The Protective Effect of Exogenous Putrescine in the Response of Tea Plants (*Camellia sinensis*) to Salt Stress

Fei Xiong, Jieren Liao, Yuanchun Ma, Yuhua Wang, Wanping Fang, and Xujun Zhu¹

College of Horticulture, Nanjing Agricultural University, Nanjing 210095, People's Republic of China

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Abstract. *Camellia sinensis* cultivars were treated with 5 mM putrescine (Put) under a range of sodium chloride (NaCl) concentrations. Plant performance, as indicated by various indicators associated with plant growing condition such as photosynthetic parameters and polyamine (PA) contents, especially the content of Put, was improved by the treatment. The extent of both Na⁺ accumulation and K⁺ loss increased. The activity levels of the antioxidant enzymes related to salt stress, such as superoxide dismutase (SOD), peroxidase (POD), and catalase, were significantly altered with different salt stress levels and showed a decrease in the general trend. Besides, tea polyphenols, the tea quality indicator, increased during the salinity treatment but decreased when we applied Put. These findings suggest that treatment with Put might constitute an effective means for alleviating salinity stress-induced injury through its positive effect on photosynthetic efficiency and for controlling reactive oxygen species (ROS) scavenging ability under appropriate salt stress levels.

The salt-sensitive tea plant *C. sinensis* (L.) O. Kuntze is naturally distributed in the Southeast Asian monsoon region and is a commercially valuable plant, particularly in China, where it has been cultivated for at least 2000 years (Chen et al., 2009). In some regions of the world, salinity is the most serious threat to agriculture and the environment (Golldack et al., 2014). Salt stress can cause osmotic stress, ion poisoning, and secondary stresses (secondary stresses, such as nutritional deficiency and oxidative stress, are caused by osmotic stress and ion poisoning) (Zhu, 2001). Salt stress can also destroy the cell membrane, impair its function, and disturb cell metabolism and normal physiological functioning. High salt concentrations in the soil will result in plant nutritional deficiencies. This is because competition from salt ions reduces the absorption of nutrient elements, such as potassium (K⁺), resulting in K⁺ deficiency in the plant cells (Hasegawa et al., 2000). Furthermore, salt stress can seriously disrupt the integrity of the cell membrane structure. In addition, ion poisoning can also bring negative effect on

the photosynthetic system, causing a decrease in photosynthetic efficiency, and metal ions degrade the cell performance by disturbing the ionic transport through the polymer membrane (Ehteshami et al., 2016). However, it is not known how this actually occurs in *C. sinensis*. Secondary stress causes various deleterious effects in *C. sinensis*, including yellowing and wilting of the leaves, as well as reduced leaf growth; hindering of root growth; decreases in the photosynthetic rate; and increases in energy consumption, eventually accelerating the aging of plants and even causing death (Yang et al., 2006). To deal with ionic and osmotic stresses, plants have evolved a series of strategies to minimize the toxic effects of salt stress. Oxidative stress, as one of the strategies, is invoked under salinity stress as an early stress response and is associated with the generation of ROS (Halliwell, 2006). Under normal circumstances, the stable content of ROS in plants is at a level harmless to the plant, but salt stress disrupts this stable equilibrium (Wang et al., 2004), causing peroxidation and destruction of the membrane system and metabolic functioning, leading to further damage to biological macromolecules, such as proteins and nucleic acids, ultimately resulting in cell death (Mittler, 2002). To mitigate and cope with the damages initiated by ROS, plants have evolved a complex antioxidant system that includes enzymatic antioxidants (Scandalios, 1993).

Polyamines, which mainly include spermidine (Spd), spermine (Spm), and Put, are small polycations observed in various bacteria and plants, occurring ubiquitously in plant tissues. It was recently reported that PAs function in growth and differentiation

