FOLIAR POTASSIUM FERTILISER HELPS TO INCREASE KERNEL OIL CONTENT IN *STYRAX TONKINENSIS*, A NON-FOOD FEEDSTOCK

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Styrax tonkinensis is a biofuel feedstock with potential value to produce a considerable amount of non-edible oil. Whether exogenous potassium (K) spray can boost *S. tonkinensis* seed oil content was tested. A total amount of 0.5 g L^{-1} (K1) and 4 g L^{-1} (K2) potassium D-gluconate was applied exogenously as foliar fertilisers during fruit maturation, compared with control (CK, water) and gluconate control (0.676 mL L^{-1} (G1) and 5.403 mL L^{-1} (G2) D-gluconic acid). Higher average values of net photosynthesis rate and elevated peak values of chlorophyll a/b and chlorophyll (a+b)/carotenoid were found in the leaves that received the K applications. The superior photosynthesis behaviour was accompanied by a dry mass increment in kernel (30.1% for K1, 52.4% for K2) at 137 days after flowering (DAF). Under K treatments, lipid assembled more slowly during 37–77 DAF. The final oil concentration in kernels was similar in CK and K applications. In CK, dynamic acetyl-CoA carboxylase (ACCase) activity followed an M-shaped trend, whereas in other treatments, it only had one single peak at 57 or 77 DAF. Major increases in lipid yield per kernel were identified. The collective results highlighted the role of foliar potassium, which, in suitable concentration, may boost the total oil content per kernel in *S. tonkinensis*.

Keywords: Foliar fertiliser, kernel maturation, lipid biosynthesis, potassium D-gluconate, *Styrax tonkinensis*

INTRODUCTION

Rapid industrialisation and urbanisation are now boosting global feedstock consumption and accelerating the depletion of naturally occurring fossil fuels. In the last 50 years, an increase of fossil fuel consumption (5% per year) has been witnessed (Harwood et al. 2017). As an alternative energy, renewable biodiesel is recognised as a way to mitigate the worldwide energy and environmental challenges (Xiong et al. 2018). In developing countries like China, because of population growth and food security, limited arable land is used for grain planting. Thus, the application of crop feedstocks, i.e., oilseed rape (*Brassica napus*), soybean (*Glycine max*) and maize (*Zea mays*) to the biodiesel industry would be impractical (Xiong et al. 2018). Therefore, forest-based biomass, especially from woody oil plants, is attractive to Chinese researchers and policy-makers. Moreover, manipulating the yield and quality of oil produced by these bioenergy tree species has become an important research focus in recent years (Harwood et al. 2017).

Styrax tonkinensis is widely distributed in Vietnam, Laos and the southern provinces of China. It is known for its fragrant stem-extracted resin, medicinal properties, ornamental application and timber utilisation (Pinyopusarerk 1994, Hieu et al. 2011, Xu & Yu 2015, Matsuo et al. 2016, Wu et al. 2020a). The oilseed is also famous for its rich lipid production and capability for fast growth (Shi et al. 2013). Thus, many Chinese agronomists and researchers have recognised *S. tonkinensis* as a promising woody plant to be utilised in the renewable diesel industry (Chen et al. 2015).

Extensive work has been carried out to broaden and deepen the understanding of the mechanism that regulates lipid synthesis pathway in general (Liu et al. 2014). As observed in the model plant, *Arabidopsis thaliana*, the quantitative oil content trait in seeds is controlled by a complicated transcriptional and enzymatic regulatory network (Wu et al. 2006). The substrate partitioning among all primary and secondary carbon reserves, and the deposit and transfer of maternal-derived precursors may also affect the embryonic oil bioaccumulation in plant seeds (Baud & Lepiniec 2010, Liu et al. 2014). In *S. tonkinensis*, the seed oil yield has been shown to be influenced by the carbon competition between starch and lipid synthesis in kernels, and the maternal photosynthesis status in the pericarps (Zhang et al. 2017, Zhang et al. 2018a).

Previous investigations confirm that the oil accumulation rate declines during the mid-late stage of *S. tonkinensis* seed filling (80–120 days after flowering (DAF), as shown by *in vitro* physiological and oil body ultrastructural evidences (Zhang et al. 2017, Zhang et al. 2018b). Moreover, the potassium concentration plateau in *S. tonkinensis* kernels was observed between 80 and 120 DAF, which overlapped with the 40 day reduction in the oil accumulation rate. Furthermore, the potassium concentration showed a significant correlation with crude oil (r^2 = 0.850, p < 0.01) and total fatty acid (r^2 = 0.859, p < 0.01) (Wu et al. 2019).

Potassium is an essential macronutrient for plant growth and development (Gotore et al. 2014, Kamel et al. 2016). It plays a pivotal role in many plant physiological processes, affecting photosynthesis, photosynthate transport in phloem, enzyme activation, protein synthesis, osmoregulation, plant-water relation and stress resistance (Marschner 2012). Field experiments have shown that the use of K would influence seed yield and oil contents in canola and cotton seeds (Kamel et al. 2016).

