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# EFFECT OF WATERLOGGING ON METABOLIC CONSTITUENTS IN MAIZE

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## SUMMARY

**Thirty days old seedlings of four varieties of maize (*Zea mays* L.), viz. Laxmi, Suwan, Desla Yellow and Desla Brown were subjected to waterlogging treatment for 24 and 72 h with a view to assess, the metabolic modification. Post-hypoxic studies were also made after placing the waterlogged plants to normal condition for 48 and 96 h. The results obtained on different parameters like chlorophyll, carotenoids, free aminoacids, pyruvate, glycolic acid, reducing sugar and inorganic phosphorous contents revealed that Suwan and Desla Brown were more tolerant to waterlogging than Laxmi and Desla Yellow.**

***Key words*: Chlorophyll, maize, metabolites, waterlogging.**

## INTRODUCTION

Higher plants being aerobes, require a continuous supply of oxygen from the environment. The deficiency of oxygen even for a short period brings about considerable metabolic disturbances in most higher plants followed by the destruction of fine cell organization leading eventually to death of plants. It is known, however, both from scientific and common observations that for many species of higher plants even prolonged anaerobic environmental conditions are not necessarily lethal. A great number of wild species are known to grow well on waterlogged and even marshy soil which are absolutely unsuitable for many species, especially for the agricultural plants. However, in contrast among cultivated plants, a most remarkable example is rice, which is normally grown on regularly flooded anaerobic soils. The two main strategies utilized for the adaptation of higher plants to an anaerobic environment are either to (i) avoid anaerobiosis by transporting oxygen from the aerated part to those localized in oxygen depleted medium or (ii) to get adapted on the molecular level through a substantial modification of cell metabolism at hypoxia or anoxia.

In the first case, plant cells though localized in an anaerobic medium, show no resistance to anoxia. Hence, it may be suggested that such plants should be called apparently resistant to anoxia (Vartapetian 1978). As a matter of fact, it has been experimentally shown that root cells of such plants (hydrophytes or hygrophytes) growing on anaerobic soils not only fail to exhibit higher resistance to anoxia as compared to plants growing on well aerated soils (mesophytes) but are indeed more sensitive to $O_2$ deficiency than mesophytes (Vartapetian 1973, 1982, Webb and Armstrong 1983). It has also been shown that, with regard to root carbohydrates metabolism under anaerobic conditions, plants growing in a waterlogged environment are quite similar to those growing on dry aerobic soil (Smith and apRees 1979, apRees and Wilson 1984). On the whole, it has been shown that the cells and tissues of the first category plants lack molecular mechanism for adaptation to anoxia.

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The second category of plants has been called "truly resistant to anoxia" (Vartapetian 1978). Such plants are not only capable of surviving long term anoxia with the cell ultrastructure remaining intact, but grow vigorously with no oxygen in the medium. This category includes in particular, rhizomatous shoot in some wild species (Barclay and Crawford 1982) and germinating seed of rice (Opik 1973). Most other species like maize is intolerant to an oxygen free medium and is therefore, not resistant to anoxia lacking the adaptation mechanisms at a molecular and organism level or possessing them to a far less extent than the resistant plants. The third category plants show, in absence of oxygen, disorganization of metabolism and degradation of cell ultrastructure, which is followed by the death of whole organism. The imbibing maize seeds and seedlings used in the present study, belong to the third category as described above.

## MATERIALS AND METHODS

The experimental materials consisted of four maize (*Zea mays* L.) varieties, viz. Laxmi, Suwan, Desla Yellow and Desla Brown. Laxmi and Suwan varieties are composite varieties developed by Rajendra Agricultural University, Pusa, Bihar whereas Desla Yellow and Desla Brown are local varieties grown in Naugachia subdivision of Bhagalpur district of Bihar. Laxmi and Suwan varieties are grown in whole of Bihar whereas Desla Yellow and Desla Brown are grown at the banks of Ganga and Koshi where maize plants are exposed frequently to waterlogged condition due to flooding as well as during heavy rains. These maize varieties (Desla Yellow, Desla Brown) are commonly grown by local farmers in the Kharif seasons. Certified seeds of Laxmi and Suwan were obtained from Maize Breeding Section, Dholi, a unit of All India Co-ordinated Maize Research Project whereas the seeds of Desla Yellow and Desla Brown were obtained from local farmers of Naugachia sub-division.

