Respiration and activity of respiratory enzymes in okra (*Abelmoschus esculentus*) under seasonal environmental conditions of West Bengal, India

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**Abstract:** *Abelmoschus esculentus* var. Parbani Kranti (okra) was studied in summer, rainy and winter seasons in pot culture and 28 and 58 day old leaves were collected to study the seasonal environmental impact on their respiratory metabolism. The rate of respiration along with the activity of three respiratory enzymes viz. fructose 1,6- bisphosphate aldolase, succinate dehydrogenase, glucose-6-phosphate dehydrogenase was highest in the summer season. Rainy and winter seasons showed comparatively low respiration rate and enzyme activity. Respiration rate and activity of the three above enzymes were also higher in the younger leaf samples, 28 days after sowing (DAS) as compared to the older leaf samples, 58 DAS in all the three seasons in the order of summer> rainy> winter. Since respiratory metabolism is an index of energy demand, summer is therefore a season of higher physiological activity as compared to rainy and winter. The present study indicates the alteration in respiratory activity in different seasons and developmental stages in okra, besides giving an idea of the energy demand under these changing environmental conditions, which can in turn, by correlated with the physiological behaviour of the plant.

**Key words:** Respiration, Respiratory enzymes, Okra plant, Seasonal variation, Abelmoschus esculentus.

**Introduction**

In the course of development, plants adjust to the seasonal variation of day length and the range of insolation, temperature and precipitation. Respiratory activity is not constant and depends upon the availability of substrate, the phase of development, the state of activity and also the environmental conditions.

In this paper, rate of respiration along with the activities of three important respiratory enzymes were measured in three different seasons of the year viz. summer, rainy and winter in two developmental stages. The three respiratory enzymes measured were fructose 1,6-bisphosphate aldolase, succinate dehydrogenase and glucose-6-phosphate dehydrogenase as they belong to three different respiratory pathways-glycolysis, TCA Cycle and pentose phosphate pathway respectively. The test material- *Abelmoschus esculentus* (L.) Moench (Okra) is an economically important crop plant of the tropical climate. This study gives an indication of the seasonal alteration in respiratory capacity in connection to adaptation to prevailing environmental conditions, besides giving an idea of the energy requirement. This in turn would be an index to the level of physiological activity in the different growing stages and seasons.

Since, very little is known about the seasonal effect on respiration and respiratory enzymes of crop plants, hence this work was taken up.

**Materials and Methods**

The work was carried out in pot culture in the experimental garden of the University Campus at Ballygunge, Kolkata (22.34 ° North and 88.24 ° East) in West Bengal, India under tropical environmental conditions. The seasons under study were summer (March-June), rainy (July-September) and winter (November-February). The meteorological data (Table 1) were obtained from Regional Meteorological Centre, Alipore, Kolkata.

Seeds of *Abelmoschus esculentus* var. Parbani Kranti were obtained (National Seed Corporation, Kolkata) and sown in sandy loam soil and farmyard manure in the ratio of 3:1. Initially 8-10 seeds were sown in earthen pots of 12 inches diameter. When the seedlings were 5-6 inches tall, only the healthy ones of more or less uniform height were maintained, while the others were removed. This process was repeated thrice at different seasons, already mentioned, before the onset of the experimental observation, to ensure uniform and healthy plant material. Finally from the remaining 4-6 healthy plants, fully expanded, penultimate (second from top) leaves were collected at two stages of development-28 days after sowing (DAS) and 58 DAS in all the three above-mentioned seasons. For all three seasons, identical water managements were maintained along with a constant nutrient status of the soil.

Rate of respiration i.e. CO$_2$ release was measured in the dark by Infra Red Gas Analyzer (IRGA). Fructose 1,6-Bisphosphate Aldolase enzyme was assayed by the method of Sibley and Lehninger (1949). Fresh leaves were extracted in 0.02 M phosphate buffer, pH 7.8 and the enzyme activity expressed in terms of optical density at 560 nm per hour per mg protein. Succinate Dehydrogenase was assayed by the method of King (1967). For the assay of this enzyme, mitochondria were isolated from leaf samples in the extraction medium containing 0.02 M phosphate buffer, pH 7.8. The enzyme activity was expressed as umole succinate oxidized per hour per mg protein. Glucose-6-Phosphate Dehydrogenase was assayed by the
Table – 1: Meteorological data from November 2000 to October 2001.

<table>
<thead>
<tr>
<th></th>
<th>Summer</th>
<th>Rainy</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>35.5</td>
<td>31</td>
<td>14.5</td>
</tr>
<tr>
<td>Sunshine Hrs (Hr)</td>
<td>13.5</td>
<td>12.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Light Intensity (Lux)</td>
<td>72000</td>
<td>65000</td>
<td>32000</td>
</tr>
<tr>
<td>Relative Humidity (%)</td>
<td>77</td>
<td>89</td>
<td>61</td>
</tr>
<tr>
<td>Rainfall (mm)</td>
<td>119</td>
<td>331</td>
<td>15</td>
</tr>
</tbody>
</table>

Fig. 1: Rate of respiration expressed as mg CO$_2$ released per hr per square dm. leaf area.

S.E. = 0.02
C.D. of season = 0.02 (5%) : 0.04 (1%)
C.D. of stage = 0.02 (5%) : 0.03 (1%)

method of Upadhyay et al. (1981). Leaves were extracted in 0.02M phosphate buffer pH 6.0 and the enzyme activity expressed in umole NADPH formed per minute per mg protein. Soluble protein content for the enzymes was estimated according to Lowry et al. (1951).

The data obtained from three replications were statistically analyzed. Standard error (S.E.) and critical difference (C.D.) values of both season and stage at 5 % and 1 % levels were calculated from the respective analysis of variance (ANOVA) tables.

