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Improved Tolerance of Teak (*Tectona grandis* L.f.) Seedlings to Low-Temperature Stress by the Combined Effect of Arbuscular Mycorrhiza and Paclobutrazol

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Abstract Low-temperature damage is a common problem for tropical and subtropical plants during their early-growth stage. In this study, an experiment with a L_{18} ($2^1 \times 3^7$) mixed orthogonal array in a greenhouse was conducted to determine whether arbuscular mycorrhizal fungi (AMF) inoculation and paclobutrazol (PBZ) application through foliar spray would enhance the chilling tolerance of teak seedlings. One-month-old seedlings of clones 8301, 7544, and 7552 from a Myanmar provenance propagated by tissue culture techniques were inoculated with *Glomus versiforme* and cultivated for 6 months. The foliar surface of both mycorrhizal and nonmycorrhizal treated plants was sprayed with PBZ at concentrations of 0, 50, and 100 mg l^{-1} once a week for 3 weeks prior to exposure to low temperatures of 6, 3, and 0°C for 12 h in an artificial climate chamber, followed by 12 h of recovery at 20°C room temperature. AMF colonization significantly promoted height and RCD growth and dry biomass accumulation of shoot and root. Under low-temperature stress, AM symbiosis increased leaf chlorophyll content by 22.8%, soluble protein content by 19.6%, superoxide dismutase (SOD) activity by 10.6%, and peroxidase (POX) activity by 9.5%, whereas malondialdehyde content was decreased by 14.1%. Both AMF colonization and the foliar spray PBZ at 50 and 100 mg l^{-1} were capable of alleviating the damage caused by low-temperature stress on teak seedlings by increasing the photosynthetic pigments, accumulation of osmotic adjustment compounds, and antioxidant enzyme (SOD and POX) activity, and by decreasing membrane lipid peroxidation. AMF colonization and foliar spraying of PBZ at 50 mg l^{-1} produced a positive interaction and appears to be a good way to enhance chilling tolerance of teak seedlings experiencing stress at 6, 3 and 0°C for 12 h.

Keywords Teak (*Tectona grandis*) · Seedlings · Arbuscular mycorrhiza · Paclobutrazol · Chilling stress · Physiological and biochemical response

Introduction

Teak (*Tectona grandis* L.f.) is one of the most valuable hardwood timber species in the world. The species occurs naturally in parts of India, Myanmar, Lao PDR, and Thailand and has been widely introduced and cultivated into tropical regions of Asia, Africa, and Central America outside its natural habitat. Teak is a tropical deciduous species and favors a fairly moist warm climate. It grows best in locations that have a seasonal temperature change between 40°C in the hottest month and 13°C in the coldest month, and that optimum temperatures in controlled environments are between 27–36°C in the daytime and 20–30°C at night (Kaosa-ard 1981; White 1991). It can endure the absolute minimum temperature of 2°C in the dry zone of central India (Subramanian and others 2000).

Motivated by attractive marketing and financial return, some private companies in China have invested in teak plantations since the 1990s. In recent years, a high priority has also been given to teak reforestation and afforestation by the forestry authority of Guangdong, Guangxi, and Yunnan Provinces. Teak generally grows well in most

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subtropical zones with monsoon climate in southern China, but often suffers from some biotic and abiotic stresses. Low temperature was one of the abiotic stresses during the early stages of its growth (Kuang and Zheng 1991). A short period of chilling currents frequently occurs during the winter season. Exposure of plants from tropical and subtropical origins to chilling temperatures may stunt plant growth, induce wilting, cause necrotic lesions on leaves, and increase susceptibility to diseases and pathogens (Korkmaz and Dufault 2001). Typical chilling injury symptoms including necrotic lesions on the apical bud and dead bark on teak seedlings and young plantations are observed in some parts of Guangdong and Guangxi. Sensitivity of teak to chilling varies depending on genotypes. Variation among different provenances in response to temperatures exists (Pinyopusarerk 1979). It is observed that teak plants from the Myanmar provenances have higher chilling tolerance than those from India in teak provenance trial plantations established in China (Li and others 2010).

