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Protein and Ascorbic Acid Changes in Shoot of Certain C_3 and C_4 Plants in Relation to Light and Dark

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Changes in soluble protein and ascorbic acid contents have been analysed at various growth stages in shoot of *Hordeum vulgare* L., *Lathyrus odoratus* L. (C_3 plants); *Sorghum vulgare* Pers. and *Zea mays* L. (C_4 plants). The quantity of ascorbic acid increased with the growth of seedlings and significantly higher concentration of this metabolite as well as protein in the shoot at flowering stage in comparison to 28-days-old vegetative stage in all the plants may indicates increased requirement for the highly-activated stage of differentiation. The amounts of protein and ascorbic acid were also found significantly higher in light than in dark-grown seedlings. Interestingly, loss of ascorbic acid in C_4 plants was significantly less than that in C_3 plants maintained in dark. Moreover, C_4 plants were found more efficient in regaining the lost ascorbic acid level on exposure to light in comparison to the C_3 plants.

Key Words: Protein, Ascorbic acid, C_3 plants, C_4 plants

Introduction

The role of ascorbic acid in germination, seedling growth, differentiation, flowering and drought resistance has been extensively studied and reviewed (Mapson 1958, Isherwood & Mapson 1962, Chinoy et al. 1971). Ascorbic acid metabolism in relation to proteins and nucleic acids was also investigated with the onset of flowering (Chinoy 1962, 1964, Chinoy et al. 1973). Since, ascorbic acid is known to have diverse functions (Mapson 1958); the present work was chiefly aimed to compare certain C_3 and C_4 plants for the total soluble protein and ascorbic acid contents of shoot at different growth stages in light and dark.

Materials and Methods

Seeds of two C_3 plants viz., *Hordeum vulgare* L., *Lathyrus odoratus* L. and two C_4 plants viz., *Sorghum vulgare* Pers. and *Zea mays* L. were selected and surface sterilized with 0.01% mercuric chloride for 1 min. These seeds, after 16 hr of water soaking (designated as 'initial' stage) were sown on absorbent paper moistened with

distilled water in Petridishes, For analysis, the seedlings were harvested after 48 and 96 hr from the 'initial' stage. The seedlings were grown in water culture upto 96 hr under (i) a fluorescent tube light (970 lux) and (ii) complete darkness. After 96 hr stage, the seedlings were transferred in earthen pots (45 × 30 cm), each containing 2.5 kg of garden soil (pH = 7.8) and 2 kg of dung manure. The pots were kept continuously in natural light and watered every third day. Four replicates of samples from the potted plants were taken 7, 14 and 28 days, after the 'initial' stage, and lastly at the initiation of flowering. At 28-days stage, plants were kept in dark for 48 hr and then exposed to natural light for 0.5, 1.0, 2.0 and 4.0 hr. Shoot (3rd nodal leaf from apex) samples were taken for analysis at these stages.

The protein estimation was done following Lowry et al. (1951) using Folin's reagent. The ascorbic acid was extracted following Bessey and King (1933). Five-hundred mg of plant tissue was crushed in 25 ml of 5% metaphosphoric acid and digested on a water bath for 10 min. The extract was cooled, filtered and final volume was raised to a specific volume. Seven ml of this filtrate was titrated against 2, 6-dichlorophenol indophenol solution until a distinct pink-rose colour persisted at least for 5 sec.

The ascorbic acid content of the extract was calculated by the formula (AOAC 1970):

$$\text{Ascorbic acid (mg)/100 g tissue} = I \times S \times D/A \times 100/W$$

where I = Indophenol reagent used in titration (ml); S = Ascorbic acid reacting with 1 ml of the reagent (mg); D = volume of the extract (ml); A = aliquot titrated (ml); W = weight of the sample (g)

Results and Discussion

Protein and ascorbic acid changes at various growth stages in all the plants have been presented in table 1. The data at these stages were statistically analysed using 't' test and the results were found to be statistically significant at $P > 0.05$

The quantity of ascorbic acid in the shoot increased with the growth of seedlings upto flowering stage except a decline at 28-days-old stage in the C_4 plants. However, the decrease was significant only in *Z. mays*. The shoot at flowering stage had significantly higher concentration of protein as well as of ascorbic acid than in the vegetative shoot of 28-days-old stage in all the plants. The production and utilization of ascorbic acid at higher levels during the period of reproductive differentiation produce a highly-activated metabolic state in the cell due to which proteins and enzymes are synthesized at faster rates (Chinoy 1962, 1964).