In this study, the effect of foliar potassium on *S. tonkinensis* kernel development was investigated. Based on the significant positive correlation between oil and K concentration, it was hypothesised that exogenous foliar K application could increase kernel oil content. Mainly, the aims of the study are to answer the following two questions: (1) Would foliar K treatments affect potassium concentration in the developing *S. tonkinensis* kernel? (2) Would the seed oil accumulation process, kernel oil content and composition be influenced by K application? Since upregulation of seed oil yield through genetic manipulation might not be as effective as originally expected, data from the present study may provide a non-genetic-based method for boosting *S. tonkinensis* seed oil yield in a much simpler way (Ohlrogge & Jaworski 1997).

MATERIALS AND METHODS

Plant materials

Two-year-old *S. tonkinensis* seedlings were purchased from Jishui (Jiangxi Province, PR China) and planted in 2011 on the east side of Hewangba Reservoir, Luhe District, Nanjing, PR China (32° 54′ N, 118° 50′ E), and the trees grew under natural conditions. Open pollination occurred after the plants' juvenile phase ended in 2012. In late May 2016, three blocks (40 m × 50 m) were randomly chosen in the field. Ninety trees with similar height (3.2 ± 0.5 m) and number of flowers (3320 ± 911) were tagged in these blocks (30 trees each) for *in vivo* treatment and further sampling. The highest/lowest temperatures and cumulative monthly precipitation from April to October in Luhe District were recorded (Figure S1).

Plant foliar fertilisers

To rule out the influence of inorganic anions, 0.5 and 4 g L^{-1} potassium D-gluconate solution were used as foliar potassium fertiliser. These two treatments were assigned as K1 and K2 respectively. Then, because of the possible effects of gluconate on this experiment, 0.676 and 5.403 mL L^{-1} D-gluconic acid solution were used as the gluconate control. These two treatments were assigned as G1 and G2. The control plants were only treated with water (CK). Polysorbate was added as a surfactant to promote K absorption. Its final concentration in the foliar fertiliser was 4%. The potassium and gluconate concentration in each treatment are given in Table S1.

Fertiliser spray experiments

The fertiliser was applied onto the whole tree crown using a manual sprayer (total volume of 16 L, spray rate of 15 mL m^{-2} s^{-1}). The application

was always performed in the morning (6:30 to 8:30 a.m.) right after sampling (on 38, 58, 78, 98 and 118 DAF). A randomised complete block design was performed in this study. A similar treatment (K1, K2, G1, G2 or CK) was applied on trees with similar growth conditions in the field (n = 18). Approximately 900 mL foliar liquid fertiliser was used on each sampled tree, corresponding approximately to a dose of 0.075 or 0.599 g potassium per tree in foliar K treatment, and 0.377 or 3.015 g D-gluconic acid per tree in the gluconate control treatment.

Photosynthetic activity

Photosynthetic parameters were measured in the field on 26 July (57 DAF) using a portable photosynthesis system. Measurements were performed in the morning (08:00 to 11:30 a.m.). The leaves remained attached to the stem during measurement. The chamber temperature was kept at 24.9 ± 0.6 °C, the intensity of light 1000 µmol m^{-1} s^{-1}, ambient CO_2 concentration about 400 µmol mol^{-1}, and humidity 31.7 ± 0.9%. Data of each treatment represented the means obtained from three plants in three blocks. The values of photosynthetically active radiation (PAR) were 1045 ± 220 (CK), 1204 ± 147 (K1), 1192 ± 69 (K2), 1155 ± 99 (G1) and 1163 ± 61 (G2) µmol m^{-2} s^{-1}, respectively.

Chlorophyll content of leaves

The pigment content of leaves was determined using leaf discs. The chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid (Car) contents were measured in the samples upon extraction in buffered aqueous 80% acetone (Porra 2005). Absorbance at 644, 663 and 470 nm of the supernatant was measured with a spectrophotometer. Leaves from all treatments were rinsed in distilled water prior to analysis.

Kernel dry mass measurements

For each treatment, fresh fruits were harvested at approximately the same tree height. Fruit samples were hand-picked randomly in different crown directions from 6 July (37 DAF) to 14 October (137 DAF), with a 19-day interval between each harvest. Drupes were sealed in plastic bags immediately and embedded in an insulation ice box (-10 °C). Kernels, from three

replicates of 30 randomly selected fruit, were dried at 70 °C for 72 hours and weighed (dry matter (DM)). The remaining fruits were frozen in liquid nitrogen and stored at -80 °C for later analyses.

Lipid extraction and determination

Kernel lipid was extracted as described by Zhang et al. (2017). In brief, 30 de-coated seeds were weighed after drying at 70 °C for 72 hours. The oil in the grounded kernels was then extracted in petroleum ether (30–60 °C) using Soxhlet apparatus. The flask temperature was kept at 65 °C and the extraction lasted 12 hours. The oil was then separated from the petroleum ether phase using a rotary vacuum evaporator. After extraction, the grounded kernels were weighed again and the total crude lipid content was expressed as the percentage of oil on a DM basis.