Teak can be infected by arbuscular mycorrhizal fungi (AMF) in the field (Raman and others 1977; Mohan Kumar and Mahadevan 1987; Verma and Jamaluddin 1995; Gurumurthy and Sreenivasa 1998). Mohanan and Sheeba (2005) recorded 85 species of Glomalean fungi belonging to six genera, and *Glomus* and *Acaulospore* were the most predominant genera in rhizosphere soils of teak plantations in the Kerala State of India. A sampling investigation conducted over seven sites of Guangdong, Guangxi, and Yunnan Provinces indicated that teak growing under different edaphic and climatic conditions exhibited a high arbuscular mycorrhizal association (Gong and others 2002). AMF colonization has been reported to enhance the growth and mineral nutrient uptake of teak seedlings (Durga and Gupta 1995; Verma and Jamaluddin 1995; Rahman and others 2000; Gong and others 2002). Mycorrhiza were capable of increasing tolerance of some plants subjected to low-temperature stresses (Charest and others 1993; Paradis and others 1995; Zhu and others 2010), but there is no specific information for teak. Paclobutrazol (PBZ), a member of the triazole family of plant growth regulators, has been found to protect several plant species from low- and high-temperature stress by reducing oxidative damage (Lee and others 1985; Wang 1985; Pinhero and Fletcher 1994; Fletcher and others 2000; Lin and others 2006; Baninasab 2009). However, information on physiological and biochemical changes provoked in teak seedlings by AMF inoculation and PBZ treatment is lacking. Thus, the application of AMF and PBZ may effectively protect teak seedlings from chilling damage in the nursery and produce a positive interaction.

The objectives of this experiment were (1) to determine whether the arbuscular mycorrhizal fungus *Glomus versiforme* (Karsten) Berch (Gv9004 isolate), or PBZ improve chilling tolerance of teak seedlings, (2) to ascertain the effect of the combined application of Gv9004 and PBZ on three teak clones, and (3) to determine the optimum PBZ concentration that would provide the best protection against chilling stress.

Materials and Methods

Experimental Design

A factorial experiment involving four variables (AMF, PBZ, clone, and temperature) was set up by using a L_{18} ($2^1 \times 3^7$) mixed orthogonal array in a greenhouse at the Research Institute of Tropical Forestry (RITF), Chinese Academy of Forestry (CAF) in Guangzhou. The 18 treatments as shown in Table 1 were replicated in three blocks. Each treatment plot consisted of 12 seedlings. In total, 648 seedlings were involved in this experiment.

Plant Material and Experimental Methods

The young in vitro plantlets of teak clones 8301, 7544, and 7552 from a Myanmar provenance propagated by tissue culture techniques were transplanted to a sterilized sand

Table 1 Experimental design using an L_{18} ($2^1 \times 3^7$) mixed orthogonal array to test chilling tolerance of teak seedlings

Treatment	AMF status	PBZ concentration (mg l^{-1})	Clone	Temperature (°C)
1	A_0	P_1 (0)	C_1	T_1 (6)
2	A_0	P_1 (0)	C_2	T_2 (3)
3	A_0	P_1 (0)	C_3	T_3 (0)
4	A_0	P_2 (50)	C_1	T_1 (6)
5	A_0	P_2 (50)	C_2	T_2 (3)
6	A_0	P_2 (50)	C_3	T_3 (0)
7	A_0	P_3 (100)	C_1	T_2 (3)
8	A_0	P_3 (100)	C_2	T_3 (0)
9	A_0	P_3 (100)	C_3	T_1 (6)
10	A_1	P_1 (0)	C_1	T_3 (0)
11	A_1	P_1 (0)	C_2	T_1 (6)
12	A_1	P_1 (0)	C_3	T_2 (3)
13	A_1	P_2 (50)	C_1	T_2 (3)
14	A_1	P_2 (50)	C_2	T_3 (0)
15	A_1	P_2 (50)	C_3	T_1 (6)
16	A_1	P_3 (100)	C_1	T_3 (0)
17	A_1	P_3 (100)	C_2	T_1 (6)
18	A_1	P_3 (100)	C_3	T_2 (3)

