# ACTIVITY OF PEP-CARBOXYLASE AND TWO GLYCOLATE PATHWAY ENZYMES IN $C_3$ AND $C_4$ PLANTS GROWN AT TWO ALTITUDES

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ABSTRACT

Leaves of two $C_4$, two non-leguminous and two leguminous $C_3$ species grown at 1700 and 12,000 ft altitudes were analysed for the activity of PEP-carboxylase, glycolate oxidase and NADH-hydroxypyruvate reductase. The results revealed that the plants belonging to same photosynthetic group differ in the pattern of activity of individual enzymes when grown in two contrasting environments. While the activity of PEP-carboxylase in *Eleusine coracana* decreased when grown at high altitude, in *Amaranthus paniculatus*, it decreased under the same environment. NADH-hydroxypyruvate reductase also showed similar differential pattern of activity at two altitudes. However, the activity of glycolate oxidase showed identical pattern of changes in plants belonging to same photosynthetic group with three fold reduction in $C_4$ when these were grown at high altitude. In leguminous $C_3$ plants the activity of this enzyme increased at higher altitudes as compared to that in the same plant grown at lower altitudes. Significance of the study in finding out the adaptive potential of plants at different altitudes has been discussed.

## INTRODUCTION

HIGHER plants have been classified as $C_3$, $C_4$ and CAM types depending on the path of carbon during photosynthesis. It is considered that plants with differential photosynthetic pathways have adaptive significance and in terms of performance under stress, $C_4$ plants sustain higher photosynthetic rates than $C_3$ plants. Low $O_2$ and high $CO_2$ is mandatory for optimal rates of photosynthesis in $C_3$ plants, whereas $CO_2$ fixation in $C_4$ plants is insensitive to $O_2$ concentration[1]. The difference in the carbon flux through glycolate pathway, which has gluconeogenic function in plants, is considered to be the cause of variable performance of $C_3$ and $C_4$ plants under diverse climatic conditions. These conclusions are based on the study of change in the level of activity of different photosynthetic and photorespiratory enzymes and metabolites in plants when grown under different environmental conditions[2]. Unfortunately, in these studies, only one environmental factor has been changed at a time and, therefore, the conclusion arrived at in these studies have proved to be more of academic interest and fail to explain fully the mechanism of adaptation in plants with different photosynthetic pathways, under natural environmental conditions, where many factors change simultaneously. Keeping this in view, the present study was undertaken to understand the changes in the level of some important enzymes in $C_3$ and $C_4$ plants when grown at two contrasting altitudes. The results are presented in this communication.

## MATERIALS AND METHODS

*Sample collection*: Top young leaves of *Eleusine coracana*, *Amaranthus paniculatus* ($C_4$-species), *Fagopyrum esculentum*, *Plantago major*, *Glysine soja* and *Dolichos lablab* ($C_3$-species) were collected from the plants of same age grown on the soil from the same source at 1700 ft (Srinagar) and 12,000 ft (Tungnath) altitudes for 60 days. As the two experimental sites were 100 km apart, the leaf samples were collected and carried in ice to the laboratory where the homogenates were prepared with the help of chilled mortar and pestle using chilled 0·25 *M* sucrose in 0·1 *M* K-phosphate buffer of pH 7·5. The time range between leaf collection and preparation of homogenate was, therefore, about 6 hours during which the samples were in ice. Homogenates were passed through two layers of muslin and the preparations were spun for 10 min at 1500 × g in REMI centrifuge model T8. The supernatants were used directly for enzyme assay.

*Enzyme assay*: The PEP-carboxylase was assayed as described by Bhagwat and Sane[3]. The enzyme activity was measured by linked assay using commercial malate dehydrogenase (MDH, EC 1·1·1·37). The reaction mixture (3·0 ml) consisted of 0·5 ml of 0·1 *M* Tris-HCl buffer pH 7·8, 0·1 ml of $1{\cdot}25 \times 10^{-3}$ *M* NADH, 0·3 ml of 0·05 *M* $NaHCO_3$, 10 IU MDH, 0·1 ml of 0·04 *M* PEP. The reaction was started by the addition of PEP. The change in optical density at 340 nm was recorded for 5 min at one min interval at the EC spectrophotometer.

One unit of enzyme activity is expressed as the change in 0·1 OD which is equivalent to 0·161 $\mu$ mole of NADH oxidised.

The glycolate oxidase activity was determined according to Hess and Tolbert[4] based on the increase in absorbance at 324 nm due to the formation of glyoxylate phenylhydrazone[5]. The assay system comprised of 0·5 ml of 0·2 *M* K-phosphate buffer pH 8·0, 0·1 ml of 0·1 *M* phenylhydrazine-HCl pH 6·8, 0·1 ml of 0·2 *M* sodium glycolate enzyme and water to the final volume of 3·0 ml. The reaction was started by adding enzyme and change in OD per min at 324 nm

were recorded for 5 min. A control was simultaneously run without adding substrate.