Potassium analysis

For measuring potassium element in kernels, about 500 mg frozen fresh kernels were mixed evenly with 9 mL concentrated sulfuric acid and 1 mL concentrated perchloric acid in a Kjeldahl flask. The flask was placed at room temperature overnight. The next day, the nutritional elements were released after approximately 30 min of digestion in a electric heating oven. After cooling under running water, the colourless extract was then passed through an ash-less filter paper and brought to a final volume of 100 mL with deionized water. To quantify kernel potassium, nutritional element extract was diluted 100-fold. The K concentration was determined using an atomic absorption spectrometer (Gharaghani et al. 2018). The wavelength (776.49 nm) was set by the WinLab32 AA Flame Control software.

Acetyl coenzyme carboxylase (ACCase) activity

The kernel ACCase activity from each development stage was measured with an enzyme-linked immunosorbent assay kit according to the manufacturer's instructions. The preparation and quantification procedures were the same as described by Zhang et al. (2018b). In brief, the microtiter plates were pre-coated with an antibody specific to plant ACCase. After antibody-antigen interactions, signals were amplified and

qualified. The colour change was measured spectrophotometrically at wavelength of 450 nm against the standard curve.

Primary reserves assay

To extract soluble sugars, 300 mg kernels were homogenised in 10 mL deionised water. The homogenate was then transferred into a glass tube and kept in boiling water bath for 20 min. After cooling under running water, the sugar extract was decanted and paper-filtered into a volumetric flask. The insoluble residue was again subjected to the same extraction procedure. The combined extract was then diluted with deionised water and brought to a volume of 25 mL. The total soluble sugar content was measured using an anthrone colorimetric method with fructose as a standard (Zhang et al. 2017).

The quantification of kernel starch was adapted from Wu et al. (2020b). The residue remaining after sugar extraction was re-homogenised with 10 mL deionised water. After 15 min in boiling water bath, 2 mL $HClO_4$ (9.2 mol L^{-1}) was added, followed by a further 15 min in the bath. The diluted supernatant was prepared by the same method above. The starch content was calculated through a standard curve, prepared using a starch solution of various concentrations.

The soluble protein was quantified according to Wu et al. (2020b). A total amount of 500 mg de-coated seeds were homogenised in 5 mL extraction buffer containing 0.02 mol L^{-1} phosphate buffer (pH 7.5). After centrifuging at 6000 rpm for 15 min at 4 °C, the supernatant was diluted well before measurement against bovine serum albumin as the standard.

Statistical analysis

Values were expressed as mean ± standard deviation of three independent measurements. Excel was used to process the figures. One-way or two-way analysis of variance (ANOVA) was performed using SPSS 19.0 followed by Duncan's multiple range test. P-values less than 0.05 were considered to indicate significance within groups.

RESULTS

Effects of foliar fertiliser treatments on photosynthetic activity in leaves

Significant difference ($p < 0.05$) was found within treatments for all photosynthetic activity indexes. Under the foliar application of 0.5 g L^{-1} potassium D-gluconate and the corresponding gluconate control (0.676 mL L^{-1} D-gluconic acid), the net photosynthetic rates at 57 DAF were not significantly different with CK ($p > 0.05$) (Table 1). As the foliar potassium concentration rose from 0.083 g L^{-1} (K1) to 0.666 g L^{-1} (K2), a 44 and 69% increase were found, respectively, in the net photosynthetic rate and transpiration rate of the *S. tonkinensis* leaves. Plants under the two gluconate controls (G1 and G2) showed approximately similar photosynthetic rate and intercellular CO_2 concentration ($p > 0.05$).

With the exception of 77 and 97 DAF, little difference ($p > 0.05$) was found in the Chl a content between the K2 application and the CK (Figure 1A). The K2 resulted in boosted Chl a content which was 14 and 39% higher than CK and G2 at 77 DAF, while 41 and 31% more Chl a was detected under K2 at 97 DAF than CK and gluconate control (G2), respectively. Large

Table 1   Comparison of photosynthetic activity in leaves among treatments at 57 DAF (20 days after the first foliar spray onto *Styrax tonkinensis* trees)

Treatment	Photosynthetic rate (µmol CO_2 m² s⁻¹)	Transpiration rate (mmol H_2O m² s⁻¹)
CK	8.95 ± 0.36[a]	1.97 ± 0.53[a]
K1	9.38 ± 0.59[a]	2.74 ± 0.39[b]
K2	12.90 ± 0.22[b]	3.33 ± 0.17[b]
G1	9.68 ± 0.88[ab]	2.05 ± 0.58[a]
G2	10.60 ± 0.76[ab]	2.96 ± 0.24[b]

Data are mean values of six plants from each treatment; different letters within treatments indicate significant difference ($p < 0.05$)

chlorophyll and carotenoid content gaps were observed between gluconate controls (G1 and G2) and other treatments (CK, K1 and K2) at 57 DAF (Figure 1A–C). The Chl a/b ratio remained relatively steady under K2 at all developmental stages, whereas the Chl a/b reached its nadir at 77 DAF under all other treatments (Figure 1D). The highest Chl (a+b)/Car ratio (6.82) was found after treatment with 4 g L^{-1} potassium