The experiments were conducted with the seedlings of Laxmi, Suwan, Desla Yellow and Desla Brown which were grown in the polythene bags containing 1:1 ratio of farm yard manure and the field soil for 30 days at normal temperature and sunshine. After 30 days of seedling growth, the whole set of plants were waterlogged in big iron tray. The tray was filled with water upto the soil level of the polythene bag in which plant roots were submerged. The waterlogging was created for 24 and 72 h. Control pots were maintained under natural condition. Samples from plants were taken randomly in triplicate from waterlogged and control plants. To carryout the experiments under post hypoxic phase, the remaining plant sets were taken out from the tray and were kept under normal conditions like the control plants. After 48 and 96 h intervals, the plant samples were collected for the detailed analysis of each component during post hypoxic phase.

The respective root samples of hypoxic and post hypoxic sets of plants were separated and homogenized in a chilled mortar with pestle using 50 mM Tris-HCl buffer pH 7.6, 10 mM β-mercaptoethanol and 12.5% sucrose as extraction media. A 20% (w/v) of homogenate so prepared was centrifuged at 11000 Xg for 20 min in a refrigerated centrifuge (Remi, model C-24). The residue was discarded and the supernatant was used for estimation of reducing sugar (Somogyi 1948), amino acids (Jayaraman 1981), pyruvate (Jayaraman 1981), glycolic acid (Calkins 1943) and inorganic phosphorus (Fiske and Subbaraw 1925). Respective leaf samples were also analysed for chlorophyll and carotenoid.

## RESULT AND DISCUSSION

Most of the plants during oxygen deficiency are adapted to utilize the energy of glycolysis and at the same time, to render its toxic end products harmless. Detoxification of these end products (metabolites) can be achieved by exudation into solution surrounding the roots, transport to the above ground part, discarding into the air and enhancing its ability for secondary metabolism (Chirkova 1978). Since, very little information is available about the accumulation of these metabolic end products or their utilization during hypoxic and post hypoxic stress in maize, the investigation of such primary/secondary metabolic end products assumes high importance in understanding the molecular processes which the stress conditions assume. The metabolic status of photosynthetic pigments, and some metabolites were,

therefore, estimated during hypoxic and post-hypoxic conditions in the four maize varieties.

***Chlorophyll and carotenoids* :** To observe the overall behaviour of whole plants, the chlorophyll and carotenoid contents of leaves were determined to get some preliminary information of the above ground part of the plant during stress. The total chlorophyll contents of all the cultivars showed a decreasing pattern during hypoxic conditions in all the maize cultivars. However, the decrease in Chl b was more prominent than the Chl a. The chlorophyll a/b ratio of all the cultivars was increased with respect to their control value at 72 h waterlogged sample except Desla Brown in which the chlorophyll a/b ratio slightly decreased from the control value. In post-hypoxic conditions, the chlorophyll contents never reached the value of control plants but more or less stabilized at the value of 72 h hypoxic samples except in Laxmi, where chlorophyll contents decreased as compared to 72 h hypoxic samples (Table 1). The results indicated that during hypoxic and post-hypoxic conditions the overall plant metabolism was affected although even the stress was imposed only at particular plant organ. Our results are in agreement with the finding of Krishnamoorti *et al*. (1987) and Adak and Dasgupta (1997). There was no significant increase in the carotenoid contents during hypoxia in any of the cultivars under study but a sharp increase in carotenoid contents

**Table 1.** Chlorophyll and carotenoid contents (mg $g^{-1}$ fw) during hypoxic and post-hypoxic phases in maize varieties.

