

Biochemical Studies during Seedling Growth of Sweet Pea (*Lathyrus odoratus* L.)

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Changes in certain biochemical constituents were studied during the seedling growth of *L. odoratus* in light and dark. Total nitrogen, soluble proteins and % dry mass recorded a decline while total amino acid content increased from the initial values of soaked seeds in light and dark. Lower values of soluble proteins, total nitrogen and % dry mass in shoots of dark grown seedlings in comparison to those in light may indicate the slow pace of translocation in dark. The highest level of amino acids, keto acids and organic acids at 96 hr stage followed by a rapid decline at 5-day stage, point out active synthesis and utilization of these metabolites specially in growing root and shoot.

Key Words: Amino acids, Keto acids, Organic acids, *Lathyrus odoratus*

Introduction

The composition of amino acid, keto acid and organic acid pools during seed germination, seedling growth and under different photoperiods was worked out previously by many workers (Fowden & Webb 1955, Towers & Mortimer 1956, Singh & Singh 1965, Mukherjee & Laloraya 1974, 1979). Proteins and peroxidase activity during germination were also studied earlier (Balasimha et al. 1977, Koundal et al. 1977, Srivastava et al. 1978). The present work aims at finding out the possible variations in metabolic patterns of *L. odoratus* during seedling growth in light and dark. Such differences have been reported earlier (Banerji & Laloraya 1967, Rai & Laloraya 1967).

Materials and Methods

Seeds of *Lathyrus odoratus* L. (sweet pea)

were surface sterilized with 0.01% mercuric chloride solution for 1 min., thoroughly washed, soaked in distilled water for 16 hr ('Initial') and sown on absorbent paper moistened with distilled water in Petri dishes. Seedlings were grown in water culture up to 96 hr under (a) light (960 lux) and (b) complete darkness. After 96 hr, seedlings were transferred to earthen pots (45 × 30 cm) and kept under natural light. Each pot contained 2.5 kg of garden soil (pH=8.0) and 2.0 kg of dung manure. Samples for the present investigation were taken at 48 hr and 96 hr from 'initial' followed by 5-day stage (168 hr from initial). Growth as well as analytical values are average of 3 replicates.

Soluble protein was estimated using Folin's reagent (Lowry et al. 1951). The plant sample was extracted twice with

boiled ethanol, centrifuged, supernatant discarded and the residue extracted with 5% perchloric acid followed by centrifugation. The residue was taken out in a specific volume of 1N-NaOH. This solution was used for protein estimation. Total nitrogen content of the plant tissue (dry wt. basis) was determined by the common micro-Kjeldahl method.

The method of Steward et al. (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 (Hais & Macek 1963) was employed for separation of organic acids as mentioned elsewhere. Keto acids were extracted as 2,4-dinitrophenyl hydrazones (2,4-DNP's) according to Kaushik (1966). Amino acids, keto acids and organic acids were quantified (in mg) in terms of glycine, 2,4-DNP of α -ketoglutaric acid and citric acid respectively, using a spectronic-20 colorimeter. Total peroxidase activity was measured by the method of Maehly (1954) and it was expressed as OD (Absorbance) of the colour developed/10 min/50mg fr. wt. of plant tissue.

Results and Discussion

Shoots of dark grown seedlings showed lower values of % dry mass, soluble protein and total nitrogen contents than those grown in light (figure 1) indicating the failure of translocation to keep pace with the extension growth of seedlings in dark. The translocation appeared to influence also the roots of dark grown seedlings which recorded lower % dry mass value while cotyledons had relatively higher values than those in light. A marked decline was recorded in total nitrogen, soluble proteins and % dry mass of cotyledons from the 'initial' values of

