Oxidative Stress and Chilling Injury of Mungbean Seedlings

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Keywords: *Vigna radiata*, high oxygen, hydrogen peroxide, radicle length, chilling

Abstract

Mungbean (*Vigna radiata* (L.) Wilczek 'AC Harosprout') seedlings were used to test the hypothesis that moderate oxidative stress offers protection against chilling injury. Chilling inhibits subsequent radicle growth. Decreased radicle elongation at 25°C after 1 to 4 days of chilling at 2.5°C was used as a measure of chilling injury. Exposure of seedlings with 3-5 mm long radicles to pure oxygen (O_2) for 24 hours (during which time the radicle grew to 10-15 mm in length) induced partial resistance to 1 or 2 days of chilling, but had no effect on seedlings chilled for 3 or 4 days. Soaking seedlings with 5 mm radicle in 0.1% hydrogen peroxide (H_2O_2) for 45 minutes decreased the radicle length of chilled and non-chilled seedlings alike. The H_2O_2 treatment had no effect on chilled and non-chilled seedlings when chilling was delayed until the radicles of H_2O_2 treated seedlings had grown to 10 mm in length. These preliminary results indicated a possible role of moderate stress in inducing partial tolerance to chilling.

INTRODUCTION

Mungbean sprouts are a common ingredient in oriental cuisine and are very perishable. The recommended storage temperature of 0°C (Gross et al., 2004) causes chilling injury (Chang et al., 2001). Partial tolerance to chilling injury can be induced in other germinated seeds and seedlings by a number of pre-chilling treatments, which include abiotic and oxidative stresses (Saltveit, 2001; Wesmer, 2003). Abiotic stresses, such as moderate heat shocks, induce elevated levels of antioxidants, as do oxidative stresses (Kang and Saltveit, 2001). Oxidative stress caused by exposure to high oxygen (Zheng et al., 2003) or hydrogen peroxide (Al-Haddad and Al-Jamali, 2003) induces chilling tolerance.

Mungbean seedlings were used as a model plant to study effects of moderate oxidative stresses on resistance to chilling injury.

MATERIALS AND METHODS

Plant Material

Seeds of mungbean (*Vigna radiata* (L.) Wilzek 'AC Harosprout') were used for all experiments (Park and Anderson, 1997). Seeds were soaked in aerated water for about 24 hours. Germinated seeds with 3-5 mm radicles were placed on the surface of capillary mats in a line about 1 cm below a short edge of the mat. The mats were sandwiched between two Plexiglas sheets (130 x 70 x 3 mm) as described by Mangrich and Saltveit (2000). The units were oriented vertically with the seedlings at the top. Each unit held 6 seedlings on each of the sides, resulting in 12 seedlings per unit. Each unit constituted one replicate.

Oxygen Treatments

1. Experiment 1. Ten units were placed in a sealed 11.5 L round plastic container (27 cm diam. x 22 cm), and flushed with air or 100% oxygen (O_2) at 300 ml/min for 24 h at

Proc. 5th Int. Postharvest Symp.
Eds. F. Mencarelli and P. Tonutti
Acta Hort. 682, ISHS 2005

approximately 20°C. After flushing, two units were placed in a covered rectangular plastic container (32 x 22.5 x 17 cm) and placed in a growth chamber at 25°C (0 days chilled). The other 8 units were placed in a 2.5°C cold room. Two units were placed in the 25°C growth chamber after 1, 2, 3 or 4 days of chilling. A mark was made on the clear Plexiglas sheet covering the seedlings above the tip of each radicle. Radicle length was measured to the nearest mm periodically for the next 4 days.

2. Experiment 2. One unit each was flushed with one of four treatment combinations: (1) Air-Air, (2) Air-O_2, (3) O_2-Air, or (4) O_2-O_2 for two consecutive flushings of 12 h each. Each unit was placed in cold room at 2.5°C for 0, 2, 3, or 4 days and then at room temperature (20°C) for 4 days to observe the growth of radicles. There was one unit per replicate. The experiment was repeated once, and the data were combined.