Results and Discussion

The duration of the experimental period was from November 2000-October, 2001—a period characterized by considerable variations in temperature, photoperiod, light intensity, relative humidity and rainfall. The meteorological data (Table-1) were obtained from Regional Meteorological Centre, Alipore, Kolkata. The data indicated the monthly mean values, which have been finally expressed as seasonal mean values for the suitability of this work.

In this work it was observed that release of CO$_2$ i.e. rate of respiration was the highest in summer with gradually declining trends in rainy and winter season (Fig.1). The rate of respiration was significantly higher in the earlier developmental stage (28 DAS) as compared to the later stage (58 DAS) in all the three seasons.

The activities of all the three respiratory enzymes assayed viz. fructose 1,6-bisphosphate aldolase, succinate dehydrogenase and glucose-6-phosphate dehydrogenase exhibited identical trends in recording maximum activity in the summer season. Their values significantly decreased in the rainy season and recorded a winter minima (Figs. 2, 3 and 4). All the three enzymes recorded higher values in the preflowering stage (28 DAS) as compared to the postflowering
Fig. 2: Fructose 1,6-bisphosphate aldolase enzyme activity expressed as optical density at 560 nm per hr per mg protein of leaf sample.

S.E. = 0.01
C.D. of season = 0.01 (5%); 0.02 (1%)
C.D. of stage = 0.012 (5%); 0.018 (1%)

Fig. 3: Succinate dehydrogenase enzyme activity expressed as μ mole succinate oxidized per hr per mg protein of leaf sample.

S.E. = 0.22
C.D. of season = 0.23 (5%); 0.34 (1%)
C.D. of stage = 0.18 (5%); 0.28 (1%)
In the pre flowering samples rate of respiration decreased by 22.2% in the rainy and 86.6% in the winter while in the post flowering decreased by 34.2% and 94.7% respectively (Fig.1). Activity of the aldolase enzyme diminished by 51.2% and 87.8% in the rainy and winter pre flowering samples while in the post flowering samples decreased by 53.3% and 91.6% (Fig.2). Succinate dehydrogenase activity declined by 42.6% and 85.3% in the pre flowering rainy and winter samples while the decrease was by 30% and 80% respectively in the post flowering rainy and winter samples (Fig.3). Glucose-6-phosphate dehydrogenase activity dropped by 37.5% and 85.9% in the pre flowering rainy and winter samples while the post flowering samples dropped by 33.3% and 91.6% in the rainy and winter respectively (Fig.4).

The results clearly indicate that respiration rate and activities of the three above mentioned enzymes are the highest in the young actively growing plant parts. Ryle et al. (1979) reported dark respiration to be highest in a young plant, which declined as soon as the plant matured. Dark respiration is highest for expanding leaves and is significantly affected by temperature (Bolanos and Hsiao, 1991).

In most plant species lower rates of respiration have been correlated with sub-optimal supply of inorganic phosphate – Pi (Thorsteinsson and Tillberg, 1987, Weger and Dasgupta, 1993). In my present study dark respiration was severely diminished in the winter, which also recorded a significantly low Pi content both in the leaf and fruit samples of Okra (Sen and Mukherji, 2002, 2004). In maize leaves, the activity of aldolase enzyme was considerably diminished in Pi-deficient plants (Usuda and Shimogawara, 1992). According to Bryce et al. (1990), the absolute concentration of Pi is one of the most important factors involved in the regulation of mitochondrial respiration. Thus phosphate content seems to be an important factor in the regulation of the activities of respiratory enzymes. Hence all enzyme activities recorded a winter minima when phosphate content sharply declined (Sen and Mukherji, 2002, 2004). The overall rate of respiration is dependent on the interrelations among Pi, ATP and ADP. A high ATP and Pi content in summer (Sen and Mukherji, 2002, 2004) constitutes a high energy pool and consequently a high respiratory rate in the same season is indicative of a peak metabolic phase.

Since respiration rates are correlated with both leaf sugar and nitrogen contents during leaf senescence (Collier and Thibodeau, 1995), a decline in their contents in the older leaf samples (58 DAS) (Sen and Mukherji, 1998a, 1998b) leads to a low respiratory activity in the same. Similarly, low sugar and nitrogen contents in the winter season (Sen and Mukherji 1998a, 1998b) could severely diminish the respiratory rate. A high sugar content generally occurs in connection with high rates of CO$_2$ assimilation and causes an increase in respiration, whereas at low carbohydrate levels the respiratory rate gradually becomes lower. A high rate of CO$_2$ assimilation along with high sugar content was observed (Sen and Mukherji, 1998a, 1999a) accompanying an increased respiratory rate in the summer season. Thus respiratory activity is seen to be
lowered with poor substrate availability in the winter season (Sen and Mukherji, 1998a).

Thus summer season is the physiologically most active for *Abelmoschus*. Summer season has maximum substrate availability due to increased photosynthates (Sen and Mukherji, 1998a) resulting from high photosynthetic efficiency (Sen and Mukherji, 1999a) resulting in promotive effects on growth, yield and yield quality (Sen and Mukherji, 1998c, 1998d, 1999b). Greater ion uptake in this particular season (Sen and Mukherji, 1997) also calls for greater energy and thus a significantly higher respiratory activity meets this demand. All these aspects lead to higher respiratory metabolism in the summer season.

Seasonal variations in plant metabolism are thus able to match with the seasonal shifts in climatic factors. The critical steps in the plant life cycle are the modifications or variations to adjust to suitable/unsuitable seasonal periods as poor synchronisation between periods of plant activity and the rhythmicity of the climate can drastically reduce its yield and even restrict its survival in stressful environments.

**References**


