

# Thermogenesis in skunk cabbage (*Symplocarpus renifolius*): New insights from the ultrastructure and gene expression profiles

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**Abstract:** Floral thermogenesis has been found in several plant species. The spadix of one thermoregulatory plant, the Eastern Asian skunk cabbage (*Symplocarpus renifolius*), can maintain its temperature at approximately 22-26°C for several days, even when the ambient temperature falls below freezing. There are two major stages in skunk cabbage inflorescence development: the thermogenic female stage and the non-thermogenic male stage; in the former the spadix can produce massive amounts of heat, whereas in the latter, thermogenesis is undetectable. Based on previous studies, there is a positive correlation between heat production and the abundance of mitochondria in plant tissues and cells, and genes involved in cellular respiration and mitochondrial function are significantly enhanced at the female stage. Taken together, these findings suggest that the increased respiration or mitochondrial abundance observed in thermogenic tissues may be attributable to the high expression of specific genes. This review summarizes new insights into the changes in intracellular structures and gene expression profiles of skunk cabbage spadices during the female-male transition and proposes possible processes that are essential for each stage during floral development.

## 1. Introduction

Floral thermogenesis occurs in several plant taxa including gymnosperms (Cycadaceae), as well as eudicots (Nymphaeaceae) and monocots (Araceae). Thermogenesis begins when these plants bloom, and heat production terminates when pollen is released from the anthers. One thermoregulatory plant, the Eastern Asian skunk cabbage (*Symplocarpus renifolius*), can keep the spadix temperature between 22-26°C for several days even when the ambient temperature falls below freezing (Fig. 1A) (Knutson, 1974; Uemura *et al.*, 1993; Seymour, 2004). Other thermoregulatory plants studied to date include *Phyllodendron sellom* (Nagy *et al.*, 1972; Seymour *et al.*, 1983) and *Nelumbo nucifera* (Seymour and Schultze-Motel, 1998; Seymour *et al.*, 1998). Many species, which are thermogenic but not thermoregulatory, are generally able to produce heat for only 24 h at best. The robust thermoregulation observed in *S. renifolius* and other species makes these plants great models for unraveling the mechanism underlying floral thermogenesis. In several species of Araceae, floral thermogenesis has been proposed to serve the physiological role of spreading odor to attract pollinators (Meeuse and Raskin, 1988), whereas thermoregulation in *S. renifolius* is not closely associated with cross-pollination (Seymour and Blaylock, 1999).

*S. renifolius* produces only a faint aroma in early spring when few insects are active. Thus, heating may promote early flowering or protect the *S. renifolius* inflorescence from damage by freezing.

In *S. renifolius*, thermogenesis is closely associated with three stages of inflorescence development: female, bisexual, and male (Fig. 1B). At the female stage, which lasts until the stamens emerge from the surface of the spadix, the spadix can produce massive amounts of heat. At the bisexual stage, the stamens begin to release pollen and thermogenesis fluctuates. Finally, at the male stage, pollen is released from nearly all stamens and thermogenesis is undetectable. Microscopic analysis revealed that structural changes in the stamen are significant, and extensive anther development occurs during inflorescence development (Ito-Inaba *et al.*, 2009 a). In addition to the structural changes in stamens, the ultrastructure of petals and pistils also significantly change. These tissues accumulate a larger number of mitochondria during the female stage than during the male stage. Also, large cytoplasmic vacuoles develop during the male stage. In our recent gene expression analysis, expression of genes involved in cellular respiration and mitochondrial function was significantly enhanced during the thermogenic female stage, whereas genes involved in stress responses and protein degradation were significantly up-regulated during the non-thermogenic male stage (Ito-Inaba *et al.*, 2012 a). Therefore, changes in the intracellular structure observed in

