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Stress Effects of Chlorate on Longan (*Dimocarpus longan* Lour.) Trees: Changes in Nitrogen and Carbon Nutrition

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A B S T R A C T

Three-year-old potted longan (*Dimocarpus longan* Lour. cv. Shixia) trees were treated with potassium chlorate and effects on nitrogen and carbon nutrition were examined. The results showed that potassium chlorate at 10 and 20 g per pot failed to induce flower but suppressed shoot growth and caused leaf chlorosis and drop. The treatment significantly inhibited nitrate reductase but increased nitrogen concentration in the leaves and buds. Concentration of soluble amino acids in the leaves of treated trees increased within 14 days and then declined to the control level, while it increased constantly in buds. In both organs, the amino acid increase was an all-round one, with all the tested 21 amino acids increased. However, soluble proteins in the leaves were slightly increased by chlorate, indicating that *de novo* synthesis of amino acids was activated. Chlorate reduced photosynthetic rate and stomatal conductance but slightly increased CO_2 concentration in the mesophyll, suggesting that chlorate treatment damaged photosynthetic apparatus. The damage was reflected by the destruction of thylakoids and grana in the chloroplasts. Chlorate also caused depletion of starch with significant accumulation of soluble sugars in the leaves. Accumulation of sugars and soluble amino acids indicates osmotic adjustment in response to the stress caused by chlorate treatment.

Keywords: longan; potassium chlorate; nitrogen nutrition; carbon nutrition

1. Introduction

Chlorate is a strong oxidant and was historically used as a herbicide and a defoliant in agriculture (Duke, 1985; Vanwijk and Hutchinson, 1995). Great concern is paid upon the toxicity of chlorate because of heavy introduction of this chemical into the environment from paper making industry (Vanwijk and Hutchinson, 1995). Since the late 1990s, this chemical has been heavily input into longan (*Dimocarpus longan* Lour.) orchard due to the discovery that chlorate is effective to induce year-round flowering in longan, a subtropical evergreen tree that normally demands winter chill for flower induction (Yen et al., 2001; Manochai et al., 2005). Surprisingly, such effect has not been found in litchi (*Litchi chinensis* Sonn.), a close relative of longan in the family of chlorate by flowering. In fact, the chemical is so effective in inducing flowering in longan that Manochai et al. (2005) suggested using the species as a model plant for studying the regulation mechanisms of flower induction in perennial tree species using chlorate.

A number of authors have examined the effects of chlorate on nitrogen and carbon nutrition, which is related to the basic metabolism in plant. Subhadrabandhu, the first researcher to disclose the technology of off-season longan production with potassium chlorate in Thailand (Subhadrabandhu and Yapwattanaphun, 2001), assumed that chlorate, as an analog of nitrate, might inhibit nitrate reductase and reduce nitrogen level in the tree, resulting in a higher C/N ratio, a nutritional status that favors flower induction in longan (Lu et al., 2006). However, a number of authors (Charoensri et al., 2005; Sritontip et al., 2005a, 2005b; Wangsin and Pankasemsuk, 2005; Matsumoto et al., 2007) showed no significant changes in the concentrations of nitrogen, non-structural carbohydrates or

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in C/N ratio in longan buds or leaves. Hegele et al. (2008) observed a drop in photosynthetic rate after chlorate treatment, while Sritontip et al. (2005a) reported an increase in photosynthesis and efficacy of photosystem II. It seems that the available reports about chlorate effects on nitrogen and carbon nutrition are still controversial.

While chlorate is able to specifically induce flowering in longan, it also causes toxic effects on the crop (Huang et al., 2006; Lu et al., 2006) as it does on many other plants, where the chemical serves as a herbicide or a defoliant (Åberg, 1947; Vanwijk and Hutchinson, 1995; Mackown et al., 1996; Stauber, 1998; Borges et al., 2004). The toxicology of chlorate to plants has been well explored. The nitrate reduction system plays a key role in the toxicity of chlorate in plant, where chlorate is reduced by nitrate reductase into highly toxic chlorite or hypochlorite and then to non-toxic chloride (Åberg, 1947; Hofstra, 1977; Vanwijk and Hutchinson, 1995; Stauber, 1998; Borges et al., 2004). Due to this mechanism, chlorate has been used to screen or discriminate mutants or genotypes which are defective in nitrate reduction and thus resistant to chlorate (Singh et al., 1977; Nelson et al., 1983; Teng et al., 2006). The toxic effect of chlorate on longan can be significantly reduced by application of nitrate (Huang et al., 2006), which suppresses chlorate reduction by competing for nitrate reductase (Hofstra, 1977). Preliminary results obtained by Sritontip et al. (2005b) showed that chlorite and hypochlorite were also effective to induce flowering in longan. These studies suggest a close relationship between chlorate actions and nitrogen metabolism. In this study, the toxic effects of chlorate on longan and on nitrogen and carbon nutrition were examined in order to unravel some basic physiological events associated with its effects.