| | Control | Hypoxic | | Post hypoxic | |
|---|---|---|---|---|---|
| | | 24 hour | 72 hour | 48 hour | 96 hour |
| | | **Laxmi** | | | |
| Chl a | 0.89±0.03 | 0.84±0.02 | 0.79±0.03 | 0.53±0.03 | 0.53±0.03 |
| Chl b | 0.51±0.01 | 0.47±0.03 | 0.26±0.02 | 0.26±0.02 | 0.26±0.02 |
| Chl Total | 1.40 | 1.32 | 1.06 | 0.79±0.03 | 0.79±0.03 |
| Chl a/b ratio | 1.75 | 1.77 | 3.05 | 2.05 | 2.05 |
| Carotenoids | 0.22±0.02 | 0.13±0.03 | 0.12±0.02 | 0.31±0.02 | 0.39±0.01 |
| | | **Desla Yellow** | | | |
| Chl a | 0.88±0.01 | 0.84±0.00 | 0.71±0.00 | 0.71±0.00 | 0.67±0.01 |
| Chl b | 0.46±0.01 | 0.36±0.01 | 0.21±0.00 | 0.22±0.01 | 0.21±0.00 |
| Chl Total | 1.34±0.01 | 1.20±0.01 | 0.92±0.00 | 0.93±0.01 | 0.88±0.00 |
| Chl a/b ratio | 1.97 | 2.35 | 3.36 | 3.22 | 3.15 |
| Carotenoids | 0.15±0.03 | 0.11±0.02 | 0.12±0.02 | 0.47±0.02 | 0.41±0.02 |
| | | **Suwan** | | | |
| Chl a | 0.79±0.01 | 0.70±0.01 | 0.68±0.01 | 0.66±0.01 | 0.61±0.01 |
| Chl b | 0.58±0.00 | 0.56±0.00 | 0.34±0.01 | 0.52±0.01 | 0.48±0.01 |
| Chl Total | 1.37±0.00 | 1.26±0.01 | 1.02±0.01 | 1.18±0.01 | 1.09±0.01 |
| Chl a/b ratio | 1.36 | 1.25 | 2.00 | 1.26 | 1.27 |
| Carotenoids | 0.22±0.03 | 0.22±0.02 | 0.20±0.00 | 0.46±0.01 | 0.38±0.05 |
| | | **Desla Brown** | | | |
| Chl a | 0.86±0.02 | 0.82±0.03 | 0.657±0.03 | 0.64±0.03 | 0.64±0.03 |
| Chl b | 0.48±0.02 | 0.50±0.03 | 0.47±0.03 | 0.47±0.03 | 0.47±0.03 |
| Chl Total | 1.34±0.02 | 1.33±0.03 | 1.13±0.03 | 1.11±0.02 | 1.11±0.03 |
| Chl a/b ratio | 1.80 | 1.66 | 1.39 | 1.36 | 1.36 |
| Carotenoids | 0.11±0.03 | 0.11±0.02 | 0.12±0.00 | 0.16±0.03 | 0.14±0.03 |

was observed during post-hypoxia in all the cultivars except Desla Brown which exhibited a marginal difference in carotenoid contents during post-hypoxia (Table 1). In addition to transferring excitation energy to the chlorophyll, carotenoid plays an important role in protecting the cell against reactive oxygen species at high light intensity (Zubey 1989). The carotenoids are long chain polymers, which are very effective free radical scavengers (Goodman 1994).

***Reducing sugar content*** **:** All the maize cultivars under study exhibited an increase in reducing sugar content in 24 h hypoxic samples as compared to control value. In 72 h hypoxic samples, the reducing sugar contents were found to be identical to control values in Desla Yellow and Desla Brown whereas the contents increased in Suwan as compared to control as well as from 24 h hypoxic samles. Only 72 h hypoxic samples of Suwan composite exhibited a decrease in the reducing sugar contents as compared to control as well as 24 h hypoxic sample. A slight decrease in reducing sugar content was observed in the post hypoxic samples of maize cultivars except Desla Brown in which the value of sugar contents fairly stabilized and was at par with the control value (Table 2). These study revealed that the plants with shoots in air and roots subjected to waterlogging or to $O_2$ deficiency have higher concentration of carbohydrates than plants grown in totally aerobic conditions. The higher carbohydrate level is presumably a consequence of reduced growth of the roots. The increase in carbohydrate level, therefore, results from reduced utilization in growth and/or accelerated breakdown of carbohydrates in anaerobic conditions.