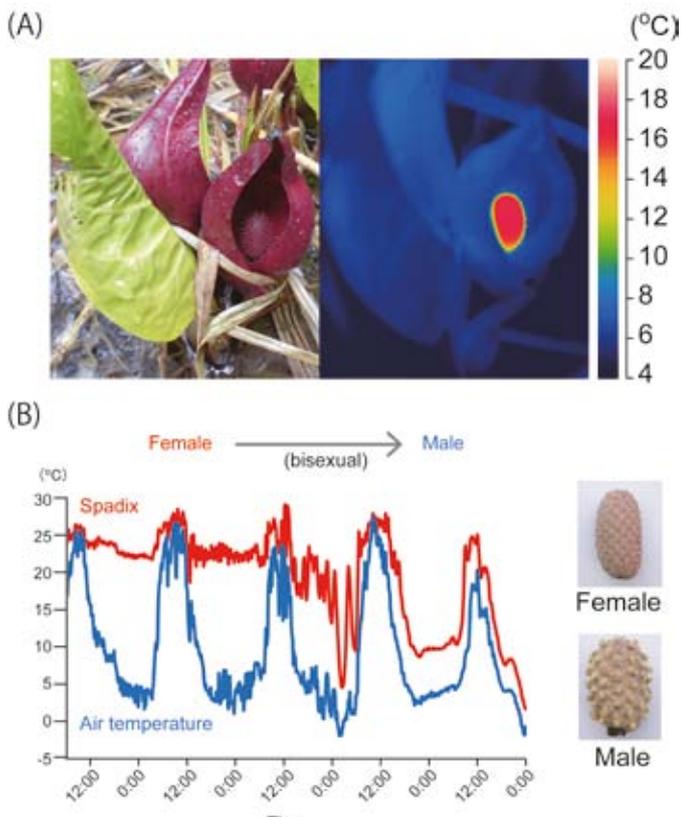


Fig. 1 - Thermoregulation in skunk cabbage (*S. renifolius*). (A) Skunk cabbages were photographed using a camera in the visible (left panel) and infrared spectra (right panel). The thermal image was taken with Thermotracer SC620 (FLIR). Heat production was observed in the spadix during the female stage of floral development. (B) The sequential changes in spadix (red) and air (blue) temperatures during floral development from the female to the male stage. Spadices at the female stage can maintain internal temperature at approximately 22–26°C, whereas spadices at the male stage cannot produce heat. Spadices at the bisexual stage between the female and male stages show unstable thermogenesis. Photographs of a female- and a male-stage spadix, are shown in the upper right and lower right panels, respectively. (B) was partially extracted from figure 1 in our previous paper (Ito-Inaba *et al.*, 2009 a).

petals or pistils during the female-male transition are well supported by changes in the transcriptome during inflorescence development.

Two processes may be important for thermoregulation in skunk cabbage (Ito-Inaba *et al.*, 2012 a). First, short-term mechanisms that depend on increased cellular respiration with the help of energy dissipating proteins, such as alternative oxidase (AOX) or uncoupling protein (UCP), may play an essential role in which AOX may have a more major function than UCP. Secondly, long-term effects of mitochondrial biogenesis on the number and structure of mitochondria probably are also involved. Following much effort to characterize the activity or expression of AOX during floral development, the pivotal role of this enzyme in floral thermogenesis was revealed (Watling *et al.*, 2006; Grant *et al.*, 2008; Wagner *et al.*, 2008; Ito-Inaba *et al.*, 2009 b; Miller *et al.*, 2011). However, the presence

of additional genes that are co-expressed with AOX and that may function directly or indirectly in thermogenesis remains to be clarified. In addition, although it has been hypothesized that heat-producing floral tissues contain many mitochondria, quantitative and comparative studies on mitochondrial content are lacking. In this review, we summarize our recent progress in describing changes in the ultrastructure and gene expression profiles during skunk cabbage floral development.