One unit of enzyme activity is expressed as the amount which will form one $\mu$ mole of glyoxylate phenylhydrazone in one min in the test. The molar extinction coefficient $17 \times 10^3\, cm^{-1}\ M^{-1}$ at 324 nm has been used for calculating the concentration of glyoxylate phenylhydrazone.

The assay for NADH hydroxypyruvate reductase was conducted as described by Tolbert *et al.*[6]. The assay system comprised of 1·0 ml of 0·2 *M* K-phosphate buffer pH 6·5, 0·1 ml of $1{\cdot}25 \times 10^{-3}$ *M* NADH, 0·2 ml of 0·4 *M* sodium glyoxylate or 0·1 ml of 0·1 *M* $\beta$-hydroxypyruvate, Na salt, enzyme and water in a total volume of 3·0 ml. Reaction was started by adding enzyme and the decrease in OD at 340 nm at room temperature was recorded every min. up to 5 min. The control was run with all the above components except substrate (glyoxylate or $\beta$-hydroxypyruvate).

The enzymatic activity is expressed in terms of fresh weight of the plant material or on the basis of soluble protein (specific activity) for which protein was estimated by the method of Lowry *et al.*[7]. Two replicates were run for each assay and identical results were found in both the replicates.

## Results

The activity of PEP-carboxylase in leaf extracts of plants of $C_3$ and $C_4$ species grown at low (1700 ft) and high (12,000 ft) altitudes are shown in Table I. The activity on leaf fresh weight basis in $C_4$ plants (*Eleusine coracana* and *Amaranthus paniculatus*) was 8–14 fold higher than that in $C_3$ plants (*Plantago major, Fagopyrum esculentum, Glycine soja* and *Dolichos lablab*). But for *Amaranthus paniculatus* and *Glycine soja* in rest of species the activity decreased considerably with increase in altitude.

When the specific activity (activity per mg soluble protein) was compared at two altitudes, there was three fold decrease with increase in altitude from 1700 to 12,000 ft in *Eleusine coracana* but approximately 1·5 fold increase in *Amaranthus paniculatus*. In leguminous species, *Glycine soja*, there was three fold increase in specific activity when these were grown at high altitudes.

The level of activity of photorespiratory enzymes, glycolate oxidase, and NADH hydroxypyruvate reductase in plants grown at two altitudes (1700 ft and 12,000 ft) is shown in Table II.

Among the $C_4$ plants, there was approximately three fold decrease in glycolate oxidase activity per unit leaf fresh weight at high altitude (12,000 ft), whereas in non-leguminous $C_3$ species it was unaffected and there was 1·5 to 2 0 fold increase in the activity of in leguminous plants. Similar trends but marked differences were recorded in specific activity of this enzyme with altitude. In contrast to this the activity

TABLE I

*Activity of PEP-carboxylase ($\mu$m mole/min/g fresh wt.) in whole leaves of plants grown at 1700 ft. (a) and 12,000 ft. (b) altitudes*

| Plant species | PEP-carboxylase* (a) | PEP-carboxylase* (b) | Ratio of specific activity at 1700 ft. to 12,000 ft. |
|---|---|---|---|
| 1. *Eleusine coracana* | 8·37 (0·167) | 2·58 (0 052) | 1 : 0·32 |
| 2. *Amaranthus paniculatus* | 10·30 (0·304) | 14·49 (0·483) | 1 : 1·56 |
| 3. *Fagopyrum esculentum* | 1·29 (0·121) | 8 (0) | 1 : 0 |
| 4. *Plantago major* | 0·64 (0·050) | 0 (0) | 1 : 0 |
| 5. *Glycine soja* | 0·64 (0·030) | 1·29 (0·090) | 1 : 3·00 |
| 6. *Dolichos lablab* | 1·93 (0·100) | 1·29 (0·113) | 1 : 1·09 |

* Figures in the parentheses indicate the specific activity (activity/mg soluble protein).

TABLE II

*Activity ($\mu$ mole/min/g fresh wt. of tissue) of glycolate oxidase and NADH hydroxypyruvate reductase in whole leaves of plants grown at 1700 ft (a) and 12,000 ft (b) altitudes*

| Plant species | Glycolate oxidase (a) | Glycolate oxidase (b) | NADH hydroxypyruvate reductase (a) | NADH hydroxypyruvate reductase (b) |
|---|---|---|---|---|
| 1. *Eleusine coracana* | 0·490 (0 009) | 0·110 (0·002) | 10·61 (0·210) | 9·80 (0·200) |
| 2. *Amaranthus paniculatus* | 0·490 (0·014) | 0 140 (0 005) | 13·86 (0·410) | 15·19 (0·510) |
| 3. *Fagopyrum esculentum* | 0 353 (0·034) | 0·353 (0 028) | 1·29 (0·121) | 1·29 (0 100) |
| 4. *Plantago major* | 0·353 (0·031) | 0·353 (0·025) | 1·29 (0·110) | 1·93 (0·140) |
| 5. *Glycine soja* | 0·282 (0 015) | 0·353 (0 025) | 7·73 (0 410) | 7·73 (0·550) |
| 6. *Dolichos lablab* | 0·424 (0·022) | 0·494 (0·043) | 6·44 (0 350) | 8·37 (0·740) |