2. Materials and methods

2.1. Plant materials

The experiments were done on 3-year-old air-layered 'Shixia' longan trees with even canopy size (around 1 m in diameter) grown in 50 L pots from September 2005 to January 2006 and repeated from November 2006 to March 2007 with different potted trees of the same age. The latest flush of all the trees had fully matured when they received chlorate treatments. The pots were placed in a greenhouse with temperatures of 24 °C to 29 °C and relative humidity of about 80%.

2.2. Treatments

In the season of 2005–2006, the trees were treated with potassium chlorate at 0 (control) or 10 g per pot ($n = 5$ trees). In the season of 2006–2007, trees were given 0, 10 or 20 g of potassium chlorate per pot. Chlorate for each pot was dissolved in 2 L of water before applied by soil drenching. In both seasons, leaf chlorophyll index was measured on four marked leaflets of each tree with a SPAD-502 chlorophyll meter. The number of leaflets dropping from each tree was counted in the season of 2006–2007. Shoot growth was traced by measuring the length of 3 marked new shoots in each tree until the new shoot stopped growth.

2.3. Gas exchanges

Net photosynthesis rate (P_n), stomatal conductance (G_s), transpiration rate (E) and mesophyll CO_2 concentration (C_i) were determined with a Li-Cor 6400 photosystem analyzer. Measurements were made between 09:00 and 12:00 AM on 4–5 tagged leaves each tree under a temperature of 25 °C and photosynthetic photon flow density of 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In 2005–2006, the measurements were carried out at –2, 7, 14, 28 and 56 d after treatment, while in 2006–2007, one day data of photosynthesis were collected at 24 d after treatment.

2.4. Concentrations of starch and soluble sugars

Leaf samples were taken at 14 and 28 d after chlorate treatment in 2006–2007 season for sugar or starch analyses. Soluble sugars were extracted twice with 5 mL 80% (v/v) alcohol from 0.5 g of tissue by grinding the sample into homogenate with a mortar and pestle. The homogenate was centrifuged at 5 000 × *g* for 10 min and the supernatant was collected for soluble sugar analysis using the anthrone method according to Zhang (1990). Starch in the sediment was extracted and analyzed according to the method of Xu et al. (1998), where the sediment was re-suspended with 10 mL of 80% (w/v) calcium nitrate, bathed in boiling water for 20 min, and centrifuged at 8 000 × *g* for 10 min after cooling down. 0.1 mL of 0.01 $mol \cdot L^{-1}$ I-KI was added to 2 mL of the supernatant, and absorbance of the mixture was read on a photospectrometer at 630 nm. A standard curve of starch concentration vs absorbance ($Y = 461.8X$, $R^2 = 0.9986$) was constructed for calculating starch concentration in the tissues.

2.5. Concentrations of nitrogen and carbon

One-centimeter terminal shoots (buds, 3 per tree), and leaves from the latest mature flush (new leaves, 3 per tree) and from the previous flush (old leaves, 3 per tree) were sampled at 14 d after chlorate treatment in the season of 2005–2006. The samples were surface-washed with deionized water, oven dried at 65 °C for 48 h and then ground into powder. 5 mg of the powder was weighed out from each sample for analyses of total nitrogen and carbon with flash combustion chromatography (Fisons CHNS-O elemental analyzer, Model EA-1108). In the season of 2006–2007, samples of 3 leaflets per tree were taken from the latest mature flush at –2, 7, 14, 28, 56 and 95 d after chlorate treatment. Half a gram of leaf tissue from each sample was digested in 5 mL concentrated sulfuric acid at 280 °C. Nitrogen concentration was determined with a KJELTEDC AUTO-2300 automatic nitrogen analyzer.

2.6. Concentration of nitrate

Nitrate analysis was conducted with a Dionex-120 ion chromatographer. The leaf samples were taken at –2, 7, 14, 28 and 56 d after chlorate treatment in the season of 2006–2007. Half a gram of fresh tissue was ground into homogenate in a mortar with 5 mL deionized water and centrifuged at 12 000 × *g* for 20 min. 1 mL of the supernatant was forced through a Millipore™ filter (0.2 μm), and 25 μL of the filtrate was injected into the ion chromatographer equipped with an Ionpac AS11-HC separation column (25 °C), an Ionpac AG11-HC protection column and an electricity conductivity detector. Nitrogen was used as the carrier gas at a pressure of 0.3 kPa, and mobile phase was 6 $mmol \cdot L^{-1}$ NaOH with a flow rate of 1.08 $mL \cdot s^{-1}$.

2.7. Activity of nitrate reductase

The assay was carried out based on the method of Zhang (1990) with freshly collected leaf discs (6 mm in diameter) from the latest mature flushes at 14 d after chlorate treatment in the season of 2006–2007. Twenty leaf discs (0.2 g) were put into 4 mL of