Wolgica Poir., *Ononis arvensis* L. and *Trigonella corniculata* L.

The isolated tracheids are the result of (i) the failure of extension cells to differentiate into vascular tissue between a vein-ending (VE) and a tracheid (T) (figure 1 at arrow), (ii) uncoiling and simultaneous dissolution of a few coils of the vein-ending just below the tracheid (figures 2-4 at arrows), (iii) degeneration of the vein-ending bearing the tracheid (figure 5 at arrows) and (iv) rupture of the parenchymatous bundle sheath (BS) between (a) a vein and a tracheid (figure 6 at arrow), (b) vein-ending and a tracheid (figure 7 at arrow) and (c) tracheid and a tracheid (figure 8 at arrow). Such tracheids may occur scattered in the mesophyll (figure 9). The last three types do not appear to have been described earlier.

Thanks are due to the Botanical Garden and Museum, Berlin, for supplying the seeds (asterisk marked in the text) and to the Government of Gujarat for financial assistance to MGR.

9 February 1982


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**RESPIRATORY METABOLISM OF SOMATIC EMBRYOGENESIS IN CALLUS CULTURES OF DIOSCOREA DELTOIDEA WALL**

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SOMATIC embryogenesis in *in vitro* cultured cells provides a useful system to study the process of differentiation and development at the cellular level. Although earlier experiments have shown biochemical changes during embryogenesis\(^8\), information on its respiratory metabolism is scanty. It was considered worthwhile to investigate the respiratory metabolism of somatic embryogenesis in the callus tissue of *Dioscorea deltoidea* Wall.

The callus tissue of *Dioscorea deltoidea* Wall used in this experiment was obtained from its tuber and maintained as stock through more than 7 sub-cultures on Murashige and Skoog's (MS) medium\(^9\) supplemented with 2 mg/l of 2,4-D (2,4-Dichlorophenoxy acetic acid) and 0.75% of agar adjusted to pH 5.8 before autoclaving. Such a medium allows callus proliferation which is referred to as the non-embryogenic callus. For embryogenic callus, the level of kinetin was raised to 9 mg/l. Sterilization and other cultural conditions were as reported by Singh\(^10\). Callus pieces (0.50 mg each) were inoculated in each test tube which contained 20 ml of medium solidified by Difco Bacto agar. For measuring the rate of respiration, 1 g of embryogenic and non-embryogenic tissues was randomly placed in each manometric flask after 15 days of growth period. Respiration was measured in dark at 27°C in Warburg reaction vessels with a side arm using conventional manometric technique\(^11\). The required material of the manometric flasks and shaking speed of the manometers were the same as described by Singh and Singh\(^12\).

The effect of sodium fluoride (NaF), malonic acid, sodium azide (NaN\(_3\)) and 2,4-dinitrophenol (DNP) on the rate of respiration was also studied. Preparation of these chemicals, measurement of normal rate of respiration, tipping of these chemicals, observations after tipping and other precautionary measures were followed\(^13\). The inhibition or acceleration obtained has been presented as the percentage of the average normal rate of respiration. In sodium azide, separate manometers were used for control\(^14\). The concentration of inhibitors and the DNP used were: NaF, 0.02 M; malonic acid, 0.05 M; sodium azide, 0.002 M and DNP, 5 × 10\(^{-5}\) M. NaF, NaN\(_3\) and DNP were dissolved in phosphate buffer (0.02 M) of pH 5.3. Malonic acid was dissolved in a small quantity of distilled water, the pH of the solution was adjusted to 4.7 with sodium hydroxide and made to volume with phosphate buffer of pH 4.7.

Non-embryogenic callus tissue showed a proliferated growth, yellow in colour, whereas embryogenic callus tissue turned into slight green with slow growth. Embryogenic tissues had a higher rate of respiration as compared to non-embryogenic tissues. This seemed to be due to the increased protein synthesis in embryogenetic tissues as compared with that of non-embryogenetic one. Since protein synthesis takes place at the expense of metabolic energy with concomitant increase in ADP and Pi, their ability to stimulate O\(_2\) uptake\(^15,16\) indicates a clear possibility for the stimulated O\(_2\) uptake through increased protein synthesis during embryogenesis. An increased content of protein has also been reported by Fujimura *et al.*\(^8\) in embryo genesis of carrot cell suspension culture. In the present study protein synthesis was not determined but the possibility of greater protein synthesis during embryogenesis is not ruled out. Lower sensitivity to DNP treatment during embry-
ogenesis in the present study further supported the above view.

Sodium fluoride inhibited the respiration of embryogenic tissues more than the non-embryogenic (table I). There were always more fluoride resistant components of respiration in non-embryogenic tissues as compared to embryogenic tissues. An increased respiratory inhibition due to sodium fluoride has also been reported by Shaw and Singh in healthy hypocotyl and normal root callus of Citrus aurantifolia, respectively which suggested that glycolytic pathway was more active in healthy hypocotyl and normal root callus of Citrus aurantifolia as compared to rust infected hypocotyl and habituated Citrus root callus. The results of the present experiment indicated that embryogenic callus tissue of Dioscorea deltoidea Wall may also have more active glycolytic pathway.