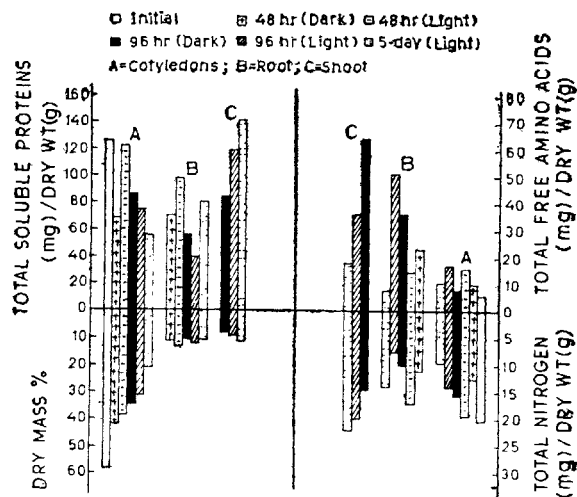


Figure 1 *L. odoratus* showing levels of total soluble proteins, total free amino acids, total nitrogen content and % dry mass at various stages of seedling growth

soaked seeds in light and dark. These values were, however, found to increase in shoots in light during the advancement of seedling growth. It is well known that reserve proteins present in the cotyledons are rapidly broken down and the soluble nitrogenous components obtained by this hydrolysis are translocated to the growing axis (Oota et al. 1953).

Samples of *L. odoratus* showed maximum amino acid concentrations in shoot of 96 hr (Dark) followed by 96 hr root in light. α -Alanine was one of the dominant amino acids witnessed in these samples (figure 3). Histidine and γ -aminobutyric acid were also present in large amounts in 96 hr stage of root while shoot could accumulate methionine and threonine. Dark shoots were unique in showing a large accumulation of total amino acids whose value slightly declined with further seedling growth as compared with light grown shoot samples. Both the amides, asparagine and glutamine,

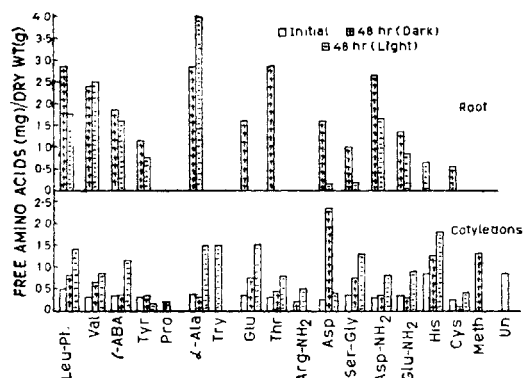


Figure 2 *L. odoratus* showing levels of free amino acids at initial and 48 hr stages of seed germination were observed in *L. odoratus* but the level of glutamine in most of the root and shoot samples was lower than that of asparagine. The cotyledons and shoots in dark had a lower and higher amount of asparagine respectively as compared to those in light (figures 2 and 3). In light, various amino acids appear to be used up for developing shoot and root while in dark, they accumulate due to their slow rate of utilization as was also shown in earlier studies (Mukherjee & Laloraya 1979).

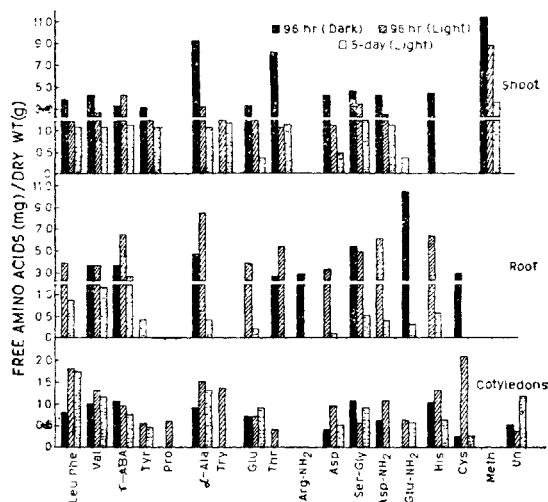


Figure 3 *L. odoratus* showing levels of free amino acids at 96 hr and 5-day stages of seedling growth

Pyruvate, phosphoenolpyruvate (PEP), oxaloacetate (OAA), glyoxylic acid and an unidentified spot (Un) were the major keto acids of *L. odoratus* (figure 4). The concentration of keto acids was much higher in light than in dark.

