

Full Length Research Paper

## Effects of solid matrix priming with *Trichoderma harzianum* on seed germination, seedling emergence and photosynthetic capacity of eggplant

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The effects of solid matrix priming with strain T22 of *Trichoderma harzianum* on seed germination, seedling emergence and photosynthetic capacity in two eggplant varieties were evaluated. Seed were primed by mixing with vermiculite and the *T. harzianum* wettable powder (WP) suspension at a rate of 1:3:3.2 (fresh weigh basis, respectively) at 15°C for 7 days in the dark, then primed seed were dried. Solid matrix priming (SMP) with *T. harzianum* increased seed germination vigor, germination index and seedling emergence, decreased mean emergence time, enhanced seedlings quality and photosynthetic characteristics of eggplant. The result indicates that SMP with *T. harzianum* could serve as an effective way to improve seeds germination and seedling emergence and promote plant growth of eggplant.

**Key words:** Solid matrix priming, *Trichoderma harzianum*, germination, seedling vigor, photosynthetic capacity, eggplant.

### INTRODUCTION

Optimum eggplant seed germination and seedling emergence occur at relatively high temperatures (25 to 30°C). Poor germination and delayed seedling emergence are common phenomenon at sub-optimal temperatures which is a great concern of growers that grow eggplant especially in late winter and early spring in China. Rapid seed germination and seedling emergence are important determinants for successful establishment of plants for crop production. In order to improve germination and seedling growth, several approaches including hardening,

seed priming, seed soaking and seed coating have been used to precondition seeds (Guo et al., 2012). Seed priming is widely used to enhance seed vigor in terms of germination potential and increased stress tolerance.

*Trichoderma* species have been known as biocontrol agents and plant growth stimulators that are widely used as seed treatments to control diseases and to enhance plant growth and yield (Sophie and Rudy, 1996; Nagaraju et al., 2012). Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic and physiological stresses in

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seed germination and seedling development (Mastouri et al., 2010). The beneficial effects of seed primed with *T. harzianum* have been reported in sunflower, tomato, carrot and onion seed (Nagaraju et al., 2012; Mastouri et al., 2010; Harman and Taylor, 1988). The effect of eggplant seed primed with *T. harzianum* has not been reported.

Solid matrix priming (SMP) simulates the natural imbibition processes taking place in the soil by the treatment of seeds with organic or inorganic solutions (TeKrony, 2001). The seed is placed on or mixed with the hydrating substrate which gradually moisturizes the seed. SMP has several advantages including ease of handling, better aeration, reduced costs and the possibility of integrated by adding chemical or biological agents such as *Trichoderma* spp. known to improve seed performance (Paparella et al., 2015). Strain T22 of *T. harzianum* generally increases plant growth and development and controls diseases and has been in commercial use in China. The objectives of this study were to evaluate seed germination, seedling emergence and subsequent growth, and photosynthetic parameters in eggplant seeds combining SMP with strain T22 of *T. harzianum*.

## MATERIALS AND METHODS

### Plant

Two eggplant cultivars 'Moxige' and 'Sanyue Zaoqie' were evaluated in this study. Seeds of the cultivars were supplied by the Shanghai Ruiqi Seed Corporation and Shanghai Agricultural Science and Technology Seed Co. Ltd., China. Seeds were disinfested in 0.3% v/v sodium hypochlorite solution for 5 min to eliminate seed-borne pathogens and then washed thrice thoroughly with distilled water, and air-dried by spreading the seed on absorbent paper at room temperature.

### Solid matrix priming

Strain of *T. harzianum* was supplied by the Beijing Coway BioWorks Biotech Co., Ltd. as a wettable powder (WP) with an active ingredient concentration of  $3 \times 10^8$  CFU/g. For SMP, the *T. harzianum* WP was suspended in water at  $4.9 \times 10^8$  CFU/L. Seeds for the two eggplant cultivars were mixed with vermiculite and the *T. harzianum* WP suspension at a rate 1:3:3.2 (fresh weigh basis, respectively) and placed in individual boxes. SMP was conducted at 15°C for 7 days in the dark, and then primed seed were dried at 25°C for 3 days. Non-primed seed was used as control.

### Seed germination

Primed and non-primed seeds for each cultivar were spread on two layers of filter paper and placed in covered 12×12 cm germination boxes. Treatments were arranged in a completely randomized design with four replications of 50 seeds. Germination tests were conducted in a germination cabinet at 30°C light/25°C dark under a 14 h photoperiod for two cultivars. For Moxige, germination tests also were conducted at 25°C light/20°C dark and 20°C light/15°C dark under a 14 h photoperiod. Radical protrusion to 1 mm was scored as germinated. Germination was recorded daily for 14 days. At the 14th day, a total of 40 plants were randomly selected from

each treatment (10 plants replicate<sup>-1</sup>) uprooted and weight, and finally measured. From the total number of seeds that germinated, the germination vigor (GV), final germination percentage (FGP), germination index (GI), and vitality index (VI) were calculated as described subsequently.

$$GV (\%) = (n_t/N) \times 100$$

Where,  $n_t$  is the cumulative number of germinated seeds at the 3rd day (30°C light/25°C dark) and 5th day (25°C light/20°C dark and 20°C light/15°C dark). N is the number of seeds for germination.

$$FGP (\%) = (n_{14}/N) \times 100$$

Where,  $n_{14}$  is the cumulative number of germinated seeds at the 14th day.

$$GI = \sum (Gt/Dt)$$

Where, Gt is the number of germinated seeds in t days; Dt is the number of corresponding germination days.

$$VI = GI \times S$$

Where, S is the average weight of 10 fresh seedling.