***Free amino acids*** **:** There was a decrease in root free amino acid contents in all the maize cultivars during different periods of waterlogging (hypoxia) and also during post-hypoxia except Desla Brown in which the free amino acid contents during hypoxic and post-hypoxic conditions were almost identical to the control (Table 3). The observed decrease in free amino acids is likely utilized by stress system for the ultimate conversion into pyruvate during anaerobic conditions or as result of the damaged cellular integrity. The free amino acids may be lost in surrounding medium as observed in the wheat roots (Bertani *et al*. 1987).

***Pyruvate content*** **:** The root pyruvate contents in all maize cultivars increased in 24 and 72 h waterlogged samples but the increase was more in 24 h samples as

**Table 2.** Root reducing sugar content (mg/100 mg dw) during hypoxic and post-hypoxic phases in maize varieties.

| Variety | Control | Hypoxic | | Post hypoxic | |
|---|---|---|---|---|---|
| | | 24 hour | 72 hour | 48 hour | 96 hour |
| Laxmi | 0.38±0.02 | 0.40±0.04 | 0.26±0.02 | 0.20±0.04 | 0.27±0.01 |
| Desla Yellow | 0.35±0.01 | 0.45±0.05 | 0.36±0.02 | 0.31±0.01 | 0.26±0.02 |
| Suwan | 0.33±0.01 | 0.39±0.01 | 0.53±0.02 | 0.45±0.01 | 0.32±0.02 |
| Desla Brown | 0.27±0.01 | 0.34±0.02 | 0.25±0.01 | 0.30±0.02 | 0.33±0.03 |

**Table 3.** Root free amino acid content (mg/100 mg dw) during hypoxic and post-hypoxic phases in maize varieties.

| Variety | Control | Hypoxic | | Post hypoxic | |
|---|---|---|---|---|---|
| | | 24 hour | 72 hour | 48 hour | 96 hour |
| Laxmi | 0.42±0.01 | 0.38±0.03 | 0.28±0.02 | 0.25±0.02 | 0.22±0.04 |
| Desla Yellow | 0.78±0.02 | 0.42±0.06 | 0.33±0.03 | 0.43±0.02 | 0.29±0.03 |
| Suwan | 0.66±0.01 | 0.43±0.01 | 0.32±0.02 | 0.27±0.03 | 0.29±0.02 |
| Desla Brown | 0.77±0.01 | 0.66±0.02 | 0.71±0.02 | 0.65±0.01 | 0.66±0.02 |

compared to 72 h samples. The pyruvate contents in 72 h waterlogged root samples of Suwan was found to be almost similar to that of control value. In post-hypoxic samples, the pyruvate contents had almost similar value as in their respective control except Desla Brown which exhibited slightly higher value from the control samples (Table 4). It is evident that during the initiation of anoxia, the root system is deprived of $O_2$, and therefore, the operation of TCA cycle is considerably diminished and hence the pyruvate accumulation is observed in 24 h waterlogged samples. By the time the ADH activity is fully induced and the pyruvate concentration is decreased as in the case of 72 h waterlogged sample. The other possibility of decrease in pyruvate contents may be the increase in transaminase activity as reported by Effer and Ranson (1967) and Sinha *et al*. (1995). Therefore, under such stress, metabolic requirements for various cellular/molecular purposes would restrict the accumulation of pyruvate in the plant system.

***Glycolic acid content*** **:** The glycolic acid contents marginally increased in 24 h waterlogged root samples of Laxmi and Suwan cultivars whereas in 72 h waterlogged root samples, Suwan and Desla Yellow exhibited an increase in glycolic acid contents as compared to control value. In 48 h post-hypoxic root samples, the increase in glycolic acid contents in Laxmi and Suwan was 56% and 70%, respectively. There was a marginal decrease in glycolic acid contents in 96 h post-hypoxic root samples of all the cultivars except Delsa Brown which exhibited approximately 45% decrease from control values (Table 5). A number of organic acids, e.g. malic, pyruvic, lactic, succinic and glycolic acids have been reported to get accumulated during anaerobiosis (Crawford 1978). However, the role of glycolic acid is not very clear because glycolic acid is the main substrate of photorespiratory pathway which is metabolized mainly by a set of photorespiratory enzymes localized in peroxisomes with an association of the enzymes of mitochondria and chloroplast. In roots, the possibility of glycolate accumulation is most likely initiated during gluconeogenesis and glycolate starts accumulating during disturbed or unregulated glucose metabolism.