## 2. Thermogenesis and mitochondrial abundance

In mammalian cells, the positive correlation between metabolic activity and the number and size of mitochondria within a tissue is well established (Ghadially, 1988). Mammalian brown adipose tissue (BAT), which is the main site for non-shivering thermogenesis, contains considerable numbers of large mitochondria with abundant cristae. In contrast, these relationships are not well characterized in plants, and there are very few published papers that have examined the intracellular structure of thermogenic tissues by electron microscopy. In a well-known thermogenic plant, *Sauromatum guttatum* (voodoo lily), ultrastructural changes in the inflorescence during the transition from the pre- to post-thermogenic stages were extensively studied, and clear details of mitochondrial morphology were obtained (Skubatz *et al.*, 1993). In addition, during the thermogenic stage of *S. guttatum* floral development, mitochondria accumulated osmophilic material between the inner and outer membranes (Skubatz and Kunkel, 2000). In another thermogenic plant, *Philodendron selloum*, large lipid bodies present in sterile florets before heating were progressively depleted during heat generation, and the mitochondria often contained enlarged cristae during maximum heating (Walker *et al.*, 1983). However, there are no conclusive data indicating a relationship between heating in plant tissues and mitochondrial features, such as content or morphology.

We first analyzed the detailed changes in mitochondrial content and morphology during floral development of thermogenic skunk cabbage, *S. renifolius* (Ito-Inaba *et al.*, 2009 a). As shown in figure 2A, petal cells at the female stage contained a large number of mitochondria. By contrast, petal cells at the male stage contained only a small number of mitochondria but had large central vacuoles. In the pistil cells, likewise, a large number of mitochondria were present at the female stage but few mitochondria persisted to the male stage. Furthermore, stamens at the female stage, especially in the microspore and plasmodium, had high densities of mitochondria. The sizes and morphologies of mitochondria observed in all tissues varied. To evaluate the mitochondrial content quantitatively between the female and male stages in each floral tissue, the average mitochondrial density (mitochondrial numbers  $\mu\text{m}^{-2}$  cytosol) in thin sections of cells were analyzed in five to 10 cells. These data also revealed that both petals and pistils at the female stage contained larger numbers of mitochondria compared with the male stage. Details of the ultrastructure and the quantitative

data on mitochondrial content are described in our previous paper (Ito-Inaba *et al.*, 2009 a). We next compared the mitochondrial protein content recovered from thermogenic and non-thermogenic stages or tissues (Ito-Inaba *et al.*, 2009 a, b). As shown in figure 2B, the mitochondrial protein content of female-stage spadices (0.54 mg g<sup>-1</sup>) was two-fold higher than that of males (0.29 mg g<sup>-1</sup>), a value consistent with our electron microscopic data. In addition, mitochondrial protein content of non-thermogenic skunk cabbage, *Lysichiton camtschaticensis* (0.011 mg g<sup>-1</sup>), was much lower than that of *S. renifolius*. Since *L. camtschaticensis* has no ability to produce heat but has a close relationship with *S. renifolius* in morphology and phylogeny, this result suggests that a lower mitochondrial content may correlate with the lack of thermogenesis in *L. camtschaticensis*. Taken together, these results reveal that there is a positive correlation between heat production and the abundance of mitochondria in plant tissues and cells. These are the first quantitative data indicating differences in mitochondrial content between thermogenic and non-thermogenic stages or tissues. Therefore, plants might produce the massive heat from their tissues by increasing their mitochondrial density in a manner similar to mammalian BAT.

### 3. The quantitative gene expression profile in female- and male-stage spadices of *S. renifolius*

To understand the molecular basis of floral thermogenesis, we examined the gene expression profiles of female- and male-stage spadices of *S. renifolius*. Since the complete genome sequence of *S. renifolius* is not available, we took advantage of the super serial analysis of gene ex-

