Effects of long-lasting brassinosteroid TS303 and propyl dihydrojasmonate on enhancing peanut resistance to chilling

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Abstract: The effects and the action mechanism of long lasting brassinosteroid(coded as TS303)and propyl dihydrojasmonate(PDJ)on peanut resistance to chilling were studied. The enhancement of chilling tolerance was indicated by the reduction of malondialdehyde and electrolyte leakage. Chilling stress decreased superoxide dismutase(SOD)and catalase(CAT)activities and relative water content while it increased the peroxidase(POD)activity and soluble saccharide and proline contents. TS303, PDJ and their mixture TNZ ameliorated the injury caused by chilling stress through preventing decreases in CAT and SOD activities and relative water content by enhancing the increases in proline and soluble saccharide contents. TS303 exhibited better effect than PDJ on preventing decreases in SOD and CAT activities, meanwhile, PDJ exhibited better effect than TS303 on enhancing the increases in soluble saccharide and proline contents, and it might be the difference in action mechanism that resulted in the additive or synergistic protective effect on cell membrane system.

Key words: brassinosteroid; chilling; peanut (*Arachis hypogaea*); propyl dihydrojasmonate

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Brassinosteroids can protect plants from injuring induced by biotic and abiotic stresses (Zhou *et al.*, 2002). However, nature Brassinosteroids usually exhibit a short-lived(2—3 d)or sporadic effect when used in field, Attempts to prolong their effect have been made and one chemically modified brassinosteroid (TS303), which display long-lasting effect has been reported(Sasse, 1997). TS303, whose two active hydroxyl groups are combined by ethyl groups, has no physiological effect itself. However, it can be hydrolyzed to active plant BR showing long term effect from the 5th day after it's used(Takstsuto *et al.*, 1996).

Jasmonic acid(JA)and its esters(e. g. Methyl Jasmonate, MeJA) are linolenic acid derived cyclopentanone-based compounds of wide distribution in plants, which play an important role in plant defense. They can activate genes involving in pathogen and insect resistance(Creelman *et al.*, 1997)as well as those encoding osmotins(Xu *et al.*, 1994)which are correlation to abiotic stress. Jasmonates usually exhibited better physiological effects than abscisic acid but used at two orders of magnitude lower concentration, so it would be a potential substitute for the expensive abscisic acid (Miyamoto *et al.*, 1997). Propyl dihydrojasmonate (PDJ), a kind of synthetic jasmonate whose C9-C10 vinyl bond is saturated by hydrogen and carboxyl group

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esterified by n-propyl, has stronger penetrability and steadier effect than other jasmonates.

Peanut, originated in South America, is susceptible to chilling injuries when exposed to nonfreezing temperatures in the range of 0-15 ℃. In subtropical temperature and high altitude areas, such as western-southern of China, the frequent occurrences of cold snaps in spring cause damage to peanut. Therefore chilling stress appears to be a major limiting factor for spring- peanut growth and agronomic productivity. In this study, peanut seeds were pretreated with modified chemical regulators TS303, PDJ and their mixture, the resulting seedlings were subsequently stressed with chilling, and then some resistance-related indices were investigated at different stressed days with the aim to substantiate the ameliorating effects and to further explore the mechanism.

1 Materials and methods

Peanut(*Arachis hypogaea* L. "Guihua 17") seeds were selected and surface sterilized with 30% H_2O_2 for 5 min and washed thoroughly with de-ionized water, soaked in distilled H_2O(as control), 0. 1 mg/L TS303 (Tama corporation, Japan), 1 mg/L PDJ(Zeon corporation, Japan) and TNZ(0. 1 mg/L TS303 + 1 mg/L PDJ) for 12 h, respectively, sown into plastic pots containing vermiculite in a growth chamber with a light/dark temperature of 28/20 ℃, 800 μmol · m^{-2} · s^{-1} light 10 h/dark 14 h cycle, 70-85% relative humidity, watered with one-fifth strength Hoagland solution(pH 5. 5—6. 0) every 3 d. The temperature was switched to 6 ℃ for low temperature stress for 9 d after the plants' second compound leaves were fully expanded (12-day-old), while other cultivation conditions were similar to those before stress. Peanut leaves of 0 d, 3 d, 6 d and 9 d under chilling stress were harvested to determine the following indices.