processes in plants, as well as in the plant response to any stress condition (Gill and Tuteja, 2010). The primary material of the PA biosynthetic pathway is Put (Slocum et al., 1984). Following Put biosynthesis, methionine donates ammonia propyl, resulting in the gradual formation of Spd and Spm. The PA transport system has feedback regulation mechanisms in the cells (Khan et al., 1991). Free PA levels in plant cells depend not only on their synthesis but also on their transport, degradation, and conjugation (Groppa and Benavides, 2008). Under salt stress, applying an appropriate concentration of exogenous PAs can alleviate the effect of salt to a certain extent (Groppa and Benavides, 2008). A previous study showed that exogenous Put treatment improved the K⁺/Na⁺ ratio in broad bean under NaCl stress and could alleviate part of the damage resulting from the accumulation of Na⁺ and Cl⁻ (Zeid, 2004). PAs, under physiological conditions, exist in the form of cationic polymers. In addition, PAs exhibit features of combined with low molecular or polymer in the cell, also can be used as small-molecule osmotic solutes to directly regulate osmotic adjustment (Masson et al., 2017). Bigo et al. (2001) reported that PAs can be covalently bonded with thylakoid membrane proteins under glutamine transferase catalysis and play an important role in the process of plant adaptation to salt stress. Another study (Tang and Newton, 2005) reported that PAs, especially Put, reduce callus stress and lipid peroxidation damage in pine seedlings subjected to salt stress by improving antioxidant enzyme activity. In summary, the positive role of PAs in stress relates to their influence on membrane structure stability, ROS scavenging capacity, their reaction with large biological molecules, and their role in signal transduction. Under salt stress, PA contents and varieties in plants change rapidly, which alters related physiological and biochemical reactions in plants and adjusts their resistance to salt stress (Tonon et al., 2004). The adaptation of plants to salt stress is not only associated with the PA content but also relates to the interaction between PAs. A study has shown that the ratio of (Spm + Spd)/Put under salt stress only increases in strongly salt-resistant tomatoes, not in salt-sensitive varieties, suggesting that a high level of accumulation of Spd and Spm can alleviate salt stress damage (Santacruz et al., 1997). Salt stress makes changes in PA contents related to salt resistance closely in plants has been basically reached a consensus. But the physiological function of Put is also unclear under salt stress and requires further research. Furthermore, no studies have investigated the role of exogenous Put in *C. sinensis* under salt stress. In the present work, we tested the hypotheses that 1) the growth of *C. sinensis* can be protected by exogenous Put on a short-term basis, even after only a few days of treatment, and 2) the impact of exogenous Put on salt-treated *C. sinensis* depends on the concentration of salt in relation to the influence of exogenous Put on endogenous PA metabolism.

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¹Corresponding author. E-mail: zhuxujun@njau.edu.cn.

Materials and Methods

Plant material and chemical treatments. Cutting seedlings of 2-year-old tea plants (Longjing-changye) with one bud and seven or eight leaves were grown in a chamber at the Nanjing Agricultural University (Nanjing, China). We chose plants with consistent growth as treatment materials. The plants were grown in Hoagland nutrient solution (HS) (pH 5.6), and the growth conditions were maintained at 23 ± 2 °C temperature and $70\% \pm 10\%$ relative humidity. Salinity stress was imposed by adding 50 mM NaCl and 100 mM NaCl to the HS, whereas the control treatment comprised only the HS. To deliver Put, four foliar applications were given, Put solution was made up in water (pH 9.20). The Put concentration (5 mM) was determined through preliminary experiments and referred to Yiu et al. (2009). The treatment was started on the first day of salinity stress and repeated every other day. One bud and two leaves of the plants were harvested 1 and 8 d after treatment; some samples were frozen in liquid nitrogen and stored at -80 °C until further use, whereas the remaining leaves were dried and crushed into tea powder. The tea powder was subjected to six distinct treatments of HS with the addition of 1) 0 mM NaCl/0 mM Put, 2) 0 mM NaCl/5 mM Put, 3) 50 mM NaCl/0 mM Put, 4) 50 mM NaCl/5 mM Put, 5) 100 mM NaCl/0 mM Put, and 6) 100 mM NaCl/5 mM Put.

PA extraction and tissue content determination. The techniques for extraction of PAs and for ultra high performance liquid chromatography (UHPLC) analysis were described by Zhu et al. (2015) with slight modifications. The modifications are as follows: n-butyl alcohol was added together with perchloric acid for better extraction rate and then centrifuged at $3600 g_n$ for 5 min, and after standing for 1 min, the upper organic phase was transferred to a new tube for determination.

Antioxidant enzyme extraction and assays. The lyophilized leaves were used for the enzyme assays. The enzymes were extracted from 0.25 g lyophilized leaves with the grinding medium consisting of 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, and 1% polyvinylpyrrolidone, and the homogenate was centrifuged at $13,000 g_n$ for 10 min. The supernatant was collected for the measurement of SOD and POD activities. SOD activity was assayed as described by Giannopolitis and Ries (1977), that of POD by following Pundir et al. (1999), and that of Catalase (CAT) by following Aebi (1984).