D-gluconate (K2) at 77 DAF (Figure 1E). Values of the average ratio of Chl a/b and Chl (a+b)/Car under potassium D-gluconate (2.98 and 5.46 respectively) were higher than those under gluconate controls (2.69 and 5.10 respectively) and CK (2.82 and 5.24 respectively), which indicated that the foliar K application might increase the photosynthetic activity in *S. tonkinensis.*
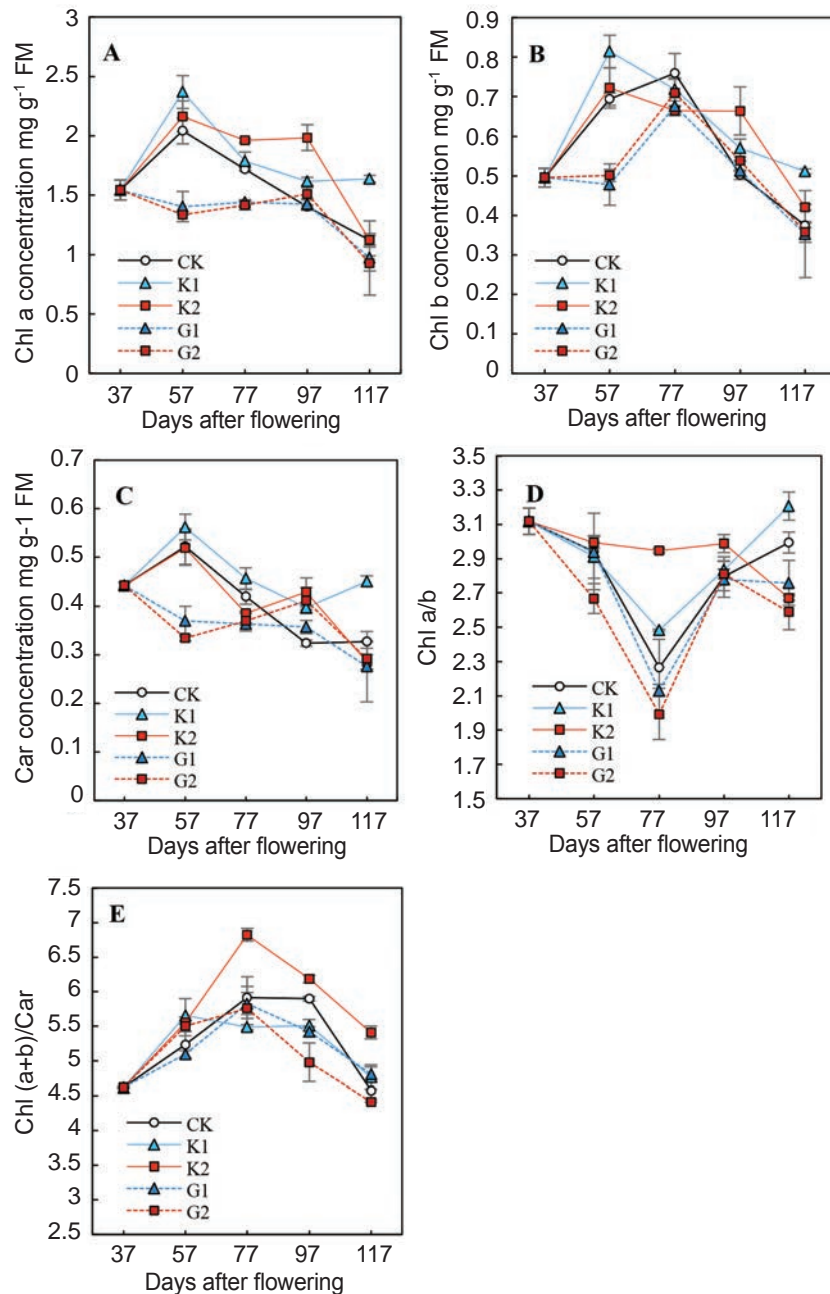
Figure 1   Dynamic chlorophyll and carotenoid concentration in *Styrax tonkinensis* leaves during fruit maturation under treatments; A = chlorophyll a concentration under CK, K1, K2, G1 and G2, B = chlorophyll b concentration under CK, K1, K2, G1 and G2, C = carotenoid concentration under CK, K1, K2, G1 and G2, D = chlorophyll a/b ratio under CK, K1, K2, G1 and G2, E = chlorophyll (a+b)/carotenoid ratio under CK, K1, K2, G1 and G2; Chl = chlorophyll, Car = caratenoid, FM = fresh matter

