Changes in the Composition of Xylem Sap during Development of the Spadix of Skunk Cabbage (Symplocarpus foetidus)

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The spadix of skunk cabbage, Symplocarpus foetidus, is thermogenic and maintains an internal temperature of around 20 °C even when the ambient air temperature drops below freezing. This homeothermic heat production is observed only during the stigma stage, and thereafter ceases at the male stage when pollen is shed. To clarify the regulatory mechanism by which the stigma stage-specific heat production occurs in the spadix, sugars, organic acids, and amino acids in xylem sap were analyzed and compared with those of post-thermogenic plants. Interestingly, no significant difference was observed in the total volume of xylem sap per fresh weight of the spadix between thermogenic and post-thermogenic plants. Our results indicate that the composition of the xylem sap differs during the development of the spadix of S. foetidus.

Key words: skunk cabbage; spadix; heat production; energy source; xylem sap

Certain primitive plants increase their own temperature by considerably accelerating aerobic respiration. These thermogenic plants include several species of the arum lily family, Symplocarpus foetidus, palms, lotus, cycads, and water lilies. To date, however, only three plant species, Symplocarpus foetidus, Philodendron selloum, and Nelumbo nucifera have been shown to be homeothermic. Interestingly, homeothermic heat production exhibits an inverse relationship between the level of respiration and changes in ambient temperature. The spadix of S. foetidus has been shown to increase its respiratory rate under low ambient temperature. Furthermore, oxygen consumption in P. selloum has also been shown to increase as ambient temperature decreases. These results suggest that a large amount of respiratory substrate is required to increase heat production, which is induced by decreased ambient temperatures. Indeed, the major energy source for S. foetidus and P. selloum have been shown to be carbohydrates and lipids, respectively. Therefore, a continuous supply of substrates from the xylem sap, which is produced and blended in the root and transported via xylem vessels, appears to play a crucial role in homeothermic heat production in plants.

Interestingly, when the spadix of S. foetidus is removed from the rest of the plant, heat production terminates immediately. This observation suggests the presence of a pathway for the translocation of an energy source from the roots to the spadix in S. foetidus. Specifically, it is probable that carbohydrates, such as sugars that are catabolized from starch in the roots, are transported to support homeothermic heat production and the development of the spadix. However, whether the root controls the physiological state of the thermogenic spadix via compositional changes in the xylem sap in S. foetidus is poorly understood. Moreover, no evidence has been presented for changes in carbohydrates or amino acids in xylem sap, both of which are potentially utilized for energy metabolism in the spadix of S. foetidus.

To clarify the mechanism for maintaining stigma stage-specific heat production in S. foetidus, we analyzed the levels of sugars, organic acids, and amino acids in xylem sap from the roots using thermogenic (stigma stage) and post-thermogenic (male stage) plants. Because the protogynous spadix of S. foetidus exhibits stigma stage-specific homeothermic heat production, our analysis of the xylem sap from various spadix stages enabled us to show the potential cross-talk between the roots and the spadix in S. foetidus. This is the first report to identify the developmental changes in the composition of xylem sap in thermogenic plants.

Materials and Methods

Plant materials, collection of xylem sap, and measurement of temperature. The experiments with Symplocarpus foetidus plants were carried out on a natural population in the village of Hakuba, Nagano Prefecture in central Japan (36°39’N, 137°50’E) between 18:00 and 19:00 on April 22, 2002. Plants with thermogenic
(stigma stage) and post-thermogenic (male stage) spadices were selected. Neither thermogenic nor post-thermogenic plants had expanded leaves. Xylem sap was collected over a 1-h period immediately after cutting spadices at the upper part of the central stalk using stainless-steel razor blades. Measurements of the spadix and ambient air temperatures were made just before the spadix was cut. The total volume of the collected xylem sap was measured and each sample was stored at −20 °C until analysis. The temperature was measured using an automatic recording thermometer (TR-52; T and D, Nagano, Japan), as described in our previous report. The mean air temperature in the present study was 9.1 ± 0.2 °C.