Determination of ion (Na^+ , K^+ , and Ca^{2+}) content. Na^+ , K^+ , Ca^{2+} determination method of extracting reference Wada et al. (2017), slightly improved. The leaves were quickly rinsed in sterile deionized water to remove ions from the free space and gently blotted dry with a paper towel. For the ions quantification, the leaves were oven-dried at 80 °C for 4 h and 0.5 g dry weight was digested. Na^+ , K^+ , and Ca^{2+} were resuspended in 5 mL of 75% HNO_3 and the solutions were filtered. The ions were quantified by an Optima 2100DV inductively

coupled plasma emission spectrometer (PerkinElmer, Waltham, MA).

Determination of the tea quality indicators. The compositions of tea polyphenols and caffeine in leaves were determined by high-performance liquid chromatography (SPD-20A; SHIMADZU, Kyoto, Japan) analysis, and the techniques for extraction described by Doi et al. (1981) were followed.

Three hundred milligrams (dry weight) of tea leaves (finely powdered) was extracted with 40 mL of distilled water in a boiling water bath for 45 min. After centrifugation at $10,000 g_n$ for 10 min, the supernatant was removed and the volume made up to 50 mL with distilled water. Total content of free amino acids was determined by the ninhydrin method. Ten milliliters of supernatant was obtained and mixed with 5 mL of 2% ninhydrin reagent and 5 mL of pH 8.0 phosphate buffer. The reaction mixture was heated in a boiling water bath for 15 min. After cooling to room temperature, 5 mL of distilled water was added. The mixture was allowed to stand for 10 min. Absorbance was recorded at 570 nm (MetashUV-5200 ultraviolet–vis spectrophotometer; Shanghai Metash Instruments Co., Ltd., Shanghai, China). L-Glutamic acid was used to make the standard curve. Each amino acid content was determined as described by Mei et al. (2016).

Measurements of chlorophyll fluorescence parameters. Leaves were adapted in the dark for 30 min before measurement of the chlorophyll fluorescence transient. Each measured O-J-I-P induction curve was analyzed according to the JIP test (Strasser and Strasser, 1995). PAM-2100 portable optical instrument (Walze, Germany) was used to measure the photochemical chlorophyll fluorescence parameters: efficient photosynthesis (PIABS), photochemical efficiency (F_v/F_m), photosystem II (PSII) potential photosynthetic rate, and electron transport activity (ψ_o , ϕE_o , and ϕP_o). Fully expanded leaves at the third or fourth position from the seedling apex were used for these determinations.

Statistical analysis. Each value was expressed as the mean $\pm$ SE of the three independent experiments. All data were analyzed using SPSS 20.0 (Windows). Significance was determined by Duncan's test and analysis of variance. A probability level of 5% ($P \leq 0.05$) was considered as statistically significant.

Results

Effects of exogenous Put on endogenous PA levels under salt stress. Following exposure to salinity stress, there were no significant changes in the PA content in both salinity stress treatments compared with the control by day 1. By day 8, the PA content showed a contrasting trend in both salinity stress treatments (Fig. 1). We noticed that the (Spd + Spm)/Put ratio decreased under the 50 mM NaCl treatment and increased under the 100 mM NaCl treatment at both days 1 and 8.

As shown in Fig. 1, a substantial increase was observed in the Put content at the

exogenous Put treatment by day 1, including the cultivar under the salinity stress. But compared with the salinity treatment without exogenous Put, endogenous Put did not show the significant change. Besides, the Put content of the cultivar with 50 mM NaCl and 5 mM Put treatment at day 8 have the same level compared with that at day 1, but with a large increase at day 8 with 100 mM NaCl and 5 mM Put. The reason may be that the salt stress with 50 mM NaCl has already been relieved by the application of Put at day 8, so the level of Put content reaches back to normal gradually, and the CAT activity (Fig. 2) supported the point. By day 8, the content of Spd declined slightly under the treatments of 50 mM NaCl, 100 mM NaCl, and exogenous Put, whereas the content of Spm increased slightly at the exogenous Put treatment by day 8.