pression (SuperSAGE) methodology as this method can provide quantitative and comprehensive gene expression profiles (Ito-Inaba *et al.*, 2012 a). In our study, 26 bp tags (SuperSAGE tags) expressed from female- and male-stage spadices were prepared and sequenced using a 454 Life Sciences Genome Sequencer 20 System. Since the length of 26 bp tags is sufficient to identify the origin of a tag using cDNA databases (Matsumura *et al.*, 2003, 2011), each 26 bp tag was annotated based on our cDNA database of the female-stage spadices using the BioEdit program. The gene expression profiles obtained were subjected to cluster analysis to identify candidate sets of co-regulated genes directly or indirectly associated with the process of female- and male-stage spadices, and were qualified as a group of female- or male-stage specific genes. To further assess the function of each gene, AGI codes of *Arabidopsis* orthologs corresponding to the identified genes were obtained from the database of The Arabidopsis Information Resource (<http://www.arabidopsis.org/index.jsp>), and the identified genes were classified based on Gene Ontology (GO) terms using the AGI codes. This analysis allowed us to predict the localization and function of the orthologs in *S. renifolius*. Each gene was weighted according to the number of corresponding SuperSAGE tags that reflected the expression level of each gene.

Based on these methods, transcripts were assigned to specific cellular components or biological processes (Ito-Inaba *et al.*, 2012 a) and the major transcriptional changes are shown in figure 3. It was of particular interest that genes encoding mitochondrial proteins were actively transcribed in female spadices but not in male spadices (Fig. 3A). In addition, the activity of genes related to electron transport or energy pathways decreased significantly during the transition from the female to the male stage (Fig. 3B). These results suggest that mitochondrial function

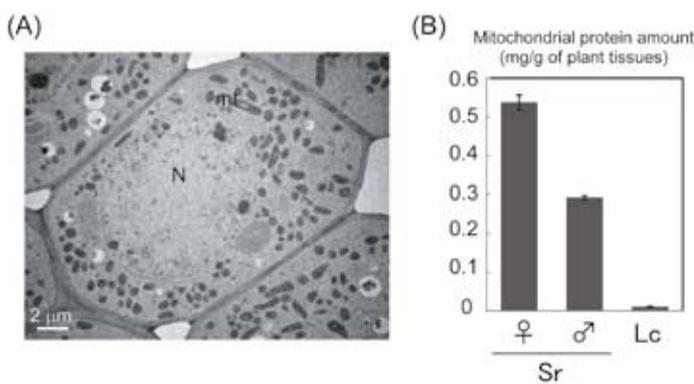


Fig. 2 - Abundant mitochondria are present in the spadix of thermogenic skunk cabbage. (A) Female spadix cells (petal tissues) contain many mitochondria. This photograph was adapted from Fig. 5A in our previous paper (Ito-Inaba *et al.*, 2009a). In this study, large numbers of mitochondria were also observed in pistils and in several tissues in stamens. (B) Quantitative comparison of mitochondrial protein amount from thermogenic and non-thermogenic stages or tissues. Female-stage spadices (♀) in *S. renifolius* (Sr) contain 2-fold and 50-fold higher content of mitochondria than male spadices (♂) and or spadices from *L. camtschaticensis* (Lc), respectively. Data was extracted from Table 2 and Table 1 in our previous papers (Ito-Inaba *et al.*, 2009 a, b, respectively).

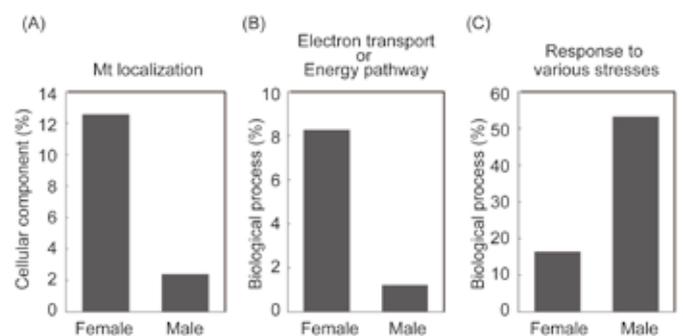


Fig. 3 - Examples of transcriptional changes of cellular components and biological processes of female- and male-stage spadices of *S. renifolius*. Genes encoding proteins localized in mitochondria (A) or that play roles in electron transport or the energy pathway (B) are highly expressed at the female stage, but not at the male stage of floral development. Genes encoding stress-responsive proteins (C) were highly expressed at the male stage, but not at the female stage. (A) was partially extracted from figure 3a (the cellular component data) in our previous paper (Ito-Inaba *et al.*, 2012 a). (B) and (C) were also partially extracted from figure 3b (the biological process data) in the same paper.