Sugar analysis. Sugars (sucrose, glucose, and fructose) in the xylem saps were analyzed by high-performance liquid chromatography (HPLC) using KS801 and KS802 columns (both columns were 8.0 × 300 mm; Shodex, Tokyo) warmed to 80 °C. Deionized water was used as the mobile phase at a flow rate of 0.4 ml min⁻¹, and peaks were detected with a refractive index detector. The detected peaks were identified and quantified using a standard solution consisting of sucrose, glucose, and fructose.

Organic acid and amino acid analysis. Xylem saps were introduced into a cation exchange column (Dowex 50 W × 4 resin, Dow Chemical, Auburn Hills, MI), and an aliquot of the effluent from the column was subsequently introduced into an anion exchange column (Amberlite IRA-400 resin, Rohm and Hass, Philadelphia, PA). Absorbed substances in the cation and anion exchange resins were eluted with 1 M ammonia and 1 M phosphoric acid, adjusted to pH 1.8, respectively. Formic acid solutions were introduced into a cation exchange column (Dowex 50 W × 4 resin, Dow Chemical, Auburn Hills, MI), and peaks were detected with a refractive index detector. The detected peaks were identified and quantified using a standard solution consisting of sucrose, glucose, and fructose.

The eluted fractions from the anion exchange resin were further analyzed for organic acids by HPLC (LC-4; Shimadzu, Kyoto, Japan) using a SCR-102 (H) column (600 mm long column; Shimadzu, Kyoto, Japan) warmed to 65 °C. Phosphoric acid, adjusted to pH 1.8, was used as an eluting solvent at a flow rate of 0.25 ml min⁻¹.

The substances eluted from the cation exchange resin were analyzed and quantitated on a fully automated amino acid analyzer (flow rate 0.1 ml min⁻¹) (JLC-500/V; JEOL, Tokyo) according to the manufacturer’s instructions.

Statistical analysis. Analysis of variance was performed using the SAS program (SAS Institute, Cary, NC). Obtained values were expressed as mean ± SD.

Results

Stigma stage-specific heat production in the spadix

To determine whether the protogynous spadix exhibited stigma stage-specific heat production when the xylem saps were collected, we measured the temperature of the spadix at various stages. Although we tried to include samples from the early stigma stage with pre-thermogenic plants, almost all pre-thermogenic plants were under the ground, and hence we could not study those plants for further analysis. The temperature of the spadix at the stigma and male spadix stages were 22.5 ± 1.1 °C and 9.1 ± 0.4 °C respectively (Fig. 1). Because the average ambient temperature during the experiment was 9.1 ± 0.2 °C (Fig. 1), these results clearly indicate that our sample plants exhibited stigma stage-specific heat production. Hereafter, we refer to plant at the stigma and male spadix stages as thermogenic and post-thermogenic respectively.

Volume of xylem sap

Figure 2 shows the mean volume of sap collected during the 1-h period after decapitation of the spadix. Because most of the plants had no expanded leaves and the spadix was the only sink organ for xylem sap in a plant, data are expressed as a volume per fresh weight of the spadix (Fig. 2). No significant difference was observed in the total volume of xylem sap per fresh
weight of the spadix between thermogenic (31.2 ± 24.7 μM h⁻¹ g⁻¹) and post-thermogenic (50.5 ± 30.4 μM h⁻¹ g⁻¹) plants. The fresh weights of the spadices of thermogenic and post-thermogenic plants were 5.6 ± 1.4 g and 8.6 ± 3.2 g respectively.

**Analysis of sugars**

As shown in Fig. 3, the concentrations of three major sugars in the xylem sap (sucrose, glucose, and fructose) were significantly higher in thermogenic plants. Other neutral sugars such as arabinose, xylose, mannose, and galactose were not detectable in our assay. Sucrose from thermogenic plants exhibited the highest concentration (4.5 ± 0.4 mM g⁻¹) among the detected sugars in xylem saps.