Electrolyte leakage was measured as described by Lutts *et al.* (1996).

Samples for determination of contents of protein and MDA, activities of SOD, POD, CAT and APX were prepared by freezing 0. 5 g of leaves in liquid nitrogen to prevent proteolytic activity, followed by grinding with 5 mL extraction buffer (0. 1 mol/L phosphate buffer, pH 7. 5, containing 0. 5 mmol/L EDTA, 10 mg/mL PVP and 1 mmol/L ascorbic acid) in a chilled pestle and mortar, the homogenate was centrifuged at 13 000 × g, 4 ℃ for 20 minutes, the supernatant was used to analyze.

MDA content was determined according to Zhao *et al.* (1994). 1 mL of the supernatant was added to 3 mL of 5 mg/mL thiobarbituric acid(TBA) in 0. 2 g/mL trichloroacetic acid(TCA). The mixture was heated at 100 ℃ for 20 min in a sealed tube and then cooled in an ice bath. After centrifugation at 5 000 × g for 10 min, the absorbance of the supernatant was recorded at 450, 532 and 600 nm with a spectrometer. The concentration of MDA was calculated by the following formula: C(μM) = 6. 45(A532-A600)-0. 56A450.

Protein content was determined according to Bradford(1976) using bovine serum albumin as a standard.

SOD activity was determined according to Giannopotitis & Ries(1977); one unit of SOD activity was defined as the amount of enzyme inhibiting photochemical reduction of NBT by 50% per minute.

Ascorbate peroxidase(APX) was assayed by recording the decrease in absorbance due to ascorbic acid at 290 nm(Nakano & Asada, 1981). Enzyme activity was computed by referring to a standard curve of known concentrations of AsA, and one unit of APX activity was defined as the amount of enzyme catalyzing the consumption of 1 μmol AsA per minute.

Catalase(CAT) activity was assayed by measuring the rate of decomposition of H_2O_2 at 240 nm in a reaction mixture as described by Chance & Maehly(1955). Enzyme activity was computed by referring to a standard curve of known concentrations of hydrogen peroxide, and one unit of CAT activity was defined as the amount of enzyme catalyzing the conversion of 1 μmol H_2O_2 into water per minute.

POD activity was determined by monitoring the increase in absorbance at 470 nm as guaiacol was oxidised, according to the method of Chance & Maehly (1955).

Relative water content(RWC) was estimated grav-

imetrically according to the method of Feng *et al.* (2003). Leaves fresh weight(FW), turgid weight(TW) and dry weight(DW) were measured and RWC was computed as RWC(%)=(FW-DW)/(TW-DW)×100.

Proline was extracted with boiling 30 mg/mL aqueous sulfosalicylic acid and determined by acid ninhydrin reagent as described by Bates *et al.* (1973).

Soluble saccharide was extracted with 80% ethanol and estimated by anthrone reagent using glucose as standard, according to the method of Yemm & Willis(1954).

Experiments described here were performed with four replicates; all parameters taken for the experiments processed by analysis of variance(ANOVA) and the means were compared by Duncan's Multiply Range Test (DMRT) at the 5% significance($P<0.05$) limits in SAS.

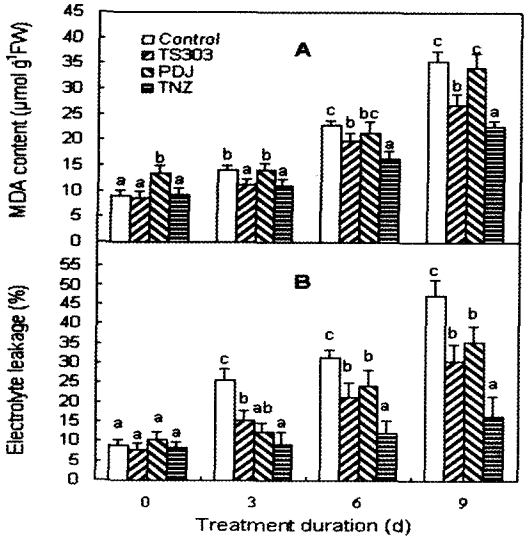

Fig. 1 Effects of TS303, PDJ and TNZ on malondialdehyde content (**A**) and electrolyte leakage (**B**) in peanut leaves under chilling stress