and/or cellular respiration play a key role in floral thermogenesis. This finding is consistent with our electron microscopic observation that the thermogenic female-stage spadix accumulates a large number of mitochondria and has an increased oxygen consumption rate. Furthermore, genes classified as stress responsive were highly expressed in male spadices (Fig. 3C). Of these genes, a gene encoding a cysteine protease in *S. renifolius*, designated as SrCPA, was the most abundant transcript in the spadices, and levels increased significantly during the female-male transition (Ito-Inaba *et al.*, 2012 b). This class of cysteine protease is involved in programmed cell death (Beyene *et al.*, 2006) and stress responses (Stevens *et al.*, 1996) in other organisms. Since our previous studies suggested that a parallel relationship exists between the increase in CP transcripts and vacuolar development in each of the various spadix tissues during the female-male transition, the high level of SrCPA expression may be correlated with vacuolar development in male-stage spadices. In addition, several stress-responsive genes and genes encoding degradative enzymes or ubiquitin-proteasome system components had increased expression levels at the post-thermogenic stage. Therefore, we hypothesize that cysteine protease and other degradative enzymes that leak from the vacuole may degrade mitochondria, thereby terminating thermogenesis at the male stage.

#### 4. Conclusions and Perspectives

Our previous electron microscopic study revealed that intracellular structures within the individual tissues change significantly during the transition from the female- to the male-stage spadix in *S. renifolius*. The mitochondrial content is reduced, especially in the petals and pistils, whereas the vacuolar volume increases during the female-male transition. Consistent with this cellular change, gene expression profiles analyzed using SuperSAGE methods indicated that the genes involved in cellular respiration and mitochondrial function are up-regulated in female-stage spadices, whereas the genes involved in stress responses and protein degradation are up-regulated in male-stage spadices. These observations suggest that the maintenance and termination of floral thermogenesis in the female- and the male-stage spadices, respectively, may be explained as shown in figure 4. At the female stage, the high expression levels of genes related to cellular respiration and mitochondrial function induce significant oxygen consumption and mitochondrial biogenesis, and activate cellular metabolism leading to substantial heat production. In contrast, at the male stage, the high expression levels of genes related to protein degradation and vacuolar metabolism induce senescence, programmed cell death, and vacuolar development, leading to the termination of heat production. After thermogenesis, the expression of several stress response genes, such as cold-inducible genes, increase because the spadix cannot produce any heat. With exposure to the cold air, the spadix cells proceed to senescence.