**Analysis of organic acids**

Figure 4 shows the levels of three organic acids (citrate, malate, and succinate) in the xylem saps. These molecules are potential substrates of the tricarboxylic acid (TCA) cycle within mitochondria. The levels of citrate concentration were lower than those of malate or succinate, and no significant difference was observed between thermogenic and post-thermogenic plants (Fig. 4). On the other hand, the concentrations of malate and succinate in the thermogenic plants were significantly higher (Fig. 4).

**Analysis of amino acids**

Among the detected amino acids, Asn and Gln, which contain amide nitrogen, were the primary amino acids in xylem sap in both thermogenic and post-thermogenic plants (Table 1). Concentrations of Asp, Asn, Glu, Gln, Gly, and Ala were significantly lower in post-thermogenic plants, whereas the levels of Thr, Ser, Val, Ile, Leu, Tyr, and Phe showed no significant changes.

**Discussion**

Thermogenesis during the stigma stage of *S. foetidus* is terminated immediately after the spadix is removed from the rest of the plant. Furthermore, because the respiration quotient of the thermogenic spadix is 1.0 and there are no leaves during flowering of *S. foetidus*, a continuous supply of carbohydrates, reserved as starch in the roots, is necessary for stigma stage-specific heat production. If the mechanism that maintains such stigma stage-specific heat production is controlled, in part, by the energy source from the roots, the composition of xylem sap, especially carbohydrates, should differ between thermogenic and post-thermogenic plants. In the present study, we focused accordingly on the composition of the xylem saps obtained from thermogenic and post-thermogenic plants. Xylem sap is often used to estimate the interaction between roots and above-ground parts, mineral absorption, and nitrogen assimilation and translocation.

In the protogynous spadix, homeothermic heat production has been shown to disappear at the male stage.

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**Table 1. Concentrations of Amino Acids in Xylem Sap from Thermogenic and Post-Thermogenic Plants of *S. foetidus***

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Thermogenic (mm/g spadix fresh weight)</th>
<th>Post-thermogenic (mm/g spadix fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>0.12 ± 0.14 a</td>
<td>0.02 ± 0.02 b</td>
</tr>
<tr>
<td>Thr</td>
<td>0.11 ± 0.07 a</td>
<td>0.06 ± 0.09 a</td>
</tr>
<tr>
<td>Ser</td>
<td>0.10 ± 0.06 a</td>
<td>0.06 ± 0.07 a</td>
</tr>
<tr>
<td>Asn</td>
<td>1.85 ± 0.89 a</td>
<td>0.61 ± 0.53 b</td>
</tr>
<tr>
<td>Glu</td>
<td>0.12 ± 0.06 a</td>
<td>0.04 ± 0.03 b</td>
</tr>
<tr>
<td>Gln</td>
<td>0.58 ± 0.36 a</td>
<td>0.22 ± 0.36 b</td>
</tr>
<tr>
<td>Gly</td>
<td>0.03 ± 0.02 a</td>
<td>0.01 ± 0.01 b</td>
</tr>
<tr>
<td>Ala</td>
<td>0.12 ± 0.13 a</td>
<td>0.02 ± 0.01 b</td>
</tr>
<tr>
<td>Val</td>
<td>0.28 ± 0.55 a</td>
<td>0.16 ± 0.17 a</td>
</tr>
<tr>
<td>Ile</td>
<td>0.02 ± 0.01 a</td>
<td>0.05 ± 0.06 a</td>
</tr>
<tr>
<td>Leu</td>
<td>0.03 ± 0.01 a</td>
<td>0.03 ± 0.04 a</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.01 ± 0.01 a</td>
<td>0.01 ± 0.01 a</td>
</tr>
<tr>
<td>Phe</td>
<td>0.02 ± 0.01 a</td>
<td>0.02 ± 0.03 a</td>
</tr>
</tbody>
</table>

