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Rudimentary leaf abortion with the development of panicle in litchi: Changes in ultrastructure, antioxidant enzymes and phytohormones

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ABSTRACT

Floral bud of litchi (*Litchi chinensis* Sonn.) is a mixed bud that consists of leaf primordia, rudimentary leaves and panicle primordia. According to field observation, the fate of rudimentary leaves depends upon the environment and development of the panicle primordia. Vigorous development of panicle results in abortion of rudimentary leaves. In this study, three types of rudimentary leaves, the upper leaves (UL) which subtended a developing panicle primordium and were bound to abscise after a period of panicle development, the abscising lower leaves (AL) which had an emerging axillary panicle and would abscise under a light external force, and the non-abscising lower leaves (NAL) which subtended a vegetative bud and would develop normally, were sampled from the terminal shoots of cvs. 'Nuomici' and 'Feizixiao'. Ultra-structure of the petiole base in the three types of rudimentary leaves in 'Nuomici' was observed. Signs of cell autolysis, e.g., tonoplast disruption and fusion of vacuole and cytoplasm, were found in localized cells in UL, while massive cell death was observed in AL, suggesting that the two types of rudimentary leaves were at different stages of abortion. The UL was at an early stage of abortion, whereas AL at a late stage of abortion. Cell autolysis was not observed in the nonvascular tissues in NAL. In 'Nuomici', AL had a significantly lower superoxide dismutase (SOD) activity compared to the UL and the NAL, while the latter two had no significant difference in the enzyme activity. In 'Feizixiao', however, no significant difference was found among the three types of rudimentary leaves, although AL had the lowest SOD activity. Peroxidase (POD) activity in AL in both cultivars was significantly higher than that in NAL. The UL had a lower POD activity in 'Nuomici', but a higher activity in 'Feizixiao' compared to NAL. Ascorbate peroxidase (APX) activity was the highest in NAL, and there was no significant difference between the UL and AL. H_2O_2 concentration was the highest in AL in the two cultivars. The NAL had higher levels of zeatin plus zeatin riboside (ZRs), auxin (IAA) and gibberellin (GA_{4+7}), and lower levels of abscisic acid (ABA) than AL. Total concentration of IAA, GA_{4+7} and ZRs (TC) and the ratio of (IAA+GA_{4+7} + ZRs)/ABA (RTC) in NAL were higher than those in UL and AL in the two cultivars. The RTC in UL was higher than that in AL. The results suggested that abortion of the rudimentary leaves caused by panicle development may involve programmed cell death, reduced antioxidant activity and increased H_2O_2 and share similar hormonal control (reduced ZRs, IAA and GA_{4+7} and increased ABA) to the senescence and abscission of mature leaves.

1. Introduction

Litchi (*Litchi chinensis* Sonn.) is an evergreen fruit tree cultivated widely in tropical and subtropical regions. Irregular bearing attributed to unreliable flowering is a pending problem to litchi producers. Floral differentiation is induced by low temperatures (Menzel and Simpson, 1988; Chaikiattiyos et al., 1994; Chen and Huang, 2005) and enhanced by autumn and winter drought (Stern et al., 1993, 1998). After a period of inductive low temperature and drought, apical buds of the shoots may break and elongate when air temperature and soil moisture increase. Panicle initiation is then visible as "whitish millets", swelled buds with dense whitish trichomes at shoot apex or at the axils of the rudimentary leaves (Huang and Chen, 2005). At this stage, the buds are with panicle, leaf primordia and rudimentary leaves. According to our observations, the fate of the mixed buds is largely depended upon environment. If air temperature is not too warm, growth of axillary panicle primordia may prevail and the rudimentary leaves will be aborted and abscise automatically. If litchi plants are exposed to high temperatures, the rudimentary leaves at lower nodes of the

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elongating bud may develop to fully expanded leaves and the axillary panicle primordia may cease to develop and shrink. However, the apical panicle primordium continues to develop, resulting in abortion of the upper rudimentary leaves. In such a case, vegetative/floral mixed shoots will be produced. Suppressing the development of the rudimentary leaves encourages panicle development, and growers remove it by hand or kill the rudimentary leaves by spraying ethephon, which induces their abortion.

Abortion is a senescence process occurring in the midway of organ development. Senescence processes, whether in aged organs or in those under abortion, involve programmed cell death (Noodén, 2004). A strong enhancement in production of reactive oxygen species (ROS) has been observed to take place in tissues during senescence process (Thompson et al., 1987), and programmed cell death has been found to be associated with ROS production (Mach and Greenberg, 2004; Sun et al., 2005). ROS including $O_2^{-\bullet}$, hydrogen peroxide (H_2O_2), hydroxyl free radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$), are normal metabolites in plant cells (Scandalios, 1993; Iannelli et al., 1999). They are scavenged by antioxidant apparatus such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), peroxidase (POD, EC 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11). In senescing tissues, ROS scavenging activities are reduced and the balance between ROS production and scavenging is destroyed, resulting in accumulation of ROS, which damages cell membrane via lipid peroxidation (Thompson et al., 1987), a characteristic event of senescence (Dhindsa et al., 1981; Pauls and Thompson, 1984).