More than 200 years ago, pioneering studies on floral thermogenesis were undertaken in the European Arum (Araceae) by Lamarck (1778). Since then, heat production by the reproductive organs of several plants has been investigated. We anticipate that the numbers of plants known to produce heat will increase in the future as the subtle temperature differences between the air and plant bodies can be measured by technical advances in temperature probes or thermography. To study the molecular mechanisms underlying floral thermogenesis, two energy dissipating systems, an alternative oxidase (AOX) and uncoupling protein (UCP), have been the principal subjects of investigation (Vanlerberghe and McIntosh, 1997; Vercesi *et al.*, 2006; Zhu *et al.*, 2011). Because of the correlation between heat production and AOX concentration, as well as activity in several thermogenic plants (Grant *et al.*, 2008; Ito-Inaba *et al.*, 2009 b; Miller *et al.*, 2011), AOX rather than UCP has been assumed to control plant thermogenesis. Recently, the crystal structure of a trypanosomal AOX was reported (Shiba *et al.*, 2013). Since the post-translational regulation of AOX has been hypothesized to regulate the thermogenic capacity of this protein (Grant *et al.*, 2009), revealing the structural features of AOX may open the door to elucidating the mechanisms underlying the post-translational regulation of AOX. Furthermore, we anticipate that recent advances in next generation sequence (NGS) technology will uncover additional genes, besides AOX and UCP, that are involved in floral thermogenesis. In *S. renifolius*, the gene expression profile has already been studied using NGS technology combined with the SuperSAGE method and could provide valuable information to define the identity of female- and male-stage spadices at the molecular level (Ito-Inaba *et al.*, 2012 a). As far as we know, this was the first study in which the molecular mechanism underlying floral thermogenesis was analyzed using NGS technology. Quite recently, the genome of the sacred lotus (*Nelumbo nucifera*), a well-known thermogenic plant, was sequenced (Ming *et al.*, 2013). We also expect that this advance will accelerate study of the molecular mechanism underlying heat production in the reproductive organ development of

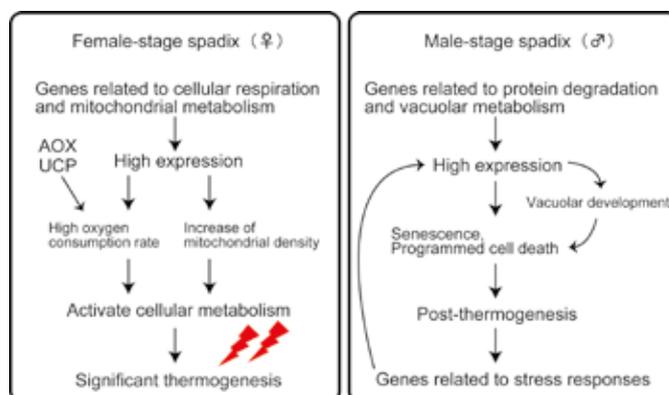


Fig. 4 - A proposed model of the possible processes in female- and male-stage spadices to maintain and terminate thermogenesis, respectively.

sacred lotus. *S. renifolius* is a monocot thermogenic plant, whereas *N. nucifera* is a eudicot thermogenic plant. Thus, comparative studies of these plants will reveal general and diverse aspects of floral thermogenesis.