### Seedling emergence

Seeds for the four treatments were planted at 1.5 cm depth into plug cell trays filled with growth medium consisting of peat and vermiculite in a ratio of 2:1. Treatments were replicated four times using 2000 seed per treatment and arranged in a randomized complete block design in the cell tray. The cell tray was placed in a glasshouse in Shanghai Nanhui and watered daily. Daily minimum and maximum air temperatures were recorded until the end of emergence. The maximum temperature ranged from 21 to 36°C whereas minimum temperature ranged from 13 to 18°C. Emergence counts and date were recorded daily. Emergence percentage and emergence time were calculated. Twenty-five days after planting, when the percentage of emergence had stabilized in all treatments, the numbers of seedlings that emerged and lived were recorded. 120 plants randomly selected from each treatment (30 plants replicate<sup>-1</sup>) were uprooted and washed with DW. After drying with filter paper, data for fresh weights and seedling height were recorded. At the 65th days after sowing, samples were dried at 70°C for 7 days and dry weights also recorded.

The final emergence percentage (FEP), mean emergence time (MET), and emergence index (EI) were calculated according to the following formulas:

$$FEP (\%) = (n_{25}/N) \times 100$$

Where,  $n_{25}$  is the cumulative emergence number at 25th days after planting.

$$MET = \sum tn / \sum n$$

Where, n is the number of seeds which were germinated on the t day.

$$EI = \sum (Et/Dt)$$

Where, Et is the number of emergence seeds in t days; Dt is the number of corresponding germination days.

### Leaf gas-exchange measurements

Net rate of photosynthesis (A), transpiration rate (E) and stomatal

**Table 1.** The effects of SMP with *T. harzianum* on seed germination for two eggplant varieties (30°C light/25°C dark).

Cultivars	Treatment	Germination vigor (%)	Final germination (%)	Germination index	Vitality index
Moxige	Control	82.7	96.7	14.5	0.6
	Primed	93.0*	99.0	16.3*	0.9*
Sanyue zaoqie	Control	42.0	85.0	10.5	0.5
	Primed	92.0*	94.0*	15.7*	0.8*

\*Significant between primed seed and the control seed at  $P < 0.05$ .

**Table 2.** The effects of SMP with *T. harzianum* on Moxige seed germination at low temperature.

Temperature	Treatment	Germination vigor (%)	Final germination (%)	Germination index	Vitality index
20°C (light)/15°C (dark)	Control	0	95.0	7.0	0.1
	Primed	84.0*	98.0	13.9*	0.4*
25°C (light)/20°C (dark)	Control	72.5	95.3	9.6	0.3
	Primed	94.0*	100.0	16.1*	0.5*

\*Significant between primed seed and the control seed at  $P < 0.05$ .

**Table 3.** The effects of SMP with *T. harzianum* on seedling emergence and growth for two eggplant varieties at 25th days after sowing.

Cultivars	Treatment	Emergence (%)	Mean emergence time (D)	Seedling emergence index	Seedling height (mm)	Fresh weight of 40 seedlings (g)
Moxige	Control	72.8 <sup>c</sup>	14.2 <sup>b</sup>	19.3 <sup>d</sup>	18 <sup>c</sup>	4.7 <sup>b</sup>
	Primed	89.5 <sup>a</sup>	12.5 <sup>c</sup>	36.9 <sup>a</sup>	25 <sup>a</sup>	5.8 <sup>a</sup>
Sanyue Zaoqie	Control	78.5 <sup>b</sup>	14.4 <sup>a</sup>	21.7 <sup>d</sup>	17 <sup>c</sup>	4.9 <sup>b</sup>
	Primed	80.8 <sup>b</sup>	12.6 <sup>c</sup>	34.1 <sup>c</sup>	21 <sup>b</sup>	6.0 <sup>a</sup>

Means within a column followed by the same letter were not significantly different at 5% level.

conductance ( $g_s$ ) were measured on the penultimate fully expanded leaf using a portable infrared gas analyzer based photosynthesis system (LI-6400, LiCor, Inc., USA). During the measurement interval, the ambient  $CO_2$  concentration was 300  $\mu mol/mol$ . Water use efficiency (WUE) was calculated as the ratio between net photosynthesis and transpiration rate. The 4th leaf (from top) of each plant (4 plants for each treatment) was measured.

### Statistical analysis

Analysis of variance was carried out with the data from all experiments using SPSS. Data expressed as percentages were arcsine transformed prior to analysis of variance. Means separation was performed by least significant difference (LSD) test if F-test was significant at  $P = 0.05$ .

