

research highlights

LEAF DEVELOPMENT

Hole creation

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The Madagascan laceleaf plant (*Aponogeton madagascariensis*) is an aquatic plant popularly used in aquariums due to its decorative leaves consisting of a meshwork of holes. These holes form by the death of regular patches of cells leaving an edge about five cell layers wide around the leaf veins. Adrian Dauphinee of Dalhousie University, Halifax, Canada, and colleagues, have investigated how a balance between antioxidants and reactive oxygen species (ROS) creates this patterning.

At first all the cells in an *A. madagascariensis* leaf are darkly coloured due to the presence of anthocyanins. However, the cells farthest from the veins progressively lose their pigmentation forming transparent 'windows' before dying completely leaving the characteristic holes. Cells closer to the veins retain their pigmentation and continue to



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develop normally creating a sharp boundary at the hole's edge. Dauphinee *et al.* discovered that growing *A. madagascariensis* in the presence of the antioxidants acetic acid and cysteine greatly reduced the number of holes that formed in its leaves and that the presence of holes was somewhat recovered by the further addition of hydrogen peroxide to the growth medium.

Staining of developing cells with nitro blue tetrazolium (NBT) revealed higher levels of superoxide in cells destined for death, while immune assays showed that these cells are deficient in two antioxidant enzymes: superoxide dismutase 1 (SOD1) and catalase (CAT). These enzymes, and the high concentrations of anthocyanins that can be seen by eye, protect cells closer to the leaf veins from oxidative stress. Lacking sufficient protection, cells further from the veins mount a programmed cell death response involving breakdown of mitochondria and chloroplasts that further increases the oxidative stress.

It remains unclear whether the leaf veins are releasing some form of mobile signal to establish this pattern of antioxidants, but this study establishes that the positive feedback inherent in programmed cell death creates the sharp border around the holes and thus the tracery of these elegant leaves.

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