AMF status: A_0 uninoculated, A_1 inoculated with *G. versiforme*, C_1 clone 8301, C_2 clone 7544, C_3 clone 7552, P_i PBZ concentration, T_i temperature, where $i = 1, 2, 3$

bed in the greenhouse. One month later, healthy and uniform seedlings about 6 cm in height were transplanted into 2.0 l plastic pots filled with a mixture of lateritic red soil, black peat, sand, and zeolite (4:3:2:1, v/v/v/v) previously sterilized by autoclaving (121°C, 120 min), one plant per pot. Ten g of inoculum of Gv9004 isolate was added near the roots of the seedlings of inoculation treatments 10–18 (Table 1). Both mycorrhizal and nonmycorrhizal inoculated seedlings were watered once a day and fertilized with a solution of NPK compound (N:P:K 15:15:15) at 1% concentration every 2 months.

Six months after cultivation all seedlings were sprayed once a week for 3 weeks with PBZ solution at the three concentrations as listed in Table 1 until the foliar surface was completely wet. One week after the last spray, three seedlings randomly selected from each treatment were subjected to each temperature regime (6, 3, and 0°C) for 12 h in a growth chamber and then moved to a room at 20°C for 12 h. The content of chlorophyll (CHL), soluble protein (SPRO), and malondialdehyde (MDA) and the activities of superoxide dismutase (SOD) and peroxidase (POX) of leaves were measured for the seedlings treated with 12 h of chilling and 12 h of post-chilling. Leaf samples originated from three replicates per treatment. For the remaining nine seedlings in each treatment, root colonization and dry biomass of shoots and roots (oven-dried at 70°C for 48 h) were measured. Prior to low-temperature stress, seedling height and root collar diameter (RCD) of all seedlings were recorded.

Assay and Data Analysis

Mycorrhizal colonization was estimated using the root fragment method (Brundrett and others 1996). Root samples were cut into 1 cm long segments, soaked in 10% (w/v) KOH at 90°C for 1 h in a water bath, rinsed three times in tap water, acidified in 2% (v/v) HCl, and stained with trypan blue (Grace and Stribley 1991). For each replicate, 30 root segments from each treatment were examined under the microscope.

CHL was extracted from 300 mg of fresh leaves in 95% ethanol and assayed by the method of Bruinsma (1963). SPRO was extracted by the method of Bradford (1976). The MDA content was determined as described by Dhindsa and others (1981). SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of NBT. The change in absorbance was measured at 560 nm (Giannopolitis and Ries 1977). POX activity was determined by the oxidation of guaiacol in the presence of H_2O_2 at 470 nm (Chance and Maehly 1955). Analyses of variance (ANOVA) were conducted using the general linear model (GLM) procedure of SAS 8.0 (Statistical Analysis Systems Inc., Cary, NC, USA). Duncan's multiple-range test was used to detect significant differences among treatment means at $P < 0.05$ (Kuehl 2000).

Assessments of Low-Temperature Tolerance Capacity

Principal component analysis (PCA) was employed to develop a smaller number of artificial variables (called principal components) that will account for most of the variance in the observed variables (CHL, SPRO, SOD, POX, and MDA). The principal components were then used as criterion variables in subsequent assessment analyses (Jolliffe 2002). A composite indicator of low-temperature tolerance capacity (Y) was yield by the weighted linear combination (WLC) on the eigenvectors of principal components (PC_i). The weights (W_i) were given by the variance proportion of the correlation matrix of the observed variables in the PRINCOMP procedure of the SAS 8.0. The criterion for determining the number of meaningful principal components (i) to retain was that the cumulative percent of variance accounted for at least 85% of the total variance.