Senescence is a highly regulated process and involves expression of several senescence-associated genes, which are altered by hormone treatment (Buchanan-Wollaston, 1997; Weaver et al., 1998). Ethylene promotes senescence and abscission (Beaudry and Kays, 1988; Huberman et al., 1997). Abscisic acid (ABA) was thus named due to its abundance in senescing and abscising organs (Addicott, 1983), and it can lower auxin levels, enhance leaf senescence and stimulate leaf abscission (Jackson and Osborne, 1972). However, abscission of organs is not one of the major functions of ABA (Srivastava, 2001). There are studies showing that ABA increases stress resistance and reduces leaf senescence and abscission caused by stresses (Jiang and Zhang, 2002; Gómez-Cadenas et al., 2003). Cytokinin (CTK) delays senescence (Ma and Tian, 2005) and may counteract ABA, and inhibit ethylene production. Gibberellins (GAs) can effectively prevent leaf senescence (Ranwala and Miller, 2000).

Antioxidant enzymes, ROS and hormonal regulation in senescence of aged leaves have been well studies (Kar and Feierabend, 1984; Melakeselam and Zhou, 1998; Fu et al., 2000; Scebba et al., 2001; Miyamoto et al., 2001; Guo and Gan, 2005), whereas there lacks information about the physiological mechanism of the premature senescence or abortion of rudimentary leaves, which is necessary for further development of panicle in litchi. This study was to investigate the ultrastructure, antioxidant enzymes, ROS and phytohormones in the rudimentary leaves committed to abortion.

2. Materials and methods

2.1. Materials

The experiment was carried out from September 2005 to August 2007. Six- to seven-year-old litchi (*Litchi chinensis* Sonn.) trees of commercially cultivated cvs. 'Nuomici' and 'Feizixiao' grafted onto 'Huaizhi' were selected for the experiment at the experimental orchard of South China Agricultural University, Guangzhou, China, with three replicate trees of similar size and

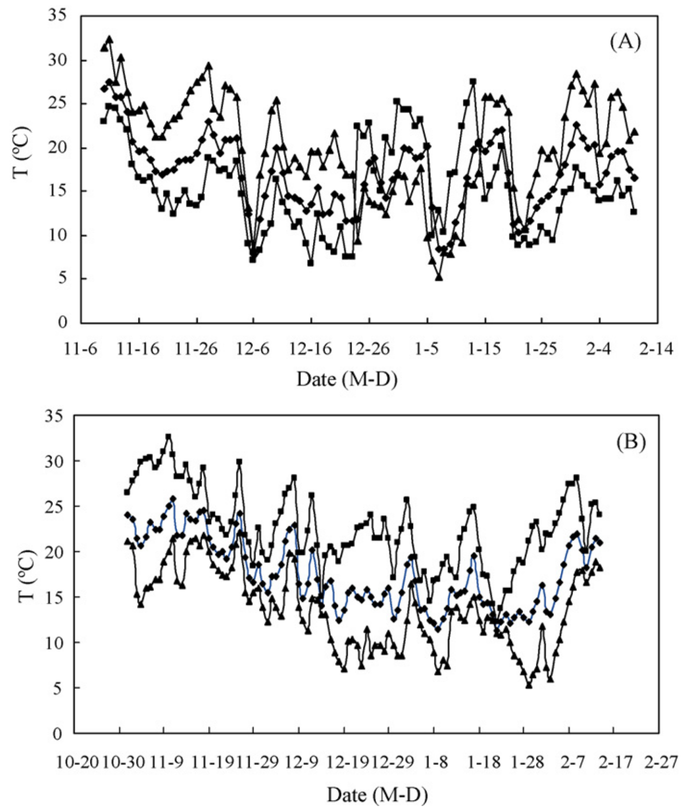

Fig. 1. Temperature changes in the winters of 2005–2006 (A) and 2006–2007 (B). (■) Diurnal maximum temperature; (◆) diurnal average temperature; (▲) diurnal minimum temperature. The latest flush matured in mid-November, after which temperature tended to drop. Panicle primordia ("whitish millets") were observable in early January in 2006 and in mid-January 2007 in both 'Nuomici' and 'Feizixiao'.

phenological stage for each cultivar. The latest flush before winter was matured in mid-November in both years, after which temperatures dropped in a fluctuating manner due to intermittent cold fronts (Fig. 1). Panicle primordia were observable in both cultivars in early and mid-January in 2006 and 2007, respectively. Rudimentary leaves were selectively sampled based on the developmental status of the panicle primordium at their axilla on February 4 in 2006 and February 14 in 2007. Three types of rudimentary leaves examined were: the upper leaves (UL), which were with an strong axillary panicle primordium and bound to abscise after a period of time (Fig. 2, Panel 1 and 4); the abscising lower leaves (AL) which had a emerging axillary panicle primordium and abscised with a light force (Fig. 2, Panel 2 and 5); and the non-abscising lower leaves (NAL), which had a vegetative bud at their axillae and would develop normally (Fig. 2, Panel 3 and 6). UL was thus at an earlier stage of leaf abortion than AL.

2.2. Electron microscopy

Sample preparation for ultrastructural study was conducted based on Huang et al. (2002). Petiole bases of the three types of rudimentary leaves from 'Nuomici' trees were excised and vacuum penetrated and fixed with 3% glutaraldehyde. After washed with phosphate buffer solution (pH 7.2, 0.2 M), the samples were fixed and stained with 1% (w/v) osmium tetroxide, dehydrated in series of ethanol solutions from 80 to 100% (v/v) and embedded in araldite resin. Ultra-thin (80 nm) sections were longitudinally cut with an ultramicrotome (Leica Ultracut UCT). The sections were double stained with uranyl acetate and lead citrate, then mounted