The gymnosperm *Pinus pinea* contains both *AOX* gene subfamilies, *AOX1* and *AOX2*

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ABSTRACT

The gymnosperm *Pinus pinea* L. (stone pine) is a typical Mediterranean pine used for nuts and timber production, and as an ornamental around the world. Pine genomes are large in comparison to other species. The hypothesis that retrotransposons, such as *gymny*, made a large contribution to this alteration in genome size was recently confirmed. However, *P. pinea* is unique in other various aspects. *P. pinea* demonstrates a different pattern of *gymny* organization than other *Pinus* subgenera. Additionally, *P. pinea* has a highly recalcitrant behaviour in relation to standard conifer protocols for the induction of somatic embryogenesis or rooting. Because such types of cell reprogramming can be explained as a reaction of plant cells to external stress, it is of special interest to study sequence peculiarities in stress-inducible genes, such as the alternative oxidase (*AOX*). This is the first report containing molecular evidence for the existence of *AOX* in gymnosperms at the genetic level. *P. pinea AOX*s were isolated by a polymerase chain reaction (PCR) approach and three genes were identified. Two of the genes belong to the *AOX1* subfamily and one belongs to the *AOX2* subfamily. The existence of both *AOX* subfamilies in gymnosperms is reported here for the first time. This discovery supports the hypothesis that *AOX1* and *AOX2* subfamilies arose prior to the separation of gymnosperms and angiosperms, and indicates that the *AOX2* is absent in monocots because of subsequent gene loss events. Polymorphic *P. pinea AOX1* sequences from a selected genetic clone are presented indicating non-allelic, non-synonymous and synonymous translation products.

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