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## References

- BEYENE G., FOYER C.H., KUNERT K.J., 2006 - *Two new cysteine proteinases with specific expression patterns in mature and senescent tobacco (Nicotiana tabacum L.) leaves.* - J. Exp. Bot., 57: 1431-1443.
- GHADIALLY F.N., 1988 - *Ultrastructural pathology of the cell and matrix.* - Butterworth-Heinemann, London, UK, 3rd Edition, pp. 194.
- GRANT N.M., MILLER R.E., WATLING J.R., ROBINSON S.A., 2008 - *Synchronicity of thermogenic activity, alternative pathway respiratory flux, AOX protein content, and carbohydrates in receptacle tissues of sacred lotus during floral development.* - J. Exp. Bot., 59: 705-714.
- GRANT N.M., ONDA Y., KAKIZAKI Y., ITO K., WATLING J.R., ROBINSON S.A., 2009 - *Two cys or not two cys? That is the question; alternative oxidase in the thermogenic plant sacred Lotus.* - Plant Physiol., 150: 987-995.
- ITO-INABA Y., HIDA Y., INABA T., 2009 b - *What is critical for plant thermogenesis? Differences in mitochondrial activity and protein expression between thermogenic and non-thermogenic skunk cabbages.* - Planta, 231: 121-130.
- ITO-INABA Y., HIDA Y., MATSUMURA H., MASUKO H., YAZU F., TERAUCHI R., WATANABE M., INABA T., 2012 a - *The gene expression landscape of thermogenic skunk cabbage suggests critical roles for mitochondrial and vacuolar metabolic pathways in the regulation of thermogenesis.* - Plant, cell & environment, 35: 554-566.
- ITO-INABA Y., MASUKO H., WATANABE M., INABA T., 2012 b - *Isolation and gene expression analysis of a papain-type cysteine protease in thermogenic skunk cabbage (Symplocarpus renifolius).* - Bioscience, Biotechnology, and Biochemistry, 76: 1990-1992.
- ITO-INABA Y., SATO M., MASUKO H., HIDA Y., TOYOOKA K., WATANABE M., INABA T., 2009 a - *Developmental changes and organelle biogenesis in the reproductive organs of thermogenic skunk cabbage (Symplocarpus renifolius).* - J. Exp. Bot., 60: 3909-3922.
- KNUTSON R.M., 1974 - *Heat production and temperature regulation in eastern skunk cabbage.* - Science, 186: 746-747.
- LAMARCK J., 1778 - *Flore Français.* - L'Imprimerie Royale, Paris, France, pp. 537-539.
- MATSUMURA H., REICH S., ITO A., SAITOH H., KAMOUN S., WINTER P., GÜNTHER KAHL G., REUTER M., KRÜGER D.H., TERAUCHI R., 2003 - *Gene expression analysis of plant host-pathogen interactions by SuperSAGE.* - Proc. Natl. Acad. Sci. U.S.A., 100: 15718-15723.
- MATSUMURA H., YOSHIDA K., LUO S., KRUGER D.H., KAHL G., SCHROTH G.P., TERAUCHI R., 2011 - *High-throughput SuperSAGE.* - Methods Mol. Biol. 687: 135-146.
- MEEUSE B.J.D., RASKIN I., 1988 - *Sexual reproduction in the arum lily family, with emphasis on thermogenicity.* - Sexual Plant Reproduction, 1: 3-15.
- MILLER R.E., GRANT N.M., GILES L., RIBAS-CARBO M., BERRY J.A., WATLING J.R., ROBINSON S.A., 2011 - *In the heat of the night - alternative pathway respiration drives thermogenesis in Philodendron bipinnatifidum.* - The New phytologist, 189: 1013-1026.
- MING R., VANBUREN R., LIU Y., YANG M., HAN Y., LI L.T., BOWERS J.E., TANG H., LYONS E., FERGUSON A.A., NARZISI G., NELSON D.R., BLABY-HAAS C.E., GSCHWEND A.R., JIAO Y., DER J.P., ZENG F., HAN J., MIN X.J., HUDSON K.A., SINGH R., GRENNAN A.K., KARPOWICZ S.J., WATLING J.R., ITO K., ROBINSON S.A., HUDSON M.E., YU Q., MOCKLER T.C., CARROLL A., ZHENG Y., SUNKAR R., JIA R., CHEN N., ARRO J., WAI C.M., WAFULA E., SPENCE A., HAN Y., XU L., ZHANG J., PEERY R., HAUS M.J., XIONG W., WALSH J.A., WU J., WANG M.L., ZHUYI J., PAULL R.E., BRITT A.B., DU C., DOWNIE S.R., SCHULER M.A., MICHAEL T.P., LONG S.P., ORT D.R., SCHOPF J.W., GANG D.R., JIANG N., YANDELL M., DEPAMPHILIS C.W., MERCHANT S.S., PATERSON A.H., BUCHANAN B.B., LI S., SHEN-MILLER J., 2013 - *Genome of the long-living sacred lotus (Nelumbo nucifera Gaertn.).* - Genome Biol., 14: R41.
- NAGY K.A., ODELL D.K., SEYMOUR R.S., 1972 - *Temperature regulation by the inflorescence of Philodendron.* - Science, 178: 1195-1197.
- SEYMOUR R.S., 2004 - *Dynamics and precision of thermoregulatory responses of eastern skunk cabbage Symplocarpus foetidus.* - Plant Cell and Environ., 27: 1014-1022.
- SEYMOUR R.S., BARTHOLOMEW G.A., CHRISTOPHER BARNHART M., 1983 - *Respiration and heat production by the inflorescence of Philodendron selloum Koch.* - Planta, 157: 336-343.
- SEYMOUR R.S., BLAYLOCK A.J., 1999 - *Switching off the heater: influence of ambient temperature on thermoregulation by eastern skunk cabbage Symplocarpus foetidus.* - J. Exp. Bot., 50: 1525-1532.
- SEYMOUR R.S., SCHULTZE-MOTEL P., 1998 - *Physiological temperature regulation by flowers of the sacred lotus.* - Philosophical Transactions of the Royal Society, B 353: 935-943.
- SEYMOUR R.S., SCHULTZE-MOTEL P., LAMPRECHT I., 1998 - *Heat production by sacred lotus flowers depends on ambient temperature, not light cycle.* - J. Exp. Bot., 49: 1213-1217.
- SHIBA T., KIDO Y., SAKAMOTO K., INAOKA D.K., TSUGE C., TATSUMI R., BALOGUN E.O., NARA T., AOKI T., HONMA T., TANAKA A., INOUE M., MATSUOKA S., SAIMOTO H., MOORE A.L., HARADA S., KITA K., 2013