Results

Root Colonization, Biomass, Height, and RCD of Seedlings

Root colonization of seedlings inoculated with AMF Gv9004 in treatments 10–18 for 6 months was 75.5–87.8%, significantly higher than uninoculated (non-AMF) seedlings, and there were no significant differences among the three clones. The AMF inoculation had a positive effect on the dry biomass accumulation in terms of shoots, roots, and the total of shoots and roots ($P < 0.05$), and height and RCD ($P < 0.001$). The root and shoot biomass, shoot height, and RCD of AMF-inoculated seedlings were 7.0 and 9.4 g plant^{-1}, 22.9 and 0.6 cm, respectively, increased by 28.5, 31.1, 17.7, and 20.5% compared to those of non-AMF plants. There were no significant differences among the three clones and among the three concentrations of PBZ used.

Leaf CHL

For total leaf CHL ($a + b$) content, ANOVA indicated significant effects from the AMF and PBZ variables ($P < 0.001$) and no effects from the clone and low-temperature variables as well as all the interactions (Table 2). Low temperatures of 6, 3, and 0°C for 12 h did not significantly modify CHL synthesis, but the CHL content was decreased for the seedlings with no application of PBZ in both AMF and non-AMF inoculation treatments (Fig. 1).

Table 2 ANOVA results of the L_{18} ($2^1 \times 3^7$) mixed orthogonal design with AMF status (A), PBZ concentration (P), clone (C), and low temperature (T), and their interactions (A × P, A × C, P × C, A × P×C × T) for CHL, SPRO, activity of SOD and POX, and MDA in the chilling stress experiment of teak seedlings

Source of variance	df	CHL	SPRO	SOD	POX	MDA
AMF (A)	1	***	***	***	***	***
PBZ (P)	2	***	***	***	***	***
Clone (C)	2	NS	***	***	NS	NS
Temperature (T)	2	NS	***	***	***	***
A × P	2	NS	***	***	***	***
A × C	2	NS	***	***	NS	NS
P × C	4	NS	***	***	NS	NS
A × P×C × T	2	NS	***	***	NS	NS

NS nonsignificant

*** Significance at $P < 0.001$ level of probability

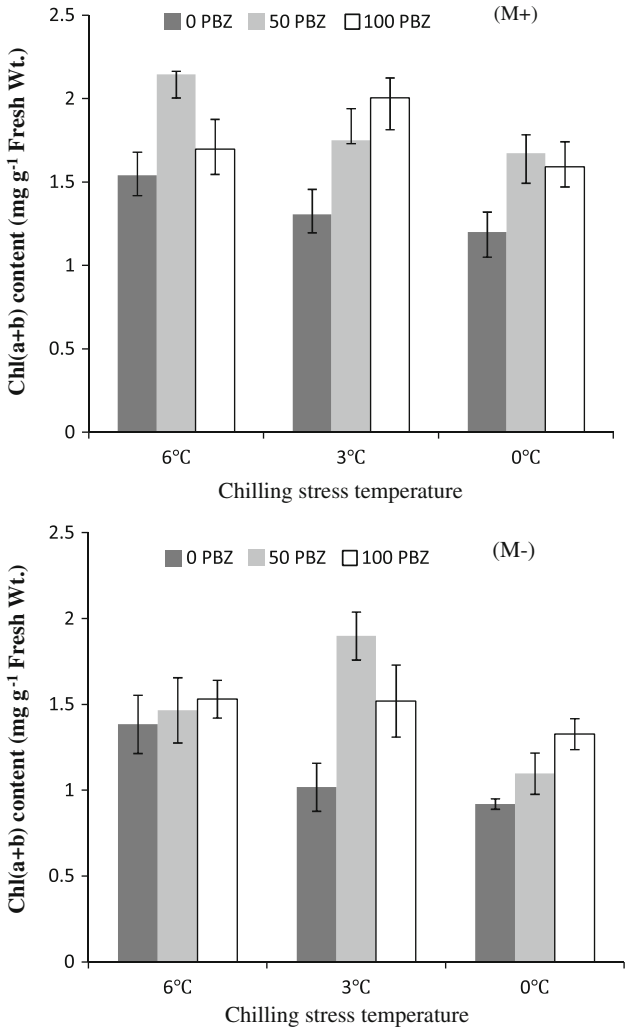

Fig. 1 Total chlorophyll (CHL $a + b$) content of teak seedlings at low temperatures of 6, 3, and 0°C. These seedlings were inoculated with mycorrhiza (M+) and nonmycorrhiza (M−) for 6 months and sprayed with three concentrations of 0, 50, and 100 mg l^{-1} PBZ solutions for three times before stress