Citric acid, malic acid and succinic acid were the major components of organic acids in sweet pea (figure 5). These were the common organic acids noticed in the normal distribution pattern in seedlings of barley, oat, white lupin and pea (Holton & Noll 1955). Most of the samples showed higher concentrations of citric acid in light than in dark which supports, similar observations made earlier by Gobis (1951).

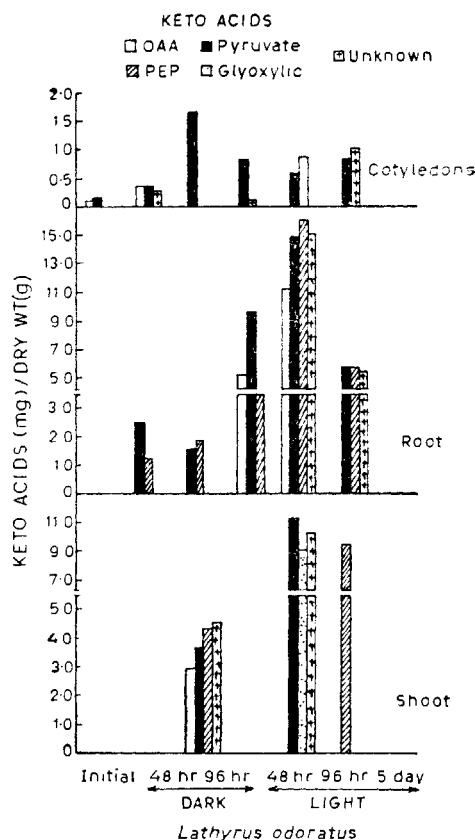


Figure 4 *L. odoratus* showing levels of keto acids at various stages of seedling growth

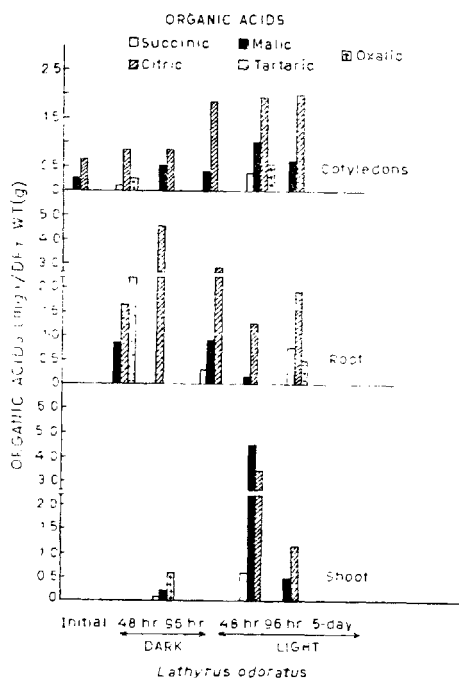


Figure 5 *L. odoratus* showing levels of organic acids at various stages of seedling growth

The maximum level of total amino acids, keto acids and organic acids in most of the seedling samples at 96 hr stage followed by a marked decline in their contents at 5-day stage may indicate active synthesis followed by their rapid utilization specially in the roots and shoots in growth reactions.

The peroxidase activity increased in shoot and root with the seedling growth both in light and dark (table 1). The increase in peroxidase activity with the growth of young seedlings had already been observed by various workers (Balasimha et al. 1977, Koundal et al. 1977, Srivastava et al. 1978).

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Table 1 Changes in the peroxidase activity in light and dark of different seedling parts of *L. odoratus* at early growth stages

Seedling parts	Growth stages	OD increase/ 10 min/ 50 mg f.w	
Cotyledons	Initial	1.00	
	48 hr } Dark	0.35	
		0.32	
	48 hr } Light	0.45	
		0.43	
	Shoot	96 hr } Dark	1.24
1.74			
96 hr } Light		1.68	
		1.92	
Root		48 hr } Dark	0.92
			0.92
	48 hr } Light	0.43	
		0.53	

*Shoot axis did not emerge out at 48 hr stage

The higher peroxidase activity of shoot in light than in dark may be due to enhancement in protein synthesis which finds support from similar studies by Graham et al. (1970).

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