- *Structure of the trypanosome cyanide-insensitive alternative oxidase*. - Proc. Natl. Acad. Sci. U.S. A., 110: 4580-4585.
- SKUBATZ H., KUNKEL D.D., 2000 - *Developmental changes in the ultrastructure of the Sauromatum guttatum (Araceae) mitochondria*. - J. Electron Microsc., 49: 775-782.
- SKUBATZ H., KUNKEL D.D., MEEUSE B.J.D., 1993 - *Ultrastructural changes in the appendix of the Sauromatum guttatum inflorescence during anthesis*. - Sexual Plant Reproduction, 6: 153-170.
- STEVENS C., TITARENKO E., HARGREAVES J.A., GURR S.J., 1996 - *Defence-related gene activation during an incompatible interaction between Stagonospora (Septoria) nodorum and barley (Hordeum vulgare L.) coleoptile cells*. - Plant Mol. Biol., 31: 741-749.
- UEMURA S., OHKAWARA K., KUDO G., WADA N., HIGASHI S., 1993 - *Heat-production and cross-pollination of the asian skunk cabbage Symplocarpus renifolius*. - American Journal of Botany, 80: 635-640.
- VANLERBERGHE G.C., MCINTOSH L., 1997 - *Alternative oxidase: From gene to function*. - Annu. Rev. Plant. Physiol. Plant. Mol. Biol., 48: 703-734.
- VERCESI A.E., BORECKY J., MAIA IDE G., ARRUDA P., CUCCOVIA I.M., CHAIMOVICH H., 2006 - *Plant uncoupling mitochondrial proteins*. - Annu. Rev. Plant Biol., 57: 383-404.
- WAGNER A.M., KRAB K., WAGNER M.J., MOORE A.L., 2008 - *Regulation of thermogenesis in flowering Araceae: the role of the alternative oxidase*. - Biochim. Biophys. Acta, 1777: 993-1000.
- WALKER D.B., GYSI J., STERNBERG L., DENIRO M.J., 1983 - *Direct respiration of lipids during heat production in the inflorescence of Philodendron selloum*. - Science, 220: 419-421.
- WATLING J.R., ROBINSON S.A., SEYMOUR R.S., 2006 - *Contribution of the alternative pathway to respiration during thermogenesis in flowers of the sacred lotus*. - Plant Physiol., 140: 1367-1373.
- ZHU Y., LU J., WANG J., CHEN F., LENG F., LI H., 2011 - *Regulation of thermogenesis in plants: the interaction of alternative oxidase and plant uncoupling mitochondrial protein*. - Journal of Integrative Plant Biology, 53: 7-13.