The CHL content of AMF-inoculated seedlings was 22.8% higher than that of non-AMF seedlings (Table 3), and with spraying of 50 and 100 mg l^{-1} of PBZ solution, CHL content was increased by 43.3 and 29.7%, respectively, compared with 0 mg l^{-1} PBZ (non-PBZ) (Table 3). There was no significant interactive effect of AMF inoculation and PBZ application, but CHL content of AMF-inoculated seedlings sprayed with 50 and 100 mg l^{-1} PBZ was 18.4 and 22.9% higher than that of non-AMF seedlings, respectively (Table 4).

SPRO

The SPRO content in the leaves of teak seedlings was significantly affected by AMF, PBZ, clone, and temperature treatments and there were remarkable interaction effects (Table 2). Lowering the temperature led to a marked decrease in SPRO content, particularly when the temperature dropped from 6 to 3°C or 0°C. Suffering from chilling stress, the SPRO content of AMF-inoculated seedlings was 19.6% higher than that of non-AMF seedlings (Table 3). Both concentrations of 50 and 100 mg l^{-1} PBZ significantly promoted protein synthesis and it was increased by 47.4 and 12.0%, respectively, compared with the non-PBZ treatment (Table 3). Furthermore, AMF and PBZ interactions also produced positive effects on the synthesis and accumulation of protein. The SPRO content of AMF-inoculated seedlings sprayed with 50 and 100 mg l^{-1} PBZ was increased by 22.7 and 25.2%, respectively, compared to that of non-AMF seedlings (Table 4). Under the chilling stress of 6°C, the interaction of A × P × C was remarkable, and the SPRO content of the combination of $A_1P_2C_3$ was significantly higher than that of the others.

Table 3 The main effects of AMF, PBZ, clone, and low temperature on the content of CHL, SPRO, and MDA, and SOD and POX activity in the chilling stress experiment of teak seedlings

Source	CHL (mg g^{-1} FW)	SPRO (mg g^{-1} FW)	SOD (U g^{-1} FW)	POX (U g^{-1} min^{-1} FW)	MDA (μmol g^{-1} FW)
AMF					
Inoculated	1.706 a	26.873 a	449.141 a	0.289 a	15.255 b
Uninoculated	1.389 b	22.464 b	406.062 b	0.264 b	17.412 a
PBZ concentration (mg l^{-1})					
0	1.241 b	20.590 c	398.830 c	0.261 c	17.227 a
50	1.778 a	30.357 a	472.177 a	0.278 b	16.332 b
100	1.609 a	23.060 b	411.798 b	0.290 a	15.552 b
Clone					
8301	1.504 a	25.147 a	431.818 a	0.282 a	16.170 a
7544	1.636 a	23.088 b	416.193 b	0.276 a	16.345 a
7552	1.497 a	25.772 a	434.868 a	0.271 a	16.558 a
Temperature (°C)					
6	1.574 a	25.860 a	460.380 a	0.287 a	15.168 c
3	1.557 a	24.365 b	426.825 b	0.278 a	16.320 b
0	1.511 a	23.782 b	395.600 c	0.265 b	17.571 a

Values followed by the *same letter* within the column of each group are not significantly different at $P < 0.05$

Table 4 The interactive effects of AMF and PBZ on the content of CHL, SPRO, and MDA, and SOD and POX activity in the chilling stress experiment of teak seedlings

AMF status	PBZ concentration (mg l^{-1})	CHL (mg g^{-1} FW)	SPRO (mg g^{-1} FW)	SOD (U g^{-1} FW)	POX (U g^{-1} min^{-1} FW)	MDA (μmol g^{-1} FW)
0	0	1.107	19.647	392.770	0.240	18.853
0	50	1.628	27.267	438.603	0.265	17.780
0	100	1.435	20.480	386.813	0.288	15.603
1	0	1.392	21.533	404.890	0.282	15.397
1	50	1.928	33.447	505.750	0.294	14.883
1	100	1.763	25.640	436.783	0.292	15.500