Data represent the mean± SE from four replicate experiments. Treatments labeled common lowercases within same treatment day show no significant difference ($P<0.05$). The same below.

2 Results

2.1 Effects of TS303, PDJ and TNZ on malondialdehyde content and electrolyte leakage in peanut leaves under chilling stress

Under low-temperature exposure, MDA contents in leaves were dramatically increased. Meanwhile TS303 significantly decreased MDA accumulation caused by chilling. PDJ alone did not show significant effect on decreasing MDA contents, and it even significantly increased (by 48.42%) before chilling stress. Similar results had been reported in peanut (Kumari *et al.*, 2006) and *Scenedesmus incrassatulus* (Fedina & Benderliev, 2000) treated with methyl jasmonate, an analog of PDJ. However, when PDJ was used in combination with TS303, a significant decreased of MDA content was demonstrated throughout the chilling period (Fig. 1:A).

Electrolyte leakage was increased both rapidly and stably with increasing days of stress. TS303, PDJ and TNZ slowed down the leaking resulted by chilling. The peanut leaves treated with TS303, PDJ and TNZ leaked 32.29%, 22.82% and 61.17% less than the control, respectively, on the 6th day of chilling; and 35.58%, 24.61% and 65.44% less, respectively, on the 9th day of chilling(Fig. 1:B).

2.2 Effects of TS303, PDJ and TNZ on activities of superoxide dismutase, ascorbate peroxidase, catalase and peroxidase in peanut leaves under chilling stress

SOD, CAT and APX activities were found to decrease gradually with chilling stress. TS303 treated, alone or in combination with PDJ, both significantly slowed down the decreasing in SOD and CAT activities induced by chilling, and they even induced increasing in SOD activity in the first three days. PDJ did not show a significant effect of slowing down on the decreasing in SOD and CAT activities when was used alone. TS303, PDJ and TNZ did not show significant effects on APX activity(Fig. 2:A, B, C).

POD activity in the control peanut leaf was rapidly increased in the first three days, while decreased in the following days. However, POD activities in the chemicals treated peanut leaves were increased slowly but consistently during the stress period. Generally, POD activities of TS303-treated peanut leaves were higher than those of PDJ-treated on the corresponding stress days(Fig. 2:D).

2.3 Effects of TS303, PDJ and TNZ on water, soluble saccharide and prolinecontents in peanut leaves under chilling stress

During the chilling stress, relative water content

(RWC) of peanut leaf was decreased, while soluble saccharide and proline contents were increased gradually. Increasing in the relative water content as well as soluble saccharide and proline were showed in TS303 and PDJ treatments used alone or in combination. These effects magnified with increasing days of stress. On the 9th day of chilling stress, TS303, PDJ and TNZ was found to increase relative water content by 4.72%, 12.09% and 17.94%, respectively; soluble saccharide content by 27.85%, 51.33% and 57.63%, respectively; and proline content by 17.29%, 38.15% and 59.05%, respectively (Fig. 3: A, B, C). Among treatments, TNZ exhibited the best effect. TS303 interacted with PDJ as additive, and even synergistic manner in some cases (e.g. action on RWC and proline content in the 9th day of chilling stress).