Effects of foliar fertiliser treatments on kernel yield and kernel oil content

The dynamics of the kernel dry weight evolved consistently among treatments at 37–77 DAF (Figure 2). After 77 DAF, K2 treatment caused the accumulation of more dry matter in *S. tonkinensis* kernels than in kernels growing in other foliar spray treatments, and the control (p < 0.05). Compared with CK, a dramatic increase (52%) in kernel dry weight, reaching 69.2 mg per fruit, was detected at 137 DAF when the *S. tonkinensis* trees were treated with foliar potassium fertiliser (4 g L^{-1} potassium D-gluconate) during fruit maturation.
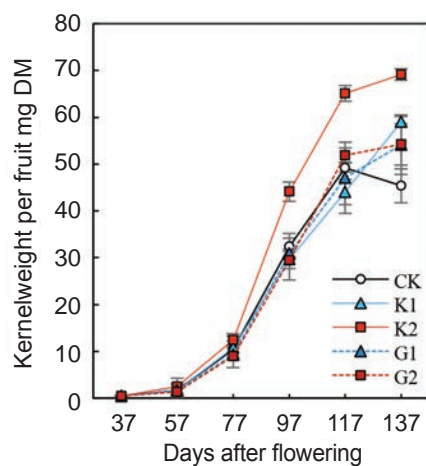
**Figure 2**   Kernel dry mass under CK, K1, K2, G1 and G2

To test whether the K application would alter the kernel oil accumulation process, the crude oil was analysed in kernels. In the CK, G1 and G2 treatments, kernel oil dynamics exhibited up-down patterns (Figure 3A). For CK, the peak concentration was found at 77 DAF, and a decreasing trend was then observed between 77 and 137 DAF (with a 13% decrease). For G1 and G2, the oil concentrations increased gradually until 97 DAF (20 days after the CK peak), and then, decreasing trends were detected for the next forty days, with a 19 and 27% decline, respectively. Interestingly, kernel oil synthesis was altered after the foliar K spray, and no oil accumulation rate reduction was found during the mid-late stage of *S. tonkinensis* seed filling. Linear regressions could be constructed for the dynamics of kernel lipid concentrations under potassium treatments (for K1, r^2 = 0.949, p < 0.01; for K2, r^2 = 0.906, p < 0.01). The change in the

oil per kernel over time displayed a sigmoidal pattern for most treatments (Figure 3B), and the total crude lipid per kernel was greatly promoted in the K applications, where it was 42% (K1) and 72% (K2) higher than CK at 137 DAF (p < 0.05). The kernel lipid composition of all treatments at 137 DAF was then further investigated. As shown in Figure 3C, 4.4–6.3% non-fatty-acid lipid (non-FA) was detected in CK, K1 and G1, whereas 9.2% non-FA was found in K2. Thus, in the one-way ANOVA, no significant difference (p > 0.05) was detected in the fatty acid concentration among K1, K2 and CK. However, when the kernel dry mass was considered, the total fatty acid content in each kernel was boosted after the foliar potassium spray, with a 39 and a 63% increase for K1 and K2, respectively (Figure 3D). Statistically, there was no significant difference within treatments among all fatty acids (p > 0.05). In CK, the dynamics of kernel ACCase activity followed an M-shaped trend. Peaks were observed along with the lipid accumulation process, at 57 and 97 DAF (Figure 3E). In other treatments, the ACCase activity expressed a single peak between 57 and 77 DAF.

DISCUSSION

Foliar K treatments may promote photosynthesis in leaves and improve fruit dry mass

