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| Author(s)   | Yazaki, Kazufumi   |
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**Isoprene emission from plants – its physiological role for plants -  
(Laboratory of Plant Gene Expression, RISH, Kyoto University)**

Kazufumi Yazaki

Isoprene is a volatile C<sub>5</sub> terpenoid that is released mainly from the leaves of many deciduous broad-leaved trees, such as *Salix*, *Quercus* and *Populus* species. The annual global emission of isoprene from these trees is estimated to be as high as 5 x 10<sup>14</sup> g of carbon, which is similar to the level of methane, the most abundant naturally emitted hydrocarbon. Isoprene has been suggested to potentially provide general protection against environmental stress, such as heat and water as well as to protect against singlet oxygen. It has been demonstrated that isoprene emission rates are correlated with photosynthetic photon flux densities and leaf development.

Recent studies have demonstrated that isoprene in plants is biosynthesized by isoprene synthase from DMAPP via MEP pathway. In poplar, foliar isoprene emission appears to depend on isoprene synthase activity, since its emission rate parallels enzyme activity according to temperature. Despite the purification of isoprene synthase proteins from several plants, isolation of its gene has only been reported from hybrid poplar (*Populus alba* x *P. tremula*) and *P. tremuloides* and *Pueraria montana*, and very little is known about its physiological role in plants; e.g. even its subcellular location has not yet been clarified.

To obtain biochemical and molecular biological insights into isoprene synthase, we cloned isoprene synthase cDNA from *Populus alba* (PaIspS) and studied gene expression in response to environmental stress [1]. Moreover, we examined the subcellular localization of PaIspS and also characterized its enzymatic function with a recombinant protein.

Isoprene synthase cDNA from *P. alba* (PaIspS) was isolated by RT-PCR. This *PaIspS*, which was predominantly observed in the leaves, was strongly induced by heat stress and continuous light irradiation, and was substantially decreased in the dark, suggesting that isoprene emission was regulated at the transcriptional level. The subcellular localization of PaIspS protein with GFP fusion was shown to be in plastids. PaIspS expressed in *Escherichia coli* was characterized enzymatically: it had an optimum pH of approximately 8.0, and an optimum temperature 40°C. Its preference for divalent cations for its activity was also studied, to show the strongest preference for Mg<sup>2+</sup> ion, while Mn<sup>2+</sup> and Co<sup>2+</sup> can be also accepted.

The predominant expression of PaIspS in leaves suggests that its enzyme activity may be positively regulated under illumination in plastids because photosynthetic electron transport results in the accumulation of Mg<sup>2+</sup> in the stroma, along with an increase in stromal pH. In addition to the transcriptional activation of PaIspS by light, this is advantageous for the production of isoprene under strong light conditions. The optimum temperature observed in this study is also in conformity with the fact that the highest isoprene emission occurred between 30°C and 40°C in nature. On the other hand, the treatment with methyl jasmonate or methyl salicylate did not influence *PaIspS* expression. This is in clear contrast to the results with pathogen-inducible terpenoid synthases such as *epi*-aristolochene synthase and indicates that isoprene emission does not play an important role as a defense reaction against insect and pathogen attack in poplar.

Isoprene is a very reactive hydrocarbon and is thought to rapidly react with hydroxy radicals in the atmosphere, which prolongs the lifetime of methane, resulting in the enhancement of a greenhouse effect in the atmosphere. PaIspS cDNA may be used as a molecular tool to suppress isoprene emission in high-emitting trees.

#### REFERENCES

- [1] Sasaki, K., Ohara, K. and Yazaki, K. (2005) FEBS Lett 579: 2514-2518.