A perusal of results showed that the amount of protein and ascorbic acid was significantly higher in light-than in the dark-grown seedlings in almost all the comparable samples studied. It has been established earlier also that protein content is higher in light-grown and low in dark-grown seedlings (Rai & Laloraya 1965, 1967, Mukherjee & Laloraya 1979).

The amount of ascorbic acid as well as of protein decreased in all the plants when kept in dark for 48 hr at 28-days-old stage. However, the decline in ascorbic acid content was more prominent in C_3 plants than in C_4 plants. Interestingly, ascorbic acid content in C_4 plants was either equal or more than the initial level of 28-days-old stage, when the final data of 4.0 hr-light stage was compared, C_3 plants, however, failed to regain it. On comparing the levels of ascorbic acid at 48 hr dark and 4.0 hr light stages, the maximum increase in light could be noticed in *Z. mays*

Table 1 *Mean values of total soluble proteins (mg/g of dry wt) and ascorbic acid (mg/100 g fr. wt) of shoot at various growth stages in certain C_3 and C_4 plants*

Growth stages		Protein				Ascorbic acid			
		H. vulgare	*L. odoratus*	*S. vulgare*	*Z. mays*	*H. vulgare*	*L. odoratus*	*S. vulgare*	*Z. mays*
		C_3 plants		C_4 plants		C_3 plants		C_4 plants	
*48 hr	Dark	36.00	—	90.64	42.56	44.00	—	28.40	32.80
96 hr	Dark	24.90	85.31	120.60	78.50	71.00	106.50	47.85	51.75
48 hr	Light	79.73	—	102.80	49.71	51.50	—	35.60	50.44
96 hr	Light	74.30	119.97	127.00	123.70	80.00	142.00	56.76	64.55
7 days	Light	149.95	141.20	66.51	90.65	85.00	148.00	58.00	66.10
14 days	Light	81.90	139.55	45.50	34.99	95.00	149.50	62.55	70.00
28 days	Light	56.34	106.70	28.14	44.80	98.00	152.00	65.60	52.78
(i) 48 hr	Dark	52.10	95.33	26.18	40.40	65.00	98.50	63.50	42.56
(ii) 0.5 hr	Light	60.50	124.66	24.80	38.70	68.50	102.00	60.46	54.50
(iii) 1.0 hr	Light	109.00	103.88	25.50	43.20	70.50	101.00	64.55	55.45
(iv) 2.0 hr	Light	96.95	97.96	24.40	35.47	74.00	120.50	68.00	57.42
(v) 4.0 hr	Light	87.70	76.40	23.00	32.85	75.00	126.00	70.60	60.55
Flowering stage	Light	119.50	125.50	65.80	86.60	111.50	198.50	74.65	77.80

* Shoot did not appear at 48 hr stage in *L. odoratus*

followed by *L. odoratus*, *H. vulgare* and *S. vulgare*. Overall results of ascorbic acid changes may indicate that its minimum loss in C_4 plants than in C_3 plants is equal or more effective than its synthesis in light in regaining the level of 28 days at 4.0 hr-light stage. Chinoy (1966) had pointed out earlier that during increased production of ascorbic acid, the carbon cycle also contributed substantially and the process of photophosphorylation and oxidative phosphorylation were further energized by the flow of electrons from monodehydroascorbic acid (MDHA) -the product of ascorbic acid oxidation. Thus, it appears that to meet the higher pool of ATP in C_4 plants, ascorbic acid is being synthesized rapidly and it gets oxidized to a powerful reducing agent-MDHA, which further activated the phosphorylation processes.

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