In the present study, foliar spray of K fertiliser (potassium D-gluconate, which is exclusive from other essential mineral elements such as nitrogen, phosphorus and sulphur) was applied to determine its effect on fruit growth and kernel lipid synthesis in *S. tonkinensis*. Numerous researchers have found that foliar applications of potassium had a positive effect on photosynthesis by stimulating the pigments and enzyme activities, which consequently encouraged the vegetative and reproductive growth in plants (Egilla et al. 2005, Jabeen et al. 2011). In the field experiment, the content of chlorophyll in leaves showed a rise and fall pattern under all treatments (Figure 1). The K spray improved the net photosynthesis rate (Table 1), as well as chlorophyll and carotenoid average values (for total Chl, CK: 2.12 mg g^{-1} fresh matter (FM), K-gluconate: 2.40 mg g^{-1} FM, gluconate control: 1.86 mg g^{-1} FM; for Car, CK: 0.40 mg g^{-1} FM, K-gluconate: 0.44 mg g^{-1}
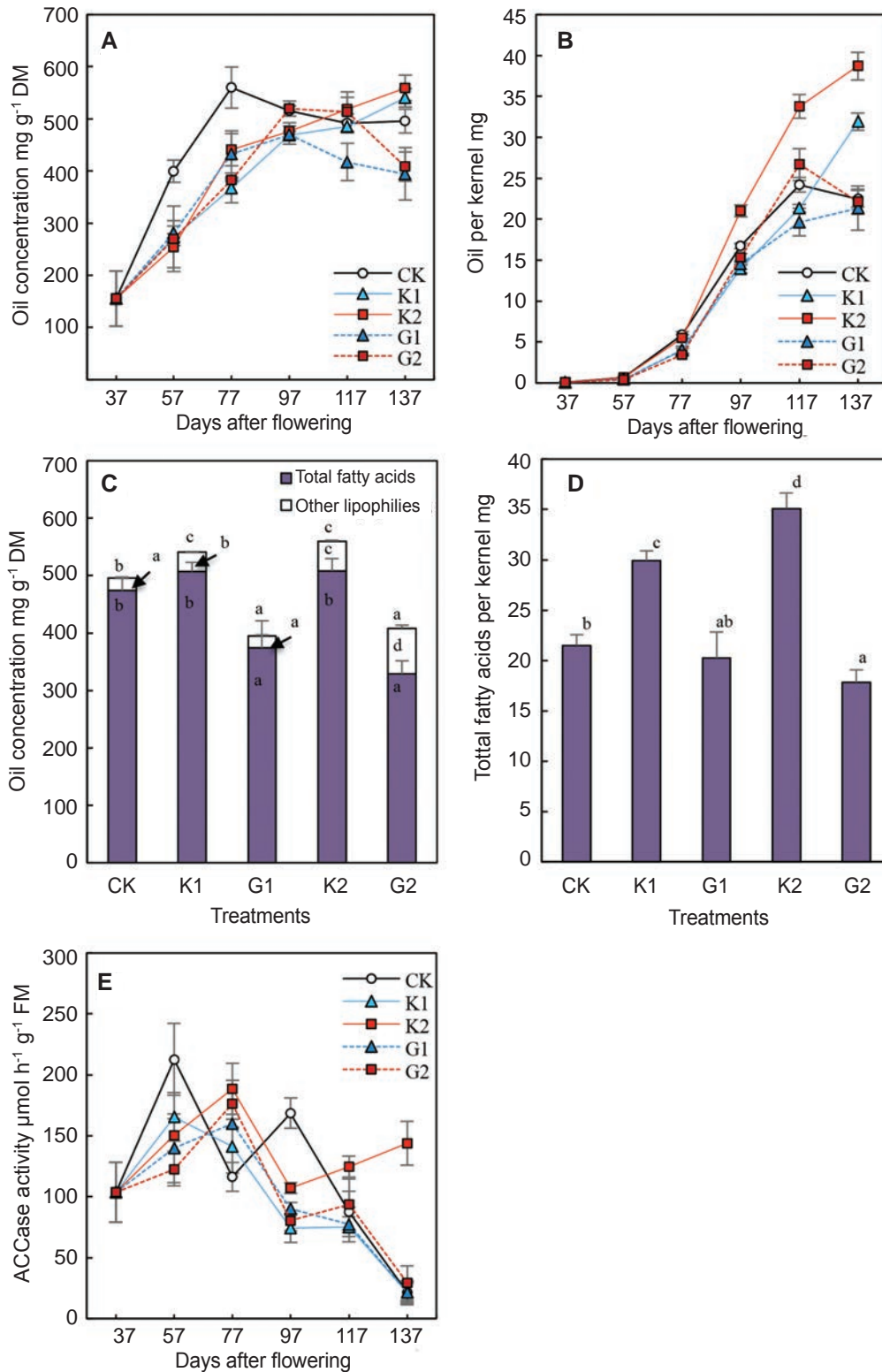
Figure 3   Lipid synthesis in developing *Styrax tonkinensis* kernels under different foliar treatments; A = oil concentration in kernels, B = oil content per kernel, C = total fatty acids and other lipophilic composition at 137 DAF; different letters within treatments indicate significant difference (p < 0.05) for total fatty acids (letters inside black bars), or other lipophilic (letters inside white bars), or total crude oil (i.e, total fatty acids + other lipophilic; letters above white bars), and different letters within treatments indicate significant difference (p < 0.05); D = total fatty acids content per kernel at 137 DAF, E = acetyl coenzyme carboxylase (ACCase) activity dynamics under CK, K1, K2, G1 and G22, DM = dry matter

FM, gluconate control: 0.36 mg g^{-1} FM) (Figure 1). It has been shown that the higher Chl a/b ratio increased the PSII performance (Li et al. 2009). Furthermore, the elevated Chl (a+b)/Car would lead to higher absorption efficiency at the blue and red spectra (Zhang et al. 2018a). The pigment evidences suggest that the activity of photosynthetic light reaction in leaves was enhanced by K spray. The present results showed that 4 g L^{-1} K-gluconate (K2) markedly enhanced the dry matter accumulation rates in the kernels (Figure 2). These findings are in line with previous studies. In chervil plant (*Anthriscus cerefolium*), K fertiliser increased herb and essential oil yield as well as photosynthetic pigments compared with control (El Gendy et al. 2015). Foliar spray of potassium increased the maize yield under rainfed conditions (Anees et al. 2016). Researchers have reached a consensus that the increment in biological yield (i.e., grain number, grain weight, etc.) could be due to the role of potassium, in both boosting of leaf photosynthetic activity and availability of photosynthates for translocation from leaves (source) to fruits (sink).