Hydrogen Peroxide Treatments

Germinated seeds with 3-5 mm radicles were placed in a Petri dish with 5 ml of water or 0.1% H_2O_2 (v/v). After 45 minutes, seedlings were placed on the capillary mats as described above, the Plexiglas sheets assembled, and the sandwich held at room temperature for 0 or 24 h. Chilling treatments and growth measurements were the same as those described above. There were two units per replicate.

Data Analysis

Radicle lengths at each growth measurement were analyzed according to a two way analysis of variance (ANOVA) with factors of treatment and duration of chilling. Growth measurements were regressed over time and the linear slope was designated as the growth rate. The decreased growth rate was used as the measure of chilling injury as described by Mangrich and Saltveit (2000). All values reported include the mean and standard deviation of each treatment.

RESULTS AND DISCUSSION

Oxygen Treatment

1. Experiment 1. The radicle growth rate was increased by the O_2 treatment in seedlings chilled for 0 to 2 days, but this induced chilling resistance was not evident in seedlings chilled for 3 or 4 days (Fig. 1). The response was variable, with O_2 flushing producing varying levels of protection or no protection in a subsequent replicate experiment (data not shown). It has been reported that high O_2 (>60%) treatments applied for the duration of a 35-day storage period increased antioxidant levels and reduced decay in blueberry (Zheng et al., 2003). In our study, however, a 24 h pre-treatment with pure O_2 produced variable results and a maximum reduction in injury equivalent to one day of chilling, which may be insufficient protection for practical application.

2. Experiment 2. The radicle growth rate of 2-day chilled seedlings was increased by either O_2-Air or O_2-O_2 (Fig. 2). The promotive effects persisted, but were less effective with 3- or 4-day chilled seedlings. The treatment of 1-day chilling was not included in this experiment, because little injury was observed with 1-day chilling in preliminary runs (data not shown). Therefore, it is impossible to compare the growth rate of these two O_2-treated, 2-day chilled seedlings with that of Air-treated, 1-day chilled seedlings. The promotive effect of the O_2 flush was more apparent in Experiment 2 than Experiment 1. This may have been attributed to the growth temperature of 20°C used in Experiment 2, which is less likely to produce a heat shock effect than the 25°C growth temperature of Experiment 1.

Hydrogen Peroxide Treatments

Growth rates of 0 to 4 day chilled radicles were reduced by H_2O_2 pre-soaking (Fig. 3a). There was no observed effect of chilling for up to four days, when the chilling was imposed on 5 mm radicles. In the replicate experiment, H_2O_2 increased the growth rate for 0-day chilling, had no effect for 1-day chilled seedlings, and reduced the growth rate

for 2- and 3-day chilled seedlings (Fig. 3b). The effect of chilling was apparent when the chilling was imposed on a radicle length of 10 mm.

There was no observed effect of H_2O_2 in our experiments. Chilling sensitive tomato seedlings sprayed with 0.5M H_2O_2 survived better and had lower death incidence as compared to control, when the experiment was conducted in a cooler (incubator) at 4°C (Al-Haddad and Al-Jamali, 2003). However, the beneficial effect of H_2O_2 spray was not observed in their repeated experiment under the same conditions. When the seedlings were stored in a large (storage) room, the H_2O_2 sprayed seedlings had less wilting and leaf necrosis than control seedlings.

CONCLUSIONS

The results of this study showed the possible benefits of an O_2 flush in prevention of chilling injury in mungbean seedlings. The prevention is not of high magnitude or consistency, with most benefit observed after 2-day chilling with the O_2 flush. In addition, the variability in radicle growth in mungbean seedlings was very apparent. Although the effect of O_2 was much more easily observed, long-term flushing would be difficult in commercial practice. Further research is needed to confirm these potential benefits.

ACKNOWLEDGEMENTS

We thank Glenn Block for highly skilful assistance in experimentation.