Figures in the parentheses indicate the specific activity (activity/mg soluble protein).

of NADH hydroxy pyruvate reductase increases with altitude only in *Amaranthus paniculatus*, *Plantago major* and *Dolichos lablab*. The specific activity of this enzyme in most of the plant species studied here increased at higher altitude but for a decrease in *Fagopyrum esculentum*.

It is interesting to note that the affinity of this enzyme to its alternate substrate glyoxylate was 2 fold higher in *Elecusine coracana* when grown at 12000 ft and that in *Plantago major* it was equal to that for hydroxypyruvate. *Dolichos lablab*, when grown at lower altitude showed lower affinity to hydroxy-pyruvate than to glyoxylate (Table III).

TABLE III

*Ratio of affinity of NADH hydroxypyruvate reductase to glyoxylate and hydroxypyruvate on specific activity at two different altitudes in different plant species. The specific activity in presence of glyoxylate as a substrate is shown one in the table*

| Plant species | Altitudes | |
|---|---|---|
| | 1,700 ft. | 12,000 ft. |
| 1. *Eleusine coracana* | 1 : 2·07 | 1 : 0·618 |
| 2. *Amaranthus paniculatus* | 1 : 3·88 | 1 : 3·740 |
| 3. *Fagopyrum esculentum* | 1 : 1·77 | 1 : 1·740 |
| 4. *Plantago major* | 1 : 1·00 | 1 : 1·000 |
| 5. *Glycine soja* | 1 : 1·29 | 1 : 2·390 |
| 6. *Dolichos lablab* | 1 : 0·91 | 1 : 1·620 |

## DISCUSSION

Present study brings out the fact that while PEP carboxylase activity in one $C_4$ plant, *Eleusine coracana* when grown at high altitude, was considerably reduced in another $C_4$ plant, *Amaranthus paniculatus* the activity of the same enzyme increased 1·54 fold under similar condition. Similarly, the affinity of NADH hydroxypyruvate reductase to its alternate substrate glyoxylate was also affected differently in these two plants at two contrasting altitudes. These results, therefore, indicate that the same enzyme system present in two different plant species belonging to same photosynthetic group reacts differently to identical environmental complex.

The specific activity of glycolate oxidase in $C_4$ plants was only one-third to that in $C_3$ species when grown at lower altitudes. These results are therefore consistent with the earlier findings reviewed by Jackson and Volk[1] and Schnarrenberger and Fock[2]. However, on fresh weight basis not much differences were recorded in glycolate oxidase activity in $C_3$ and $C_4$ plants. The interesting point which emerged in the present study is in the magnitude of change in the activity of this enzyme at two altitudes in these photosynthetic groups. While in $C_4$ plants, the activity was highly inhibited, less in non-leguminous $C_3$, in leguminous $C_3$ plants there was about 1·5 to 2·0 fold increase in the activity when these were grown at high altitudes indicating the differential response of the same enzyme from three groups of plants. The glycolate metabolism in leguminous $C_3$ plants seems to operate at a faster rate at high altitudes as compared to that at lower altitudes. In $C_4$ plants, on the other hand, it is reduced considerably. A shift in the affinity of NADH hydroxypyruvate reductase to glyoxylate in *Eleusine coracana* at higher altitude would help in plant in maintaining the equilibrium on glycolate side and reduced photorespiration. Under the environmental conditions where $CO_2$ partial pressure is considerably low a major source of $CO_2$ fixation into oxaloacetate, which provides carbon skeleton for nitrogen fixation in nodules, through PEP-carboxylase is only photorespiration. Probably to meet this $CO_2$ requirement, in leguminous plants glycolate pathway and consequently photorespiratory metabolism operates at a faster rate at higher altitudes. Further work is in progress to assess the validity of this assumption.

Although the rate of photosynthetic $CO_2$ fixation and the extent of glycolate metabolism is regulated basically by competition between $CO_2$ and $O_2$ concentration in the environment, how the magnitude of these two processes is affected in plants grown at high altitudes where multifactorial envionmental changes are involved needs an extensive study. The adaptability to high altitudes may be a function of balance between different metabolic pathways rather than simply the difference in photosynthetic pathways. Only a detailed analysis of different enzymatic regulations in various plant systems at contrasting altitudes, therefore, might lead to better understanding of the mechanism of adaptation.

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