K concertation affects oil accumulation in kernels

During *S. tonkinensis* kernel development, potassium had a significant positive correlation with oil concentration (Wu et al. 2019). The potassium concentration in kernels were not elevated under the spray of foliar K fertilisers, and the total fatty acids concentrations under K treatments had little difference (p > 0.05) when compared with those of CK at 137 DAF (Figure 3). The enhancement of total oil content per kernel is due to the positive response of fruit dry mass to K treatments, which coincides with most previous studies (Romheld & Kirkby 2010, El Gendy et al. 2015, Helmy & Mohamed 2014). Other primary assimilates (i.e., starch, sugar and protein) increased in each *S. tonkinensis* kernel as well (Figure 4A–E). Unexpectedly, the foliar K spray affected the potassium and lipid accumulation patterns in kernels (although the final oil concentration was not altered). Specifically, for CK, the potassium concentration dynamics had a 40% decrease at 77–97 DAF before it rose markedly again during the next 20 days, whereas the K concentration was well maintained (3.4–3.8 mg g^{-1} FM) during the

period from 77 to 117 DAF under K2 treatment (Figure S2). Generally, the massive potassium concentration declines and oil accumulation rate reduction at the mid-late stage can be alleviated under K treatments, nevertheless, the final oil concentration was not affected. However, the question remains as how K affect lipid biosynthesis process.

## Potassium might affect carbon partitioning among kernel primary assimilates

There is solid evidence that lipid storage is influenced by other cell metabolic pathways (Klaus et al. 2004, Weselake et al. 2009). Over-expression of *Escherichia coli* ADP-glucose pyrophosphorylase in both *Brassica napus* and *Arabidopsis thaliana* seeds resulted in the diversion of carbon precursors from lipid toward starch (Boddupalli et al. 1995, Lin et al. 2006). Consequently, a major increase in starch content and a decrease in oil content was observed. In the present study, the carbohydrate (sugar + starch) proportion among primary metabolites increased from 57 to 77 DAF (Figure 4F–J) and oil accumulation rate from 37 to 77 DAF (Figure 3A). Values from highest to lowest were CK, K2 and G2. These results showed that when more carbon accumulates in carbohydrates, the oil assembly speed would slow down. Given that cell carbon can flow into either the pyruvate pool (substrate for fatty acids biosynthesis) in plastids or other carbohydrate, the precursor competition among carbon reserves may play a role in manipulating the oil accumulation process.

After further investigation, it was found that kernel K was closely related to the oil ratio, among all primary reserves (p < 0.05) (Figure 5). The finding prompted the authors to assume that potassium may have an impact on precursor partitioning among carbon assimilation during kernel maturation, which regulates oil accumulation rate. This assumption, however, needs to be further examined.

