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Research article

# Improved tolerance to transplanting injury and chilling stress in rice seedlings treated with orysastrobin

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ABSTRACT

In addition to their fungicidal activity, strobilurin-type fungicides are reported to show enhancing effects on crop growth and yield. Previous studies suggested that the fungicide has a mitigating effect on abiotic stresses. However, there are few reports about growth enhancement through abiotic stress alleviation by strobilurin-type fungicides, but the mechanism of action of the growth enhancement is still not clear. The present study revealed that orysastrobin enhanced rice seedling growth after root cutting injury and chilling stress. We also found that orysastrobin decreased the transpiration rate and increased ascorbate peroxidase and glutathione reductase activities. This stress alleviation was eliminated by the application of naproxen, a putative abscisic acid biosynthesis inhibitor. These results suggested that orysastrobin improved tolerance against transplanting injury and chilling stress in rice seedlings by inducing water-retaining activity through the suppression of transpiration, and also by inducing reactive oxygen scavenging activity thus inhibiting reactive oxygen species accumulation.



## 1. Introduction

Abiotic stresses such as drought, salinity, and high and low temperatures decrease plant growth and crop production. Developing new technologies to overcome abiotic stresses is a great challenge in the future of crop protection. There have been several studies reporting the improvement of abiotic stress tolerance by changing phytohormone content such as abscisic acid (ABA) (Iuchi et al., 2001) and by inducing increased antioxidant capacity (Uchida et al., 2002; Badawi et al., 2004; Lisko et al., 2013). Regulation of phytohormone content and antioxidant capacity might become a new technology to reduce abiotic stress in crops.

Strobilurin-type fungicides were developed from a natural fungicidal compound, strobilurin A. This type of strobilurin fungicide has the largest market share around the world (Gerwick and Sparks, 2014). In addition to their fungicidal activity, these fungicides exhibit growth enhancement activity in field conditions (Köhle et al., 2002; Ruske et al., 2003; Nelson and Meinhardt, 2011). Grossmann et al. (1999) reported that kresoxim-methyl, a strobilurin-type fungicide, delayed the senescence of wheat through a reduction in 1-amino cyclopropane-1-carboxylic acid (ACC) content. ACC is a precursor of ethylene, which is known to mediate plant senescence. The report also indicated that kresoxim-methyl increased ABA content and reduced water consumption. Using pyraclostrobin, both the enhancement of superoxide dismutase (SOD; EC 1.15.1.1) activity and a reduction in ozone injury was reported (Jabs et al., 2002). Azoxystrobin is known to increase antioxidant enzyme activities such as SOD, catalase (EC 1.11.1.6), and ascorbate peroxidase (APX; EC 1.11.1.11) (Wu and Von Tiedemann, 2002), and trifloxystrobin was reported to enhance abiotic stress tolerance of red pepper plants (Han et al., 2012).

Considering the current understanding, strobilurin-type fungicides might have abiotic stress-alleviation effects. However, there are few reports about the alleviation effects of strobilurin-type fungicides on transplanting injury and chilling stress in rice. In addition, the relationship between physiological effects and growth enhancement by strobilurin-type fungicides is still not clear. This study was conducted to investigate the effects of orysastrobin, which is a strobilurin-type fungicides used for controlling rice blast and sheath blight, on rice growth in response to root cutting stress or chilling stress conditions and the mechanisms of stress alleviation.

*Abbreviations:* ABA, abscisic acid; ACC, 1-amino cyclopropane-1-carboxylic acid; SOD, superoxide dismutase; APX, ascorbate peroxidase; FW, fresh weight; DW, dry weight; AsA, ascorbic acid; GR, glutathione reductase; ROS, reactive oxygen species.

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## 2. Materials and methods

### 2.1. Bioassay in a nursery box

Rice (*Oryza sativa* L. cv. Koshihikari) seeds were soaked in distilled water and kept in the dark at 20 °C for 5 days followed by 30 °C for 1 day. Germinated seeds were sowed in a nursery box (10 cm × 7 cm × 4 cm) containing 150 mL of commercially available culture soil for rice. Before sowing, orysastrobin granules (7.0% (w/w)) were mixed with soil at the recommended rate. The seeds were covered with 50 mL of culture soil. The seedlings were grown in greenhouse and the pots were watered as needed. At the 4th leaf stage, the seedlings were transplanted into 500 mL plastic pot filled with soil, and watered in a manner to imitate a paddy field. The seedlings were grown to the 5th leaf stage and shoot length of the seedlings was measured.

### 2.2. Hydroponic culture and chemicals

Rice (*Oryza sativa* L. cv. Nipponbare) seeds were soaked in distilled water and kept in the dark at 30 °C for 3 days. The germinated seeds were grown with nutrient solution to the 2.5-leaf stage in a growth chamber (light/dark, 13 h/11 h, 25°C/20 °C). The composition of the nutrient solution (pH 5.5) was 8 ppm N, 8 ppm $P_2O_5$, 8 ppm $K_2O$, 8 ppm CaO, 2 ppm MgO, 1.4 ppm $Fe_2O_3$, 0.1 ppm Mn, 0.01 ppm B, 0.01 ppm Mo, 0.004 ppm Cu, and 0.01 ppm Zn or the half of this concentration (Yonezawa et al., 2015). The seedlings were transferred into plastic pots filled with nutrient solution containing orysastrobin technical and/or naproxen. The plants were grown for a further 8 days.