AMF status *0* uninoculated, *1* inoculated with *G. versiforme*

SOD

Low temperatures of 6, 3, and 0°C considerably decreased SOD activity of teak seedling leaves. AMF inoculation raised SOD activity by 10.6% compared with the non-AMF treatment condition (Table 3). A significant effect was observed by spraying PBZ. SOD activity with spraying 50 and 100 mg l^{-1} PBZ was increased by 18.4 and 3.3%, respectively, compared with the non-PBZ condition (Table 3). The interactions between AMF and PBZ and between PBZ and clone were significant (Table 2). Spraying 50 and 100 mg l^{-1} PBZ on AMF-inoculated seedlings increased SOD activity by 15.3 and 12.9%, respectively, compared to non-AMF seedlings (Table 4). SOD activity in clone 8301 and clone 7552 was higher than that in clone 7544.

POX

ANOVA indicated that POX activity in leaves was significantly affected by AMF ($P < 0.001$), PBZ ($P < 0.001$), and low temperature ($P < 0.001$), but differences among the three clones were not significant (Table 2). Low temperature led to a marked decrease in POX activity when the temperature dropped from 6°C or 3 to 0°C. Except for the significant interaction between AMF and PBZ variables ($P < 0.001$), there were no other interaction effects (Table 2). POX activity of AMF-inoculated seedlings sprayed with 50 and 100 mg l^{-1} PBZ was increased by 10.9 and 1.4%, respectively, compared to non-AMF seedlings (Table 4).

Lipid Peroxidation Level

Lipid peroxidation, expressed as MDA content, increased when the temperature was dropped from 6 to 0°C. AM inoculation and foliar spraying of PBZ at chilling stress of 0–6°C considerably affected MDA content in teak leaves. MDA content of AMF-inoculated seedlings was decreased by 14.14% compared to non-AMF plants (Table 3). Both concentrations of 50 and 100 mg l^{-1} PBZ significantly decreased MDA content compared with non-PBZ seedlings

(Table 3). Meanwhile, the interaction of AMF and PBZ variables was significant for decreasing MDA in leaves under chilling stress (Table 2). The MDA content of seedlings treated with 50 mg l^{-1} PBZ and AMF inoculation was the lowest, decreased by 19.5% compared with seedlings treated with 50 mg l^{-1} PBZ and no AMF inoculation (Table 4). There was no significant difference among the three clones (Table 2).

Comprehensive Assessment of Low-Temperature Tolerance Capacity

One-way ANOVA indicated that the CHL, SPRO, SOD, POX, and MDA values of teak seedlings were all significantly difference among the 18 treatments ($P < 0.001$). It is not possible to draw an accurate assessment on low-temperature tolerance capacity of different treatments based on a single indicator. As shown in Table 5, a composite indicator was counted based on the method of PCA and WLC. The cumulative percent of variance of the first two principal components has accounted for 87.52% of the total variance. The first component PC_1 contributed to 67.17% and was positively correlated with the original variables of CHL, SPRO, and SOD, whereas the second component PC_2 contributed to 20.35% and was positively represented mainly by the SPRO variable. A comprehensive assessment of results from the 18 treatments showed that treatment 15 ($A_1P_2C_3T_1$) had the highest capacity to tolerate the low temperature of 6°C, treatment 13 ($A_1P_2C_1T_2$) to 3°C, and treatment 14 ($A_1P_2C_2T_3$) to 0°C for 12 h. The common characteristic of the three treatments was that teak seedlings were all inoculated with AMF and treated with foliar spraying of 50 mg l^{-1} PBZ. In contrast, the values non-AMF and non-PBZ treated seedlings, such as treatment 1 ($A_0P_1C_1T_1$), treatment 2 ($A_0P_1C_2T_2$), and treatment 3 ($A_0P_1C_3T_3$), were the lowest at temperatures of 6, 3, and 0°C, respectively.

