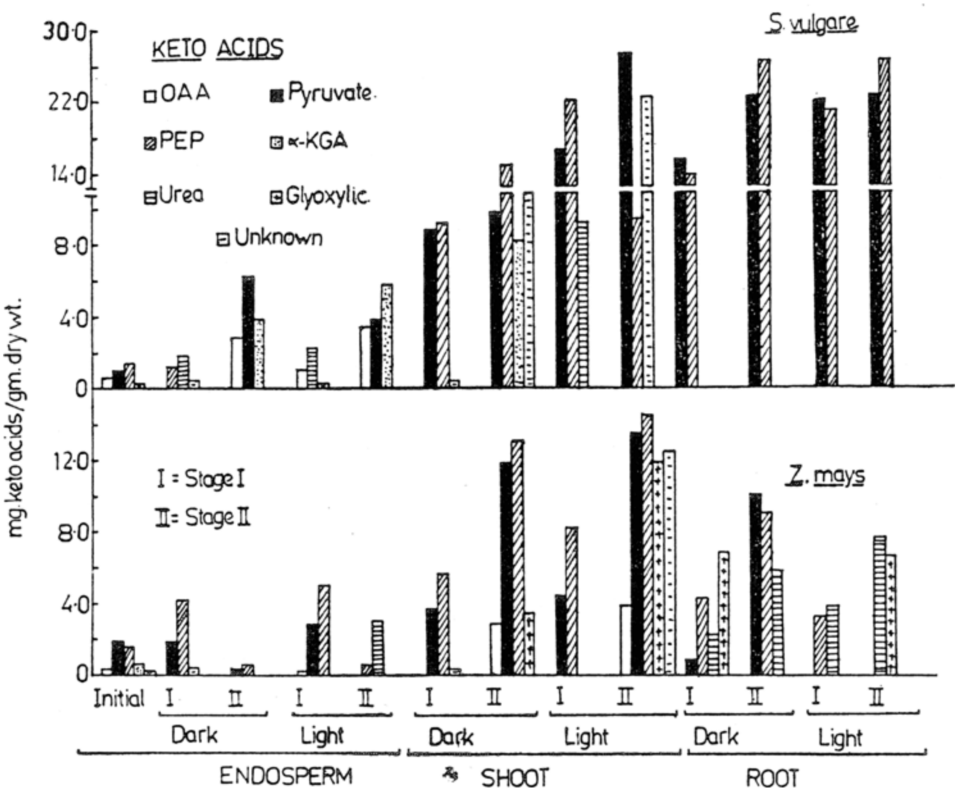

Figure 1. Changes in keto acids (mg/g dry wt) in different parts of seedlings of *S. vulgare* and *Z. mays* at stages I and II in light and dark.

including shoot, are associated with rapid synthesis of PEP. Shoots of both the plants in light exhibit comparatively higher values of pyruvic acid in light than in dark in these early stages of growth. These two keto acids show a sharp decline in the shoots at 26-day stage (table 5). Since both these plants are of C_4

Table 5. Changes in pyruvic and phosphoenol pyruvic acids (mg/g dry wt) in shoots of *S. vulgare* and *Z. mays* in light and dark.

Growth stages		*S. vulgare*		*Z. mays*	
		Pyruvic acid	PEP	Pyruvic acid	PEP
Stage I	Dark	8·90	9·20	3·70	5·87
Stage II	Dark	9·80	14·80	11·80	12·90
Stage I	Light	15·60	22·50	4·40	8·20
Stage II	Light	35·80	9·40	15·60	24·30
5-day stage	Light	5·43	6·16	..	22·20
12-day stage	Light	3·61	5·69	15·18	4·90
26-day stage	Light	4·05	..	1·85	..