### 2.3. Bioassays in root cutting and chilling stress conditions

To determine the alleviation effect on transplanting injury, roots of orysastrobin-treated seedlings were excised by retaining 1 cm, and grown with nutrient solution without orysastrobin for 3 days.

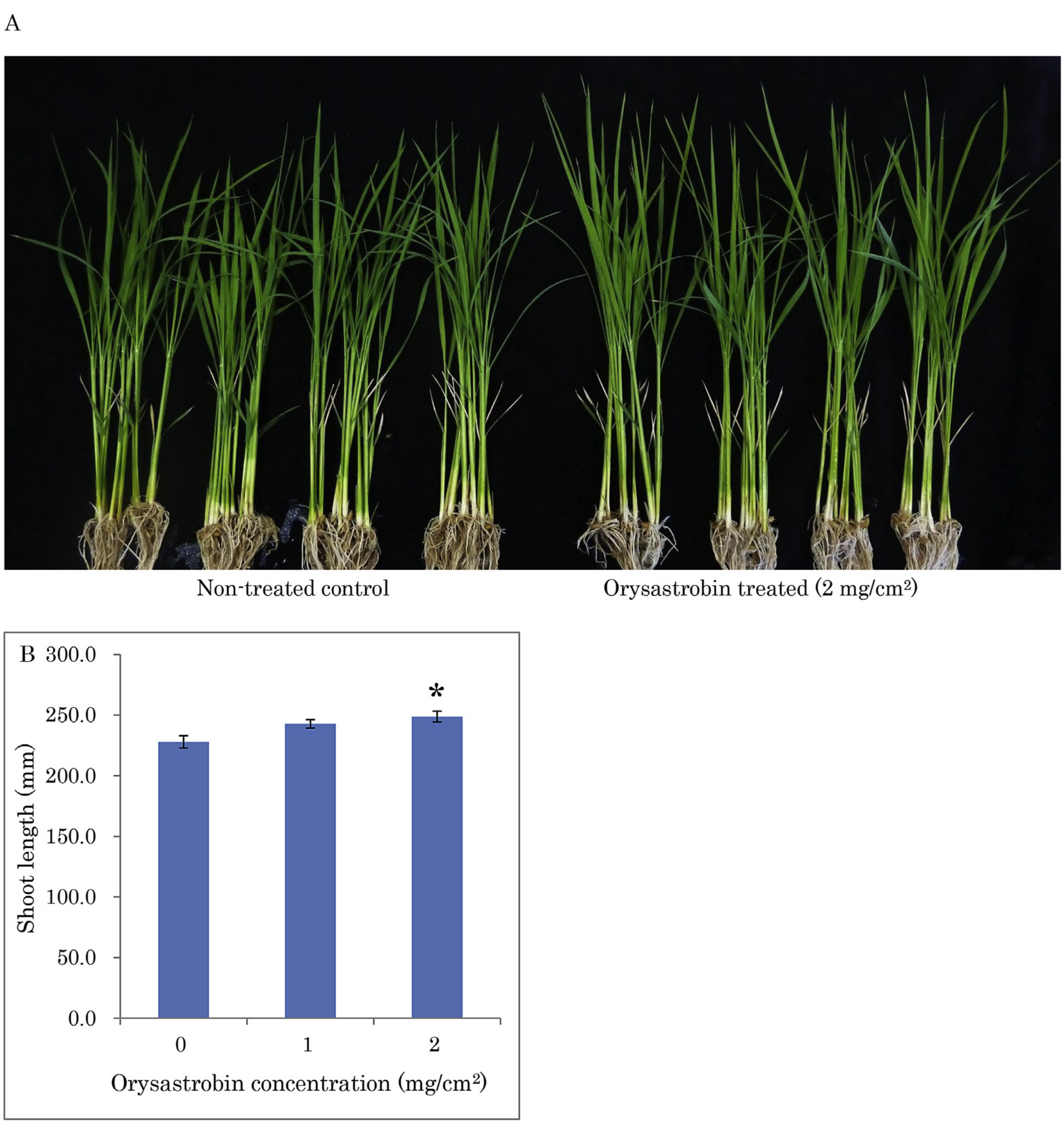


**Fig. 1.** Effect of granulated orysastrobin on shoot length of rice seedlings after transplanting.
(A) Granular formulation orysastrobin was mixed with soil. Germinated rice seeds were sown and grown to the four-leaf stage. The seedlings were transplanted into 500 mL plastic pots mimicking a rice paddy and grown to the five-leaf stage. (B) Shoot lengths of the seedlings were measured.
n = 4, Bars indicate ±S.E., $^*P < 0.05$ according to Dunnett's test (control vs. orysastrobin treatment).