CHANGE IN THE PREDOMINANCE FROM C_4 TO C_3 PATHWAY FOLLOWING ANTHESIS IN SORGHUM

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Sorghum and Pennisetum species are known to have predominantly C_4 pathway. This pathway is associated with several other characteristics. These conclusions are based on studies confined largely to seedlings. A developmental study of PEP carboxylase and RuDP carboxylase in Sorghum bicolor and Pennisetum typhoides confirmed in seedlings the predominance of PEP carboxylase, high malate: 3-phorophoglycerate ratio and 'Krantz' anatomy. However, after flowering, RuDP carboxylase was predominant in the leaves of both Sorghum and Pennisetum. This observation was associated with higher 3-phosphoglycerate:malate ratio following $^{14}CO_2$ fixation. The anatomy of the leaf remained unchanged and so was the chlorophyll a:b ratio. This change in system coincided with a slight fall in mean daily temperature. But in wheat RuDP carboxylase remained the predominant enzyme in spite of the rising mean daily temperature. Therefore, the change from C_4 to C_3 appears to be related more to the developmental stages.

The characteristics of C_4 and C_3 plants now appear to be well defined (2). The C_4 plants have C_4 acids as the major early products, predominance of PEP carboxylase and relatively higher chlorophyll a/b ratio in addition to several other characteristics. In contrast the C_3 plants have 3-phosphoglyceric acid as the early product, predominance of RuDP carboxylase and low chlorophyll a:b ratio.

We here report the developmental changes in these enzymes in Sorghum vulgare, Pennisetum typhoides and Triticum aestivum. First experiments on sorghum were conducted in 1971. They were repeated in 1972. Plants were grown under field conditions with the recommended agronomic practices. The activity of RuDP carboxylase was determined as described by Bjorkman & Gauhl (1969) and Neals, Treharne & Wareing (1971). In 1971, homogenates were not dialysed but in 1972 extracts were dialysed against the extraction medium.

The same extracts were used for determining the PEP carboxylase activity following the method of Maruyama & Lane (1964) and Khanna & Sinha (1972). This method has the advantage of avoiding the use of endogenous glutamate-oxaloacetate transaminase which can affect determinations while comparing genotypes and developmental stages. The data reported here is of 1972.

Table 1: RuDP carboxylase and PEP carboxylase activity at different developmental stages in sorghum, Pennisetum and Wheat; μmoles CO_2/g.f.w./hr.

	Seedling		Preanthesis		Anthesis		Anthesis + 10 days	
	RuDP	PEP	RuDP	PEP	RuDP	PEP	RuDP	PEP
Sorghum	44.0	302.0	302.0	882.0	479.0	297.0	618.0	144.0
Pennisetum	-	-	554.0	232.0	305.0	349.0	320.0	130.0
Wheat	543.0	34.0	1481.0	417.0	4560.0	159.0	2640.0	259.0

In sorghum, PEP carboxylase activity was higher than RuDP carboxylase in the seedling and preanthesis stages but RuDP carboxylase was predominant following anthesis (Table 1). There was a distinct change in $\frac{\text{RuDP carboxylase}}{\text{PEP carboxylase}}$ ratios in favour of RuDP carboxylase (Table 2). This behaviour was observed in other cultivars of sorghum also. A similar increase in RuDP carboxylase : PEP carboxylase ratio was seen in Pennisetum. The increase in RuDP carboxylase following anthesis was very sharp.

We examined the ratio of chlorophyll a:b and also the products of $^{14}CO_2$ fixation for one minute in full sunlight in sorghum vulgare during seedling and post anthesis stages. The soluble ethanol extracts were run in n-Butanol : acetic acid : water (4:1:5 v/v/v) and the ratio between malate and phosphoglycerate was determined (Table 3). There was almost no change in chlorophyll a:b ratio but the malate:PGA ratio was 3:1 in seedlings and 1:5 during post-anthesis. These results, therefore,

Table 2: $\frac{\text{RuDP carboxylase}}{\text{PEP carboxylase}}$ ratio at different developmental stages in sorghum, Pennsetum and wheat

	Seedling	Preanthesis	Anthesis	Anthesis + 10 days
Sorghum	1:6.8	1:2.9	1.6:1	4.2:1
Pennisetum	-	2.3:1	1:1.1	2.5:1
Wheat	15.9:1	3.6:1	28.0:1	10.0:1

indicate that in seedlings when PEP carboxylase was predominant the early product was malate as expected. With the change of predominance of PEP carboxylase to RuDP carboxylase, the early product was also 3-phosphoglycaric acid.

Table 3: Chlorophyll a:b ratio and malate/PGA ratio at the seedling and post-anthesis stages in sorghum

Stage	Chlorophyll a:b	Malate:PGA*
Seedling	2.77	3:1
Post-anthesis	2.70	1:5

The question arises whether this change is because of the developmental stage or the environmental factors, particularly the temperature. The plants of sorghum experienced a mean daily temperature of 29.7°C and 27.0°C at the seedling and post-anthesis stages respectively. This apparantly is not a significant temperature difference. Nonetheless one cannot ignore the possibility of temperature influencing the predominance of enzymes. However, in the present instance, the change in the predominance of PEP carboxylase to RuDP carboxylase was not associated with a change in leaf anatomy which was also examined. A similar question in relation to CO_2 fixation products and chloroplast structure has been raised by Laetsch & Kortschak (1972). Therefore, there is a need to re-examine

the functional and anatomical relationship between enzymes, bundle sheath and chloroplast.

In wheat, a C_3 plant, also there was stimulation in RuDP carboxylase of the flag leaf at the time of anthesis although the environmental temperatures were rising (Table 1). There was no change in the chlorophyll a/b ratio in leaves from seedling to post-anthesis stages. We, therefore, feel that the change from PEP carboxylase predominance to RuDP carboxylase is more likely due to the developmental stages rather than the environmental factors including temperature. This would make the classification of C_4 and C_3 plants arbitrary as suggested by Latzko et al (5).

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