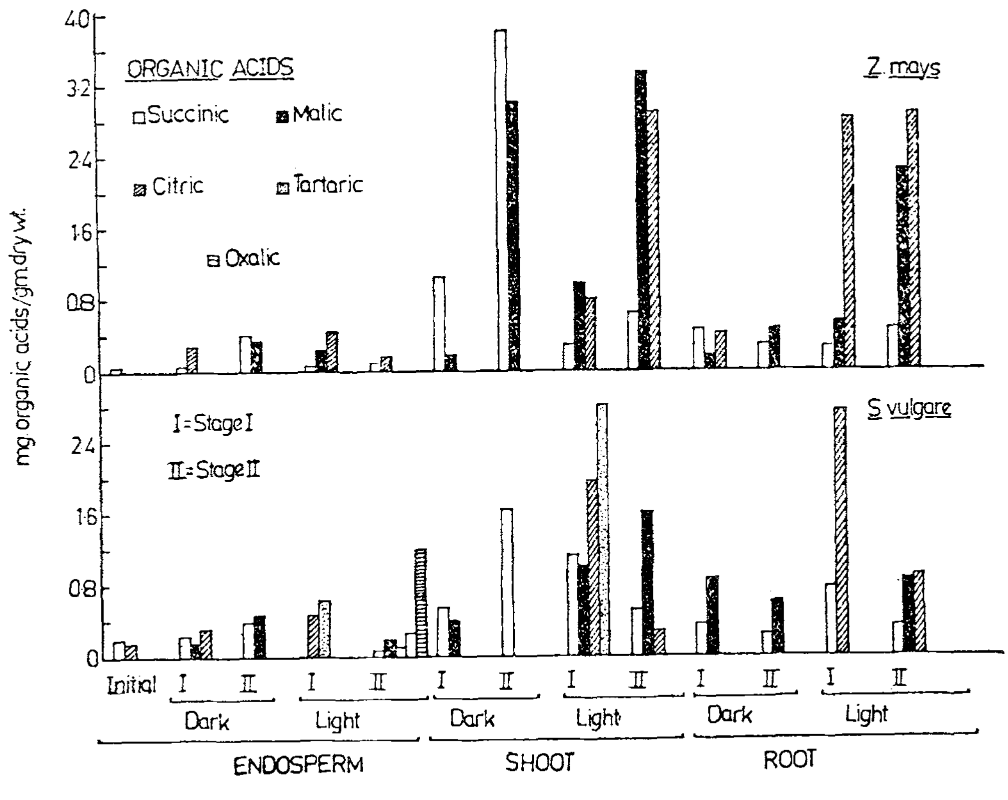

Figure 2. Changes in organic acids (mg/g dry wt) in different parts of seedlin of *S. vulgare* and *Z. mays* at stages I and II in light and dark.

type where PEP is the CO_2 acceptor, the accumulation of these keto acids may indicate sluggish CO_2 fixation in these plants at the early seedling stages. The sharp decline in PEP-concentration at later stages may indicate the onset of C_4 metabolism. It is possible that prior to this stage the plants may be of C_3 type. Indeed, it has been shown in recent years that one of these C_4 plants (*S. vulgare*) after anthesis reverts back to C_3 type (Khanna and Sinha 1973). It is likely, therefore, that the predominance of C_4 or C_3 pathway may depend upon the stages of plant growth and development. Besides these, α-KGA, an important keto acid regulating the entry of inorganic nitrogen to the organic form and which also participate in transmination reaction with different amino acids, has been traced only at certain periods of growth in light and dark and the amount is comparatively lower than PEP and pyruvate.

Paper chromatographic analysis showed that the main components of organic acids in maize and sorghum are citric, malic and succinic acids (figure 2). These acids were also recorded when Holton and Noll (1955) studied the normal distribution of organic acids in seedlings of barley, oat, white lupin and pea. The present study with *S. vulgare* and *Z. mays* also shows comparatively higher values of citric acid in light than in dark as reported earlier (Gobis 1951). Changes in malate, citrate and succinate are greater than those in tartarate and oxalate which suggest sluggish metabolism of the latter compounds.

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