Effects of exogenous Put on antioxidant enzyme activity under salt stress. After 1 d of exposure to salinity, the SOD and CAT activity levels increased by 13.9% and 8.1%, respectively, whereas the POD activity level decreased by 48.8% under 50 mM NaCl. The SOD activity level increased by 4.6%, whereas the CAT and POD activity levels decreased by 37.1% and 80.5%, respectively, under 100 mM NaCl. After 8 d of exposure to salinity, the SOD activity level increased by 7.1%, whereas the CAT and POD activity levels decreased by 4.3% and 13.8%, respectively, under 50 mM NaCl. The SOD activity level increased by 1.9%, whereas the CAT and POD activity levels decreased by 47.1% and 80.4%, respectively, under 100 mM NaCl. From day 1 to day 8, the CAT showed a significant increase at the higher salt concentration (Fig. 2).

In the presence of Put, the level of SOD activity at salt concentrations of 0, 50, and 100 mM increased by 3.4%, 2.5%, and 6.7%, respectively, by day 1 in comparison with the control. But at day 8, the SOD activity showed no significant change at 0 mM salt concentration with Put application, whereas it decreased by 18.2% at 50 mM salinity stress with Put application and increased by 2.0% at 100 mM salt concentration with Put. The activity level of CAT under 0, 50, and 100 mM decreased by 44.4%, 70.3%, and 68.5%, respectively, by day 1 compared with the control. The corresponding values by day 8 were 1.2%, 55.4%, and 47.1%. As for POD, the activity level under 0, 50, and 100 mM salt treatment with Put decreased by 68.3%, 30.5%, and 33.0%, respectively, by day 1. However, at day 8, the POD activity level showed a different trend and increased by 44.8% and 19.0% at 50 and 100 mM salt treatments, respectively, with Put application. With a large concentration, it decreased by 69.0% without salinity (Fig. 2).

Effects of exogenous Put on K^+ , Na^+ , and Ca^{2+} accumulation and the K^+/Na^+ ratio and Ca^{2+}/Na^+ ratio under salt stress. Salinity in plants results in several responses, including an increase in Na^+ concentration with a concomitant decrease in K^+ ; a modification of the plant water status linked to the osmotic

Fig. 1. The content of polyamines under exogenous putrescine (Put) application and different salinity stress ($P < 0.05$). Spd = spermidine; Spm = spermine.

Fig. 2. Antioxidant enzyme activity under exogenous putrescine application and different salinity stress ($P < 0.05$). SOD = superoxide dismutase; POD = peroxidase.

component of salt stress; and secondarily induced stresses such as oxidative stress (Zhu, 2002). Calcium is important in the cell during salt stress, for example, in preserving membrane integrity, signaling in osmotic regulation, and influencing K^+/Na^+ selectivity (Tozlu et al., 2000).

Compared with the control, the K^+ content decreased throughout the duration of the salt stress treatment. At day 1, the Na^+ content declined under 50 mM NaCl stress and increased under 100 mM NaCl stress. However, the opposite pattern was observed by day 8. The Ca^{2+} content increased under 50 mM

NaCl stress but decreased under 100 mM NaCl stress. The K^+ content increased at higher NaCl concentrations but dropped at lower NaCl concentrations. The Na^+ content increased in the absence of salt treatment and increased significantly under salt treatment. The Ca^{2+} content dropped regardless of

whether the plants were treated with salt or not. Compared with the control, the K^+/Na^+ ratio declined under salt stress, and the Ca^{2+}/Na^+ ratio dropped sharply under higher salt stress treatments (Fig. 3).

In the presence of Put, by day 1, the K^+ content in the 0, 50, and 100 mM salt treatments had decreased by 16.9%, increased by 2.1%, and decreased by 1.3%, respectively. By day 8, these values were decreased by 15.7% and 5.8%, and increased by 3.2%, respectively. The Na^+ content in the 0, 50, and 100 mM treatments decreased by 10.1% and increased by 58.3% and 88.2%, respectively, by day 1. It increased by 355.0%, 24.2%, and 29.3%, respectively, by day 8. The Ca^{2+} content in the 0, 50, and 100 mM treatments decreased by 4.6%, 3.8%, and increased by 5.7%, respectively, by day 1. It increased by 4.6%, decreased by 5.4%, and increased by 2.2%, respectively, at day 8. From day 1 to day 8, in comparison with the control, the K^+ content increased by 6.1% and decreased by 5.9% and -2.1% , respectively, at 0, 50, and 100 mM salt. The corresponding values for Na^+ content were increased by 366.9%, 818.6%, and 855.7%, and the corresponding values for Ca^{2+} were increased by 5.7% and decreased by 6.2% and 6.2%. In the presence of Put, the K^+/Na^+ ratio and Ca^{2+}/Na^+ ratio declined obviously under salt stress in comparison with the control (Fig. 3).