Each datum represents the mean ± SD (thermogenic, n = 11; post-thermogenic, n = 12). Values followed by a different letter are significantly different (P < 0.05).
thermogenic plants, concentrations of sucrose, glucose, and fructose in the xylem sap of squash have been shown to be 64.3 μM, which represents as much as 1% of the total hexose concentration found in skunk cabbage. Therefore it is probable that the fairly high levels of soluble sugars in xylem sap provide a potential energy source for stigma stage-specific heat production. This concept is also supported by the fact that the levels of sucrose, glucose, and fructose dramatically decreased when heat production was terminated (Fig. 3). A previous report indicated that sugar concentrations in xylem sap are relatively constant during the stigma stage and do not respond directly to external ambient temperature. It appears that fine control of homeothermic heat production against ambient temperature changes was conducted by uncoupling proteins and/or alternative oxidase, whose expression is potentially regulated by temperature changes. Therefore, a remarkable decrease in sugars in post-thermogenic plants as shown in this study suggest that sugars in xylem sap play an important role in supporting stigma stage-specific basal heat production of the spadix.

Oxygen consumption during the stigma stage of spadix has been shown to increase during heat production, which suggests that the respiratory pathways, including glycolysis, the TCA cycle, and the mitochondrial electron transport chain are simultaneously stimulated during heat production. As described above, the initial substrates of glycolysis (i.e., sucrose, glucose, and fructose) appeared to be imported from the roots to the spadix via xylem sap. However, whether or not substrates for the TCA cycle are also supplied from the roots is unknown. Hence we examined the level of three organic acids (citrate, malate, and succinate), which are potentially utilized in the TCA cycle within mitochondria. Concentrations of malate and succinate in thermogenic plants were significantly higher than in post-thermogenic plants (Fig. 4). Malate is catalyzed to oxaloacetate by a malate dehydrogenase in the TCA cycle. Alternatively, malate is oxidized to pyruvate, which is subsequently utilized in the TCA cycle, by NAD+ malic enzyme that exists in plant mitochondria. Furthermore, succinate is provided as a substrate for succinate dehydrogenase in the TCA cycle and a mitochondrial respiratory chain. It was also found that the concentration of citrate was extremely low, and no significant difference was observed between the concentrations in thermogenic and post-thermogenic plants (Fig. 4). These results clearly suggest that citrate in xylem sap is not preferentially utilized as a major energy source in the spadix of skunk cabbage in the thermogenic and post-thermogenic stages. Because it has been shown that the TCA cycle in the mitochondria is potentially stimulated by any organic acids constituting the pathway, it is possible that both malate and succinate are translocated from the roots and catabolized in the increased oxidative reactions in the thermogenic spadix.

Finally, our analysis of the amino acid composition of xylem sap revealed that Asn and Gln are the major amino acids. Furthermore, the levels of Asn and Gln, together with Asp, Glu, Gly, and Ala, were significantly higher in thermogenic than in post-thermogenic plants (Table 1). Because both Asn and Gln contain amide nitrogen, xylem sap appears to be a source of nitrogen for the spadix. Previous studies have shown that spadix weight increases during the period of heat production, which was also confirmed in the present study (see “Results”). Therefore, it is also probable that a supply of nitrogen in the form of Asn and Gln, together with sugars and organic acids as an energy source, is, in part, necessary for the basal growth of the spadix.

In conclusion, our results clearly indicate that the composition of xylem sap differs between thermogenic and post-thermogenic plants in S. foetidus. These results strongly suggest that developmental termination of the stigma stage-specific heat production in S. foetidus is, at least in part, controlled by root activities via decreased levels of energy source in the xylem sap. Further studies are necessary to show conclusively such a developmentally regulated cross-talk between the roots and the spadix.

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