## RESULTS

### Effects of SMP with *T. harzianum* on seed germination

The SMP with *T. harzianum* significantly improved germination vigor, germination index and vitality index for

the two eggplant cultivars evaluated at 30°C light/25°C dark (Table 1). For moxige, priming eggplant seeds significantly improved germination vigor at 20°C light/15°C dark and 25°C light/20°C dark compared with non-priming seeds which had a germination vigor of 0 and 72.5% (Table 2).

### Effects of SMP with *T. harzianum* on seedling emergence and growth

At the 25th days after sowing, SMP with *T. harzianum* significantly increased seedling emergence index, seedling height and fresh weight, and decreased mean emergence time at 25th days after sowing (Table 3). For Moxige, SMP with *T. harzianum* significantly increased the emergence percentage. For Sanyue Zaoqie, emergence percentage of SMP with *T. harzianum* has no significant differences with control. SMP with *T. harzianum* significantly increased seedling height, fresh weight and dry weight at 65th days after sowing for both

**Table 4.** The effect of SMP with *T. harzianum* on seedling growth of two varieties eggplants at 65th days after sowing.

Cultivars	Treatment	Seedling height (mm)	Fresh weight of seedlings (g)	Dry weight of seedlings (g)
Moxige	Control	67 <sup>c</sup>	2.09 <sup>b</sup>	0.28 <sup>bc</sup>
	Primed	115 <sup>b</sup>	3.48 <sup>a</sup>	0.46 <sup>a</sup>
Sanyue Zaoqie	Control	57 <sup>c</sup>	1.32 <sup>c</sup>	0.21 <sup>c</sup>
	Primed	83 <sup>a</sup>	2.42 <sup>b</sup>	0.34 <sup>b</sup>

Means within a column followed by the same letter were not significantly different at 5% level.

**Table 5.** The effect of SMP with *T. harzianum* on the net photosynthetic rate, stomatal conductance, transpiration rate, and water use efficiency in two eggplants varieties.

Cultivars	Treatment	Net photosynthetic rate [ $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ ]	Stomatal conductance [ $\text{mol}/(\text{m}^2 \cdot \text{s})$ ]	Transpiration rate [ $\mu\text{mol}/(\text{m}^2 \cdot \text{s})$ ]	Water use efficiency (A/E)
Moxige	Control	7.09 <sup>c</sup>	0.17 <sup>bc</sup>	3.10 <sup>c</sup>	2.25 <sup>ab</sup>
	Primed	10.37 <sup>a</sup>	0.29 <sup>a</sup>	4.94 <sup>a</sup>	2.11 <sup>b</sup>
Sanyue zaoqie	Control	7.43 <sup>c</sup>	0.14 <sup>c</sup>	3.03 <sup>c</sup>	2.49 <sup>a</sup>
	Primed	9.44 <sup>b</sup>	0.19 <sup>bc</sup>	3.97 <sup>b</sup>	2.41 <sup>ab</sup>

Means within a column followed by the same letter were not significantly different at 5% level.

varieties (Table 4).

### Effects of SMP with *T. harzianum* on photosynthetic capacity (leaf gas-exchange measurements)

Leaf gas-exchange measurements showed that SMP with *T. harzianum* significantly increased net photosynthetic rate and transpiration rate for the two cultivars (Table 5). For Moxige, SMP with *T. harzianum* significantly increased stomatal conductance. However, SMP with *T. harzianum* treated cultivars differed in their capacity to alter the net photosynthetic rate, stomatal conductance and transpiration rate. Maximum net photosynthetic rate, stomatal conductance, and transpiration rate were significantly increased in SMP with *T. harzianum* treated Moxige seed. SMP with *T. harzianum* had no significant effect on water use efficiency. However, the difference among different varieties in altering water use efficiency was also not significant.

## DISCUSSION

Rapid and uniform field emergences are essential for successful crop establishment, especially under adverse environmental conditions. SMP is one technique of improving seed germination, reducing the time from sowing to emergence, and improving emergence uniformity. *Trichoderma* spp. are common saprophytic fungi and have been shown to enhance seedling growth and resistance in plants. The beneficial effects of seed

treatment with *T. harzianum* on seed germination, seedling growth and alleviating abiotic and physiological stress, have been reported (Nagaraju et al., 2012; Harman et al., 1989; Mastouri et al., 2010; Harman and Taylor, 1988). The results of this study on eggplant were very consistent with the aforementioned reports.

The need for fast and uniform crop establishment is well recognized in horticultural production systems (Bennett et al., 1992). This study demonstrated that SMP with strain T22 of *T. harzianum* increased seed germination vigor and germination index, decreased mean emergence time, increased seedling emergence and enhanced seedlings quality of eggplant. The results indicate that SMP with strain T22 of *T. harzianum* could serve as an effective way to improve seeds germination and seedling emergence and promote plant growth of eggplant. This technology would be beneficial to factory seedlings and increase cultivation efficiency.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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