Effects of exogenous Put on tea quality indicators under salt stress. After 1 d of salt treatment, the free amino acid and caffeine contents decreased slightly, whereas the tea polyphenol contents evidently increased in comparison with the control. By day 8, the caffeine and tea polyphenols had risen slightly, whereas the free amino acid content decreased at a lower salt concentration and increased at a higher salt concentration. From

day 1 to day 8, the free amino acids increased by 4.4%, 0%, and 21.0% under 0, 50, and 100 mM salt treatments, respectively. The corresponding caffeine content decreased by 15.2%, 0%, and 4.4%, whereas the corresponding polyphenol content decreased by 42.3%, increased by 2.9%, and decreased by 0.4% (Fig. 4).

With the addition of Put, the content of free amino acids and caffeine rose gently under salt treatment, and the tea polyphenol content had dropped clearly by day 1. By day 8, the free amino acid content had increased with salt treatment, whereas the caffeine content had decreased. Tea polyphenols decreased at a lower salt treatment concentration but increased at a higher salt treatment concentration. From day 1 to day 8, compared with the control, the free amino acids increased by 16.7%, 12.3%, and 0% at salt treatments of 0, 50, and 100 mM, respectively. The caffeine content increased by 9.7% and decreased by 0.43% and 9.8% correspondingly, whereas the polyphenols decreased by 20.7% and 0%, and increased by 21.1% (Fig. 4).

Impact of salt stress on photosynthetic parameters and the protection of exogenous Put under salt stress. As shown in Table 1, from day 1 to day 8, the stress treatments reduced Fv/Fm, Fv/Fo, ψ_o , ϕE_o , ϕP_o , and PIABS. The six parameters were reduced to 1.3%, 7.7%, 0%, 2.5%, 1.25%, and 9.6%, respectively, of the values achieved by plants grown under nonstressed conditions in the 50 mM NaCl treatment, and by 12.5%, 39.0%, 10.2%, 22.5%, 12.5%, and 67.0% in the 100 mM NaCl treatment.

The provision of 5.0 mM Put improved Fv/Fm, Fv/Fo, ψ_o , ϕE_o , ϕP_o , and PIABS under salt stress. For Fv/Fm, Fv/Fo, ψ_o , ϕE_o , ϕP_o , and PIABS, the fold increases over the salinity

treatment were, respectively, 1.03, 1.09, 1.14, 1.15, 1.03, and 1.58 (0 mM salt); 1.01, 1.08, 1.10, 1.13, 1.01, and 1.46 (50 mM NaCl); and 1.11, 1.38, 1.11, 1.23, 1.11, and 1.14 (100 mM NaCl) (Table 1).

Discussion

The effect of salinity stress on endogenous PA levels. In general, plant species and cultivars with high stress tolerance have a significant capacity to maintain higher PA contents in response to environmental stress (Bouchereau et al., 1999). During salt stress, PA concentrations have been reported to both increase (Katiyar and Dubey, 1990) and decrease (Mei et al., 2016), with the ratio of (Spd + Spm)/Put increasing with increased salinity to protect the plants under salt stress (Fariduddin et al., 2013). A previous report indicated that salt stress could enhance PA degradation (Kongkiattikajorn, 2009). In this study, the (Spd + Spm)/Put ratio decreased under the 50 mM NaCl treatment and increased under the 100 mM NaCl treatment over the duration of the experiment, which indicated that the tea plants of the cultivar Longjing-changye tolerate low salt stress levels. A study of the germination process in a range of species indicated that the PA composition and content were altered following salinity stress, with Put decreasing and Spd, Spm, or both increasing in most cases, which is consistent with the proposed protective function of higher PAs (Zapata et al., 2004). However, endogenous PA concentrations and contents differ in different plants (Ikbal et al., 2014). Campestre et al. (2011) showed that salinity stress reduces the endogenous Put and Spd content of soybean hypocotyls, whereas at the same time promotes that of Spm. In this study, the Put

Fig. 3. K^+ , Na^+ , and Ca^{2+} accumulation under putrescine application and different salinity stress ($P < 0.05$).