Discussion

AMF are known to stimulate the growth of many plants and in some cases increase the tolerance of plants to harsh environmental conditions such as chilling stress (Charest and others 1993). In this study mycorrhizal inoculation of teak seedlings with *G. versiforme* Gv9004 for 6 months in a greenhouse resulted in a significant increase in plant height, RCD, and biomass of shoot and roots. The results are in agreement with the findings of previous studies of the isolate inoculated in *Michelia macclurei* and *Machilus pauhoi* (Li and others 2006), *Betula alnoides* (Gong and others 2000), and *Casuarina equisetifolia* (Zhang and others 2010). These results are related to AM symbiosis which may increase the absorption of nutrients by the host

Table 5 A composite assessment indicator of the low-temperature tolerance capacity of 18 treatments based on the content of CHL, SPRO, and MDA, and SOD and POX activity

Treatment	CHL (mg g^{-1} FW)	SPRO (mg g^{-1} FW)	SOD (U g^{-1} FW)	POX (U g^{-1} min^{-1} FW)	MDA (μmol g^{-1} FW)	PC_1	PC_2	Y	Order
1	1.38	19.42	418.42	0.27	17.36	2.88	1.85	2.31	14
2	1.02	17.89	379.28	0.24	18.54	1.20	2.11	1.24	17
3	0.92	21.63	380.61	0.21	21.26	0.05	3.81	0.81	18
4	1.47	32.46	466.74	0.28	16.87	4.83	3.22	3.90	6
5	1.90	22.74	438.69	0.26	17.48	3.92	2.34	3.11	7
6	1.10	26.60	410.38	0.25	18.99	2.20	3.33	2.16	16
7	1.33	21.27	389.45	0.29	15.73	3.62	0.90	2.62	12
8	1.52	20.75	348.32	0.28	16.87	2.96	0.95	2.18	15
9	1.53	19.42	422.67	0.29	14.21	4.45	0.55	3.11	8
10	1.20	23.35	380.83	0.28	15.87	3.20	1.38	2.43	13
11	1.54	19.78	427.78	0.29	14.98	4.18	0.98	3.01	9
12	1.31	21.47	406.06	0.28	14.79	3.81	1.00	2.77	11
13	1.89	33.89	518.93	0.30	15.12	6.64	3.09	5.09	2
14	2.14	29.87	437.37	0.29	15.77	5.68	2.16	4.26	3
15	1.75	36.58	560.95	0.30	13.76	7.36	3.43	5.64	1
16	1.59	20.49	416.09	0.28	16.67	3.77	1.37	2.82	10
17	1.70	27.50	465.72	0.30	14.43	5.67	1.74	4.17	4
18	2.00	28.93	428.54	0.30	15.40	5.56	1.80	4.11	5

PC_i the criterion score of principal component, Y a composite indicator

plants, especially phosphorus, nitrogen, and some micronutrients from soils (Rajan and others 2000; Smith and Read 2008).

Low temperature is one of the abiotic stresses that limit the photosynthetic activity of tropical species. CHL is vital for photosynthesis, which allows plants to obtain energy from light. It has been reported that CHL $a + b$ content decreased in plants when subjected to chilling or cold treatment (Wise and Naylor 1987; Zhang and others 2000). However, AM symbiosis could increase the CHL $a + b$ concentration of maize under low-temperature stress of 15 and 5°C (Zhu and others 2010) and that of wheat at 5°C for 1 week (Paradis and others 1995). This phenomenon is confirmed by our results. Low-temperature stress from 6 to 3°C and from 3 to 0°C induced a decrease in CHL concentration, even if there were no significant differences among three low-temperature stresses of 6, 3, and 0°C. The CHL concentration in low-temperature-stressed mycorrhizal inoculated seedlings of the three teak clones was significantly higher than that of the nonmycorrhizal plants. Arbuscular mycorrhizal root colonization could alleviate the low-temperature damage by increasing CHL synthesis, which could lead to higher photosynthesis rates and low-temperature tolerance of teak seedlings. Compared with the non-PBZ-treated plants, leaf CHL content of PBZ-treated plants exposed to chilling stress was also significantly enhanced in this experiment (Table 3), as was already reported by Pinhero and Fletcher (1994) on seedlings of corn, Berova and others (2002) on wheat, and Baninasab (2009) on watermelon under low-temperature stress. Fletcher and others (1982) showed that plants treated with PBZ could synthesize more cytokinin which in turn enhances chloroplast differentiation and CHL biosynthesis and prevents CHL degradation. Therefore, it was also possible that the CHL synthesis of PBZ-treated teak seedlings under low-temperature stress is mediated by an effect on cytokinins.