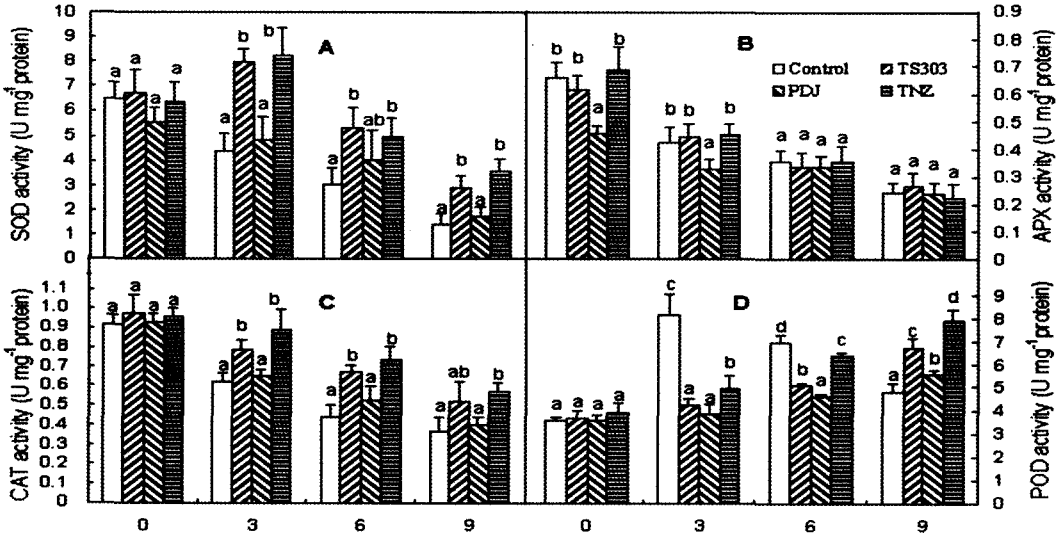

Fig. 2 Effects of TS303, PDJ and TNZ on activities of superoxide dismutase (**A**), ascorbate peroxidase (**B**), catalase (**C**) and peroxidase (**D**) in peanut leaves under chilling stress

3 Discussion

Damage caused by chilling stress is, at least in part, due to membrane lipid peroxidation (Lu & Huang, 2004). MDA is one of the main products of plant lipid peroxidation. The increasing of electrolyte leakage was considered to be a symptom of stress induced membrane damage and deterioration, and proven to be sensitive and accurate marker and thus useful for assessing the chilling damage (Simon, 1974). TS303 and PDJ could significantly decrease electrolyte leakage and MDA accumulation resulted by chilling stress, indicating an enhancement on resistance of peanuts to chilling stress.

Plants are severely affected by abiotic and biotic stresses partly because the production and quenching of reactive oxygen species (ROS) in plant cells can not be maintained in a balanced state (Bowler, 1992). ROS such as superoxide radical, hydrogen peroxide and hydroxyl radical can seriously disrupt normal metabolism through oxidative damage of lipids, proteins and nucleic acids (Imlay & Linn, 1988; Jiang *et al.*, 2002). Plants have evolved specific protective mechanisms, involving in antioxidant molecules and antioxidative enzymes such as SOD, CAT, POD and APX so as to defend themselves against oxidants (Jiang & Zhang, 2002). SOD catalyses the conversion of the superoxide anion to H_2O_2. CAT, APX and a variety of general PODs (Chang *et al.*, 1984) catalyze the breakdown of H_2O_2 in different organelles. Therefore, this enzyme system cooperatively eliminates the damaging effects of toxic oxygen species. In the present study, chilling weakened the activities of SOD, CAT and APX, but increased total POD activity. The increasing of total POD activity might be a compensative result due to CAT decreasing and useful in the defense mechanism of plants against H_2O_2. As a matter of fact, the increasing in total POD

