

## Superoxide generation in seeds of the seagrass *Zostera marina*

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### Abstract

*Zostera marina* is a flowering plant that forms seagrass beds in shallow coastal regions. Seagrass beds have an important ecological role, but have been reduced by recent changes in coastal environments. Therefore, restoration projects have initiated. The first step in the restoration process is that of collecting seeds with a high germination rate. However, the viability of *Z. marina* seeds declines rapidly during storage because of continued respiration after seed maturation. Superoxide ( $O_2^-$ ) is a factor in seed aging, although it also acts as a signal for seed germination. This study investigated the mechanism of  $O_2^-$  generation in aged and fresh *Z. marina* seeds.

Seeds from Hakata Bay, Fukuoka, were provided by TOYO Construction. Seeds obtained in 2016 to 2018 had low and high germination rates and were defined as aged and fresh seeds, respectively. Seed respiration was monitored with an optical oxygen sensor. Since aged and fresh seeds stained positively with the  $O_2^-$  indicator nitro blue tetrazolium (NBT), the metabolic process involved in  $O_2^-$  production was examined using various inhibitors.

In seeds,  $O_2^-$  is produced via three pathways: via plasma membrane localised NADPH oxidase and mitochondrial respiration; by alternative oxidase (AOX); or by cytochrome c oxidase (COX). Potassium cyanide (KCN) and salicylhydroxamic acid (SHAM) are specific inhibitors of COX and AOX, respectively. Measurements of the respiratory activity of seeds exposed to specific inhibitors revealed that fresh seeds have both COX and AOX activity. KCN and SHAM did not inhibit NBT staining of fresh seeds, while DPI, an inhibitor of NADPH oxidase, did. These results suggest that  $O_2^-$  is predominantly produced by NADPH oxidase in fresh seeds. In aged seeds, NBT staining was not inhibited by KCN or DPI, but inhibited by SHAM, suggesting that AOX respiration is involved in  $O_2^-$  production in aged seeds. In conclusion, the  $O_2^-$  generating process may change through seed storage.