***Inorganic phosphorus*** **:** There was a significant increase in Pi content in 24 h waterlogged root samples of all the cultivars except Desla Brown in which the Pi content was almost identical with the control values. In 72 h waterlogged samples, the significant increase in Pi

**Table 4.** Root pyruvate content (mg/100 mg dw) during hypoxic and post-hypoxic phases in maize varieties.

| Variety | Control | Hypoxic | | Post hypoxic | |
|---|---|---|---|---|---|
| | | 24 hour | 72 hour | 48 hour | 96 hour |
| Laxmi | 1.03±0.03 | 1.36±0.03 | 1.29±0.03 | 0.895±0.03 | 0.86±0.07 |
| Desla Yellow | 1.10±0.03 | 1.725±0.07 | 1.395±0.07 | 1.29±0.03 | 1.03±0.03 |
| Suwan | 1.36±0.03 | 1.63±0.03 | 1.32±0.07 | 1.16±0.03 | 1.19±0.00 |
| Desla Brown | 0.83±0.03 | 1.23±0.03 | 1.36±0.03 | 1.29±0.03 | 1.03±0.03 |

**Table 5.** Root glycolic acid content (mg/100 mg dw) during hypoxic and post-hypoxic phases in maize varieties.

| Variety | Control | Hypoxic | | Post hypoxic | |
|---|---|---|---|---|---|
| | | 24 hour | 72 hour | 48 hour | 96 hour |
| Laxmi | 0.25±0.01 | 0.30±0.01 | 0.21±0.00 | 0.11±0.01 | 0.20±0.02 |
| Desla Yellow | 0.22±0.02 | 0.21±0.01 | 0.29±0.03 | 0.29±0.02 | 0.03±0.05 |
| Suwan | 0.26±0.05 | 0.50±0.01 | 0.32±0.04 | 0.85±0.00 | 0.21±0.03 |
| Desla Brown | 0.35±0.01 | 0.37±0.02 | 0.38±0.02 | 0.37±0.01 | 0.19±0.01 |

**Table 6.** Root inorganic phosphorous content (mg/100 mg dw) during hypoxic and post-hypoxic phases in maize varieties.

| Variety | Control | Hypoxic | | Post hypoxic | |
|---|---|---|---|---|---|
| | | 24 hour | 72 hour | 48 hour | 96 hour |
| Laxmi | 0.369±0.04 | 0.667±0.03 | 0.56±0.04 | 0.35±0.04 | 0.321±0.01 |
| Desla Yellow | 0.33±0.02 | 0.607±0.01 | 0.547±0.05 | 0.301±0.02 | 0.333±0.05 |
| Suwan | 0.333±0.02 | 0.594±0.05 | 0.322±0.04 | 0.333±0.02 | 0.238±0.02 |
| Desla Brown | 0.357±0.02 | 0.333±0.02 | 0.274±0.04 | 0.381±0.24 | 0.322±0.04 |

contents was recorded in Laxmi and Desla Yellow whereas Suwan and Desla Brown depicted almost identical values as compared to control. The 48 h and 96 h post-hypoxic samples did not exhibit any significant change in their Pi contents as compared to their respective control values (Table 6). It is a general belief that inorganic phosphorus activates the glycolytic pathway in many respects and also regulates the phosphofructokinase activity, a key enzyme of glycolysis and is inhibited by ATP. Inorganic phosphate is an activator and able to counteract the inhibition caused by ATP. Therefore, the phosphofructokinase activity may be regulated by ATP/Pi ratio, so the greater sensitivity to activation is brought about by a decrease in the adenylate pool size accompanied by a considerable increase in inorganic phosphate.

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