Guy (1990) concluded that an increase in SPRO content during chilling appears to be an indicator of plant tolerance. This was verified in the PCA of the five original variables (CHL, SPRO, SOD, POX, and MDA) in the present study. The chilling stress induced a significant decrease in SPRO content in the leaves of teak seedlings when stressed from 6 to 3°C and 0°C. However, it was significantly enhanced in response to mycorrhizal inoculation, PBZ application, and their interaction (Table 2). A similar trend was found by Charest and others (1993) in the two hybrids of *Zea mays* L. colonized by *G. mosseae* and stressed at 10°C, and PBZ-treated mung bean seedlings stressed at 5°C for 5 and 10 h (Saleh 2007).

One of the main causes of chilling injury is the formation of reactive oxygen species (ROS) in thylakoid membranes (Huner and others 1998); they damage membrane lipids, proteins, CHL *a*, and nucleic acids and disrupt the homeostasis of the organism (Rao and others 1996). To alleviate or prevent low-temperature-induced oxidative injury, plants have evolved a cellular defense mechanism to scavenge these toxic and reactive species by antioxidant systems such as SOD (Mckersie and others 1993) and POX (Wise 1995). Our data showed that teak seedlings treated with either AMF colonization or a foliar spray of PBZ at 50 and 100 mg l^{-1} before exposure to chilling temperatures of 6, 3, and 0°C for 12 h resulted in a remarkable increase in SOD and POX activity. This was consistent with the increase in SOD and POX activity of AMF-inoculated (*G. mosseae*) tomato plants stressed at 8°C for 1 week (Abdel Latef and He 2011) and PBZ-treated (25 and 50 mg l^{-1}) mung been seedlings stressed at 5°C for 12 h (Saleh 2007). Polyunsaturated fatty acids were degraded to produce MDA under low temperature. It was observed that changes in MDA content in tissue could be a good indicator of the structural integrity of the membranes of plants subjected to chilling stress (Jouve and others 1993). In the present study, MDA content increased with the decline in temperature from 6 to 0°C. AMF inoculation and foliar spraying of PBZ at both concentrations can effectively decrease abnormally high MDA content in leaves suffering from low-temperature stress.

The chilling tolerance was different among the three clones, even if there was not much difference in the value of CHL, POX, and MDA (Table 3). A composite indicator (*Y*) of clone 8301 was higher than that of clones 7544 and 7552. In our previous research, the semilethal temperature (LT_{50}) of seven teak clones (8301, 7544, 7549, 7552, 7559, Z408, and Z602) was verified by an S-type curve of electrolytic leakage in a logistic equation. The value of LT_{50} of clone 8301 was the lowest (0.82°C), which also illuminated the chilling tolerance of clone 8301.

In conclusion, AM symbiosis had a beneficial effect on the growth of teak seedlings. Arbuscular mycorrhizal (*G. versiforme* Gv9004) colonization, foliar spraying with PBZ, and their combination positively improved the chilling tolerance of teak seedlings by increasing the content of leaf CHL and SPRO, decreasing MDA content, and enhancing the activities of SOD and POX. The combined application of AM colonization and foliar spraying of 50 mg l^{-1} PBZ was most effective. Among the three teak clones, clone 8301 showed a higher chilling tolerance than clones 7552 and 7544.

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