Fig. 4. The content of free amino acids, tea polyphenols, and caffeine under putrescine application and different salinity stress ($P < 0.05$).

Table 1. The chlorophyll fluorescence parameters under different treatments ($P < 0.05$).

Treatments	Fv/Fm	Fv/Fo	ψ_o	ϕE_o	ϕP_o	PIABS
0 mM NaCl/0 mM Put	0.80 ± 0.01 bc	4.13 ± 0.35 d	0.49 ± 0.03 ab	0.40 ± 0.03 b	0.80 ± 0.01 bc	3.03 ± 0.91 cd
0 mM NaCl/5 mM Put	0.82 ± 0.02 c	4.49 ± 0.54 e	0.56 ± 0.04 c	0.46 ± 0.04 c	0.82 ± 0.02 c	4.79 ± 0.76 de
50 mM NaCl/0 mM Put	0.79 ± 0.03 bc	3.81 ± 0.56 c	0.49 ± 0.04 ab	0.39 ± 0.05 b	0.79 ± 0.03 ab	2.74 ± 0.11 bc
50 mM NaCl/5 mM Put	0.80 ± 0.01 bc	4.12 ± 0.34 d	0.54 ± 0.04 c	0.44 ± 0.04 bc	0.80 ± 0.01 bc	4.01 ± 0.84 d
100 mM NaCl/0 mM Put	0.70 ± 0.07 a	2.52 ± 0.81 a	0.44 ± 0.03 a	0.31 ± 0.04 a	0.70 ± 0.07 a	1.00 ± 0.61 a
100 mM NaCl/5 mM Put	0.78 ± 0.02 b	3.49 ± 0.29 b	0.49 ± 0.04 ab	0.38 ± 0.03 b	0.78 ± 0.02 ab	2.37 ± 0.67 b

Different letters represent significant difference among six treatments.

Put = putrescine.

content increased obviously under salt stress, Spd content declined after 1 d with 100 mM NaCl treatment, and Spm content decreased after 8 d of salt treatment. It is inferred that the absorption and transport mechanisms of PAs in *C. sinensis* under salt stress are very complex and are probably related to the salt level and the treatment duration.

The level of free PAs is dependent on their synthesis and on their degradation and conjugation. Soluble conjugated PAs could serve as a valuable source of free PAs that protect against stress by stabilizing protein structures and preventing protein degradation through conjugation to other proteins (Roussos and Pontikis, 2007). The accumulation of Put under salt stress may be a stress response rather than a protective mechanism (Parvin et al., 2014). Spd acts to maintain high Spm levels to improve the salt tolerance of plants (Mansour and Almutawa, 1999; Peremarti et al., 2009). Therefore, in this study, the role of the form and level of the PAs in the protective mechanism of PAs on *C. sinensis* under salt stress requires further research.

PAs in general and Put specifically are considered as an antistress agent for salt-stressed plants. Salt resistance is associated with an ability to increase Put synthesis to maintain a high proportion of conjugated PAs within stressed tissues (Quinet et al., 2010). In our study, the salinity-induced changes in electrolyte leakage and photosynthesis confirmed that *C. sinensis* was compromised by salt stress. The salinity-induced generation of ROS, such as singlet oxygen, leads to increased H_2O_2 and thus lipid peroxidation, which causes membrane damage and protein

and electrolyte leakage (Mudgal et al., 2010). In this study, the activity of POD and CAT decreased by day 1 and declined under the lower salt stress treatment by day 8. This indicated that salt stress possibly influences the antioxidant system of *C. sinensis* in a short time. Key components, such as catechins, caffeine, and theanine, may be the first components used to set a discrimination standard (Obuchowicz et al., 2011). However, the quality of the tea is unclear based on the changes in the tea quality indicators.