**Table I**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Embryogenic tissues</th>
<th>Non-embryogenic tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>228.5</td>
<td>150.6</td>
</tr>
<tr>
<td>Sodium fluoride</td>
<td>137.8</td>
<td>105.2</td>
</tr>
<tr>
<td></td>
<td>(−39.9)</td>
<td>(−30.1)</td>
</tr>
<tr>
<td>Malonic Acid</td>
<td>112.7</td>
<td>45.7</td>
</tr>
<tr>
<td></td>
<td>(−50.7)</td>
<td>(−69.7)</td>
</tr>
<tr>
<td>Sodium Azide</td>
<td>92.0</td>
<td>104.8</td>
</tr>
<tr>
<td></td>
<td>(−59.8)</td>
<td>(−30.5)</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>274.7</td>
<td>202.5</td>
</tr>
<tr>
<td></td>
<td>(−20.2)</td>
<td>(−34.5)</td>
</tr>
</tbody>
</table>

(Values are mean of 3 values. Figures in parentheses represent either percent inhibition (−) or stimulation (+) over the control. Rate of respiration μl O₂ uptake/hr/g fresh wt.)

Malonic acid was used to ascertain the role of Krebs (TCA) cycle in respiration. The respiration of non-embryogenic callus was more sensitive than that of embryogenic callus. It indicates an existence of other pathway besides TCA cycle i.e. hexose-monophosphate-shunt is more dominant in respiration of embryogenic tissues. An increased level of enzymes responsible for hexose-monophosphate-shunt under shoot forming callus tissue has also been reported in tobacco callus.

The respiration of embryogenic and non-embryogenic callus tissue was strongly inhibited by azide, the inhibition being more marked in embryogenetic tissues. This clearly suggests that metal containing oxidases participate more actively in the respiration of embryogenic callus. Lower sensitivity of the respiration to azide treatment in non-embryogenic tissues, on the other hand, might be due to the mediation of other mechanisms (such as B₁ and B₇ cytochromes) in the oxidative processes of these tissues as suggested by Martin and Mortan, Bendall and Hill and Singh and Singh in silver beet, Arum maculatum and Japanese mint, respectively. Presence of these cytochromes in non-embryogenic callus tissue of Dioscorea deltoidea also seems to be a possibility. However, this needs further investigation.

Embryogenic tissues were less sensitive to DNP treatment as compared to non-embryogenic tissues. However, it is noteworthy that the actual increase in O₂ uptake by embryogenic tissues far exceeded the values recorded for non-embryogenic tissues (table I). As DNP uncouples oxidation reactions from energy conserving process of phosphorylation, the level of ADP and Pi increases in treated tissues. Consequently the O₂ uptake is enhanced. The results obtained in the present study suggest that embryogenesis itself exerts a DNP-like effect on respiration partially either due to uncoupling phenomenon or due to the synthetic reactions requiring ATP. Since an increased rate of protein synthesis would be expected during embryogenesis and this process requires utilization of ATP with concomitant increase in ADP and Pi. Their ability to stimulate O₂ uptake indicated that embryogenic tissues may have already undergone changes similar to those brought about in normal tissues after DNP treatment. Hence it seems that addition of DNP releases only residual oxidative capacity of the embryogenic tissues.

The author is grateful to Dr. T. N. Khosho, Director, National Botanical Research Institute, Lucknow, for encouragement throughout the course of investigation. He also thanks the Council of Scientific and Industrial Research, New Delhi, for the award of fellowship.

29 December 1981


**MASTOGLOIA DANSEI THWAITES—A NEW ADDITION TO THE INDIAN FLORA**

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There are only a few records\(^1\)\(^-\)\(^3\) of the freshwater diatom-flora of Andaman and Nicobar Islands. During investigations on freshwater bacillariophycean algae of Andaman & Nicobar Islands, plants of *Mastogloia dansei* Thwaites\(^4\), a species which has not yet been documented in the Indian diatom-flora, were collected. It is, therefore, intended to record this taxon in the present communication.

*Mastogloia dansei* Thwaites (figures 1 and 2).

Valve linear, naviculoid, rounded in the middle with rostrate and rounded apices; valve with loculi; loculi eight, arranged in a straight row, interposed between the connecting membrane and the valve on which they appear adherent. Valve without loculi; axial area narrow, considerably hyaline in the middle region. Striae fine, lineate very slightly radiate throughout the valve; raphe straight, thin.

Length, 22 \(\mu\)m; breadth, 8.5 \(\mu\)m; striae, 26–27 in 10 \(\mu\)m.

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**Figures 1, 2.** *Mastogloia dansei* Thwaites. 1. Frustule with loculi. 2. Frustule without loculi.

Habitat: Planktonic with other algae in a freshwater pond.

Loc.: Astinabad (Port Blair).

Coll. No.: AN 251.

Date: 28-11-1978.

One of the authors (MNS) gratefully acknowledges financial assistance from the U.G.C., New Delhi.

25 January 1982

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**A NEW EAR AND KERNEL ROT OF MAIZE CAUSED BY TRICHODERMA VIRIDE PERS. EX FRIES**

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The authors observed a new ear and kernel rot of Dent corn (*Zea mays* L.) during the field survey of maize crop in Periyapatna and Hunsur Taluks of Karnataka in the 1981 kharif season. The cultivars in which the rot was observed were Ganga-5 and Deccan-101 with 20 and 25% ear infection respectively. The observation showed that the rot of whole ear (figure 1) was common in the middle of the field especially where plant population was more and the ears of border plants showed only kernel rot owing to bird damage (figure