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Figures

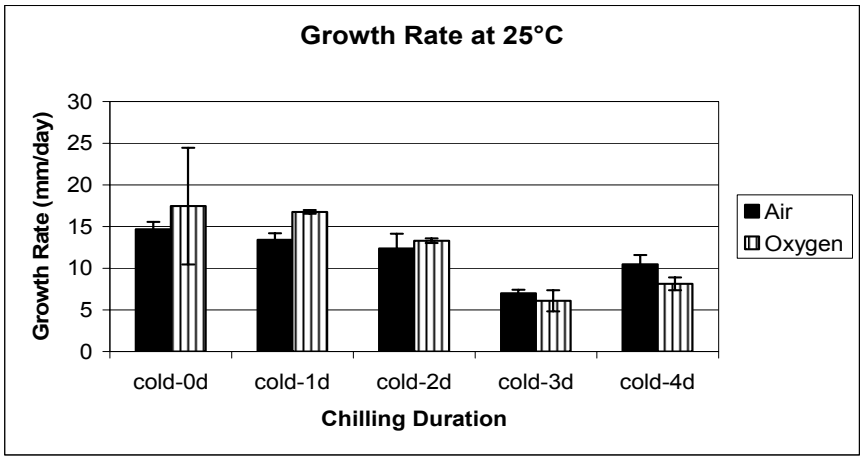

Fig. 1. The effect of O_2 flushing for 24 h prior to chilling at 2.5°C on subsequent growth rates at 25°C of radicles of mungbean seedlings (Experiment 1).

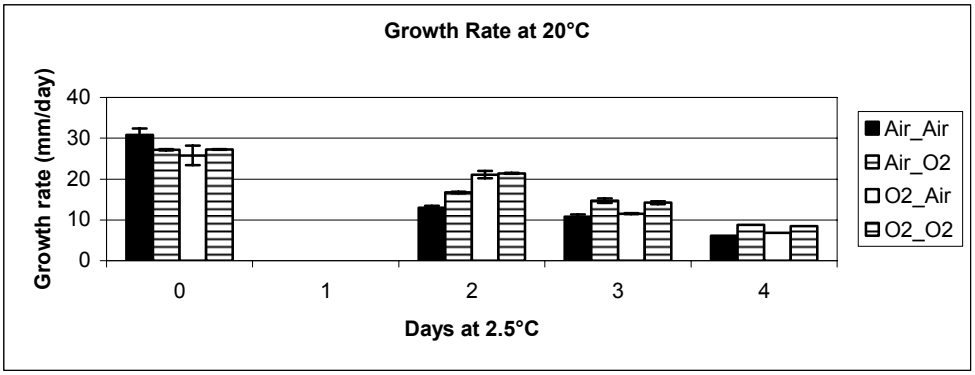

Fig. 2. Effect of O_2 flush for two periods of 12 hours each prior to chilling at 2.5°C on growth rates at 20°C of radicles of mungbean seedlings (Experiment 2).

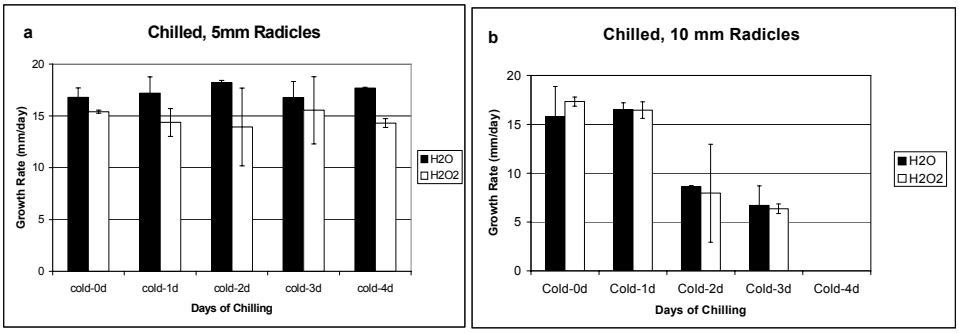

Fig. 3. Effect of hydrogen peroxide (0.1% H_2O_2, v/v) presoaking for 45 minutes prior to chilling at 2.5°C on growth rates of radicles of mungbean seedlings. Seedlings were chilled at 5 mm radicle length in the main experiment (Fig. 3a), or at 10 mm radicle length in the duplicate experiment (Fig. 3b).