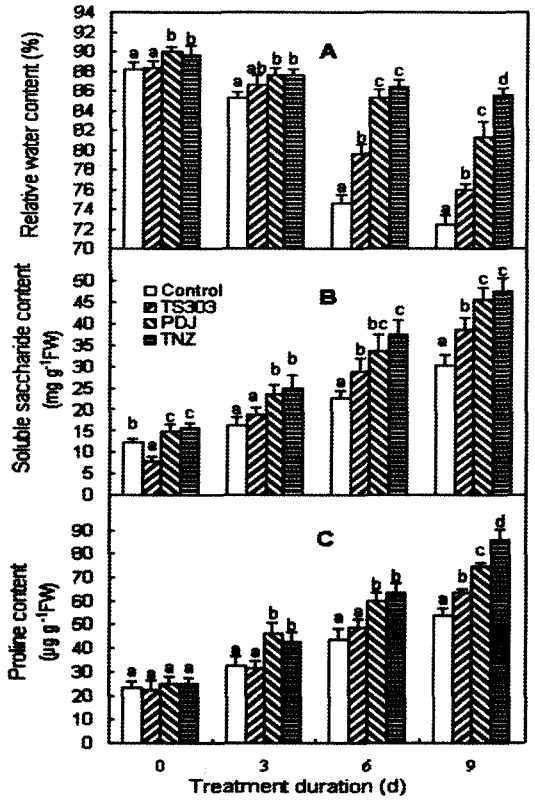

Fig. 3　Effects of TS 303, PDJ and TNZ on water (**A**), soluble saccharide (**B**) and proline (**C**) contents in peanut leaves under chilling stress

activity seemed to be a common response to various oxidative stress factors(Inshida *et al.*, 1985; Kumari *et al.*, 2006). TS303 and TNZ inhibited the decreases in activities of SOD and CAT, and enhanced the increases in activity of POD during the whole chilling period. Present results suggested that TS303 and TNZ induced stress tolerance in plants may be caused, at least in part, by increasing antioxidant activities, which in turn reducing stress-related oxidative damage to cell membranes.

It is well established that the RWC reflects the water status and is related to the growth and plant chilling resistance (Feng *et al.*, 2003), TS303, PDJ and TNZ all slowed down the decreasing in water and postponed the onset of tissue desiccation under the chilling stress. Higher content of soluble saccharide and proline had been suggested as important factors conferring chilling tolerance (Uemura & Steponkus, 1998; Flores *et al.*, 1988). Accumulation of saccharide and proline might be an adaptive change to maintain water content. Besides osmoregulation, the cryoprotective action of soluble saccharide and proline might also be involved in the stabilization of membrane system as well as provision of a store of carbon, nitrogen and energy, and act as precursors to other protective compounds (Uemura & Steponkus, 1998; Yang & Kao, 1999). PDJ and TNZ significantly enhanced the increments of soluble saccharide and proline induced by chilling throughout the stress period, and TS303 did the same on the 6th and/or 9th days.

Collectively, TS303 and PDJ could ameliorate the injury caused by chilling stress by preventing the decrease in CAT and SOD activities and relative water content by enhancing the increment in proline and soluble saccharide contents. TS303 exhibited better effect than PDJ on preventing decreases in antioxidative enzymes activities, meanwhile PDJ exhibited better effect than TS303 on enhancing the increases in soluble saccharide and proline contents, and it might be the difference in action mechanism that resulted in the additive or synergistic protective effect on cell membrane system.

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长效油菜素内酯 TS303 和二氢茉莉酸丙酯增强花生抗寒能力

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摘 要：长效油菜素内酯 TS303 和二氢茉莉酸丙酯(PDJ)浸种能增强花生对低温的忍耐能力，二者显著降低低温诱导的丙二醛含量和电解质渗漏率。低温降低超氧化物歧化酶(SOD)和过氧化氢酶(CAT)活性以及相对含水量，但增加过氧化物酶(POD)活性以及可溶性糖和脯氨酸含量。TS303 和 PDJ 以及它们的混合物 TNZ 都能延缓低温伤害引起的 SOD 和 CAT 活性下降，并能通过增加可溶性糖和脯氨酸含量来提高相对含水量。TS303 在延缓 SOD 和 CAT 活性降低方面效果比 PDJ 好，但 PDJ 在增加可溶性糖和脯氨酸含量方面效果比 TS303 强，由于 TS303 和 PDJ 作用机理不同，二者混合使用表现出加成或协同效应。

关键词：油菜素内酯；二氢茉莉酸丙酯；花生；低温

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