The protective effect of the exogenous application of Put. Soil salinity is a major environmental constraint that threatens agricultural productivity. Salt stress causes ion-specific stresses resulting from changes in the K^+/Na^+ ratio due to Na^+ uptake and K^+ loss (Yamaguchi and Blumwald, 2005). However, there is a study (Ndayiragije and Lutts, 2006) which demonstrated that although Put is efficiently absorbed and translocated to the shoots and has a positive impact on monovalent cation discrimination, increased Put did not help the plant to overcome the deleterious effect of salt stress and even reinforced the negative impact of NaCl in terms of both shoot and root growth. In our study, it was clear that the Put treatment failed in enhancing the K^+/Na^+ ratio under salt stress and even reinforced the negative impact of NaCl in the leaves. Indeed, Longjing-changye *C. sinensis* induced a strong accumulation of Put in the leaves and had a similar impact on Na^+ nutrition with exogenous Put treatment. Ca^{2+} content responds to salt stress and is associated with the membrane ion transfer balance (Zhang

et al., 2004). Dasgan et al. (2002) found that the Ca^{2+}/Na^+ ratio was closely related to tomato resistance to salt, even more so than the K^+/Na^+ ratio. Moreover, the Ca^{2+}/Na^+ ratio declined in Put-treated plants under salt stress in comparison with the control. We estimated that, in the presence of Put, such a protective effect was due to the endogenous accumulation of Put and not to the competition between cations at the site of absorption.

NaCl inhibits seedling growth by altering the activity of antioxidant enzymes and the level of free radicals in the leaves. Put might be activating antioxidant enzymes and elevating antioxidants by controlling free radical generation, thereby preventing membrane peroxidation and the denaturation of biomolecules, ultimately improving seedling growth under salinity (Verma and Mishra, 2005). In this study, although SOD activity decreased when Put was added to the plants under salt stress, it tended to increase as the salt stress level increased. The role of Put under salinity might be to protect the superoxide radicals. H_2O_2 might be detoxified by the well-organized functioning of the CAT and POD enzyme systems. In this study, compared with the control, CAT activity increased clearly by day 1, and POD activity increased obviously by day 8. Put can restrain the production of hydroxyl radicals according to the previous report (Chen and Zhang, 2000), and this could be the reason why the activity of CAT and POD decreased.

Chlorophyll fluorescence can be used as a powerful and reliable method to assess changes in the function of PSII and to examine the primary photosynthetic processes under

environmental stress conditions (Hajiboland et al., 2010). In our results, Fv/Fm, Fv/Fo, ψ_o , ϕE_o , ϕP_o , and PIABS all decreased. These results are similar to those reported by Naumann et al. (2007). Exogenous application of Put was used to compensate for salt stress, so as to confer some degree of tolerance to the photosynthetic apparatus against enhanced NaCl salinity and to permit cell growth (Demetriou et al., 2007). In our study, exogenous Put improved photosynthetic capacity and increased all of Fv/Fm, Fv/Fo, ψ_o , ϕE_o , ϕP_o , and PIABS compared with the treatments that received NaCl alone. In this study, the changes in the three chemical substances, including Put, Spm, and Spd, showed no correlation when the plants under different salt stress levels were sprayed with Put. This result indicated that the relation between salt stress and the exogenous Put application treatment is unclear.

When *C. sinensis* was subjected to salt stress, the Na^+ content increased, resulting in ionic toxicity, whereas the K^+ content dropped, leading to a loss of nutrient elements. The K^+/Na^+ ratio declined under salt stress, and the Ca^{2+}/Na^+ ratio dropped sharply under the higher salt stress treatment, which damaged cell membrane integrity and stability. The increasing POD and CAT activity and the change in SOD activity were related to salt concentration, and the endogenous PA content increased to alleviate salt injury. Moreover, the impact of exogenous Put on salt-treated *C. sinensis* was cultivar dependent in terms of the influence of exogenous Put on endogenous PA metabolism. Our results illustrated that when *C. sinensis* is subjected to salt stress and exogenous Put, the K^+/Na^+ ratio, Ca^{2+}/Na^+ ratio, POD activity, and the endogenous PA content increased, whereas the SOD and CAT activity varied depending on the salt concentration, which relieved the secondary stress damage caused by the salt. The quality of *C. sinensis* plants under salt stress with and without exogenous Put requires further assessment.

The improvement of the (Spd + Spm)/Put ratio reflects the protective role of exogenous Spd (Fariduddin et al., 2013). Yet, the reduction of the (Spd + Spm)/Put ratio did not reflect the protective role of exogenous Put. Although exogenous Put worsens ion poisoning, Put application alleviated salinity-induced photosynthetic inhibition, together with an increase in PA content and antioxidant enzyme activity in the leaves of *C. sinensis*. These effects varied in relation to the salt stress level and treatment duration.

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