Study limitations

Photosynthesis data should be measured more than once at 57 DAF, which would facilitate the evaluation on photosynthetic behavior in leaves under different K sprays. The total yield per plant is agronomically important. Unfortunately, the
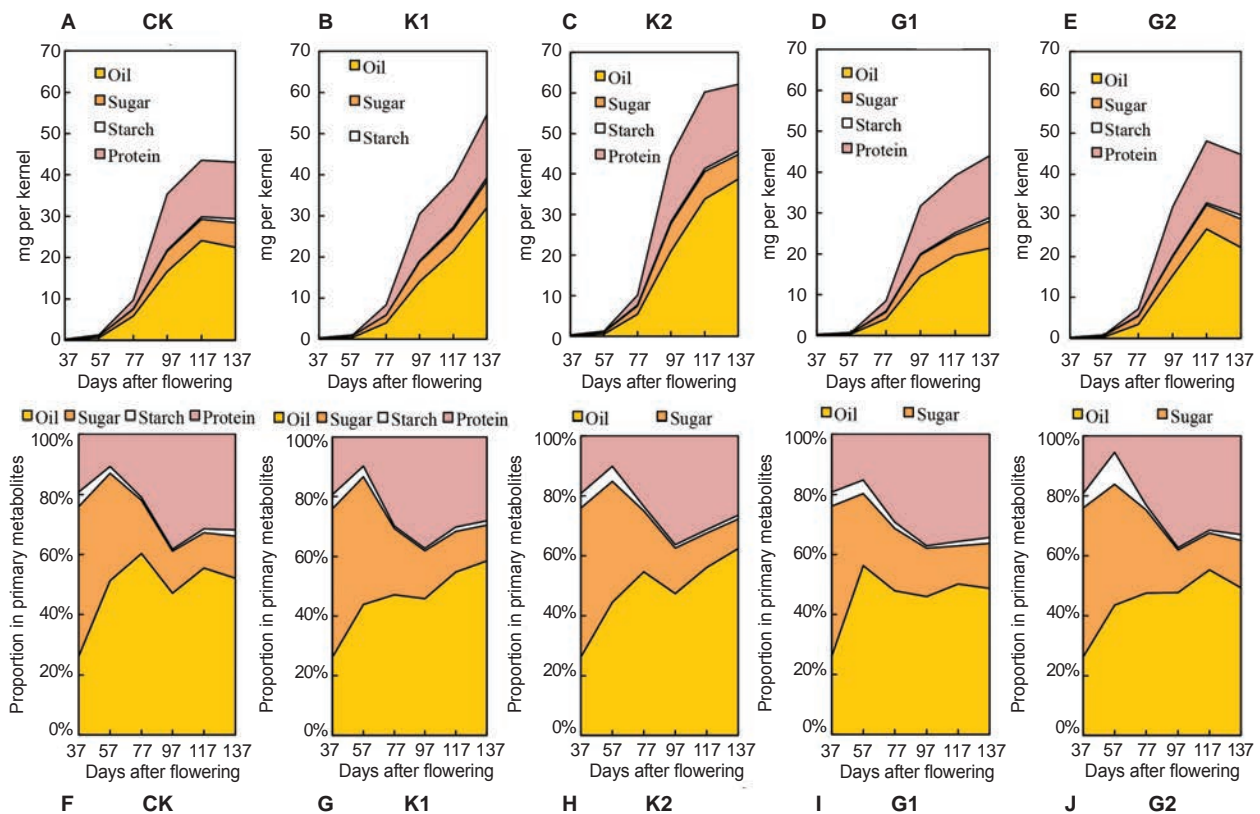
Figure 4 Reserves (lipid, starch, sugar and protein) total content per kernel (A–E) and concentration dynamics (F–J) in *Styrax tonkinensis* under different treatments
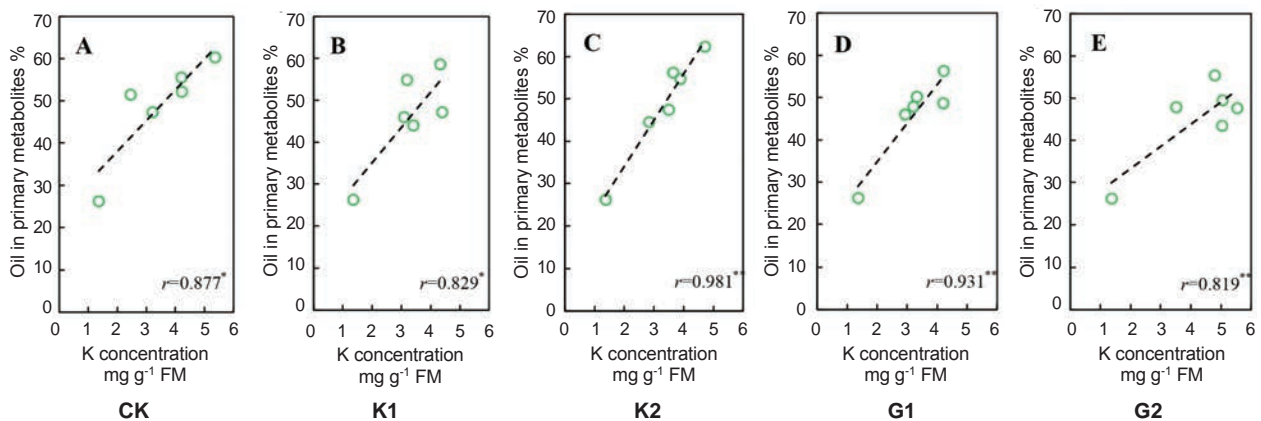
Figure 5 Relation between kernel K and oil proportion among kernel reserves under different treatments (A–E); *represents significance at 0.05 level, and **represents significance at 0.01 level

total yield of seeds, kernel oil or kernel potassium per tree were unable to be included in this paper, since the fruits were sampled over time. Potassium fertilisers in higher concentrations can be discussed in further studies. In addition, advanced proteome or transcriptome analysis can be conducted to profoundly disclose the role of K in lipid biosynthesis in *S. tonkinensis* kernels.

CONCLUSIONS

For the first time, potassium fertiliser was applied by foliar spray during *S. tonkinensis* fruit maturation season, to study the effects of foliar K on lipid biosynthesis in the kernels. During *S. tonkinensis* kernel development, foliar potassium fertiliser increased photosynthesis

and promoted the transport of assimilates to the sinks. Compared with CK, the final kernel oil concentration was not boosted under all treatments. However, major increases in the lipid yield per kernel were found under K1 (0.5 g L^{-1} potassium gluconate) and K2 (4 g L^{-1} potassium gluconate) treatments. The findings suggest that a foliar K spray, at a suitable concentration during fruit development, could be considered to increase seed and oil yields.

ACKNOWLEDGEMENTS

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Table S1 Concentration of foliar fertiliser applied for *Styrax tonkinensis* plants in each treatment and the corresponding potassium and gluconate concentrations

Treatments		Concentrations		
		Foliar fertiliser	Potassium	Gluconate
CK	Water	NA	NA	NA
K1	Potassium D-gluconate	0.5 g L^{-1}	0.083 g L^{-1}	0.417 g L^{-1}
K2	Potassium D-gluconate	4.0 g L^{-1}	0.666 g L^{-1}	3.333 g L^{-1}
G1	D-gluconic acid	0.676 mL L^{-1}	NA	0.417 g L^{-1}
G2	D-gluconic acid	5.403 mL L^{-1}	NA	3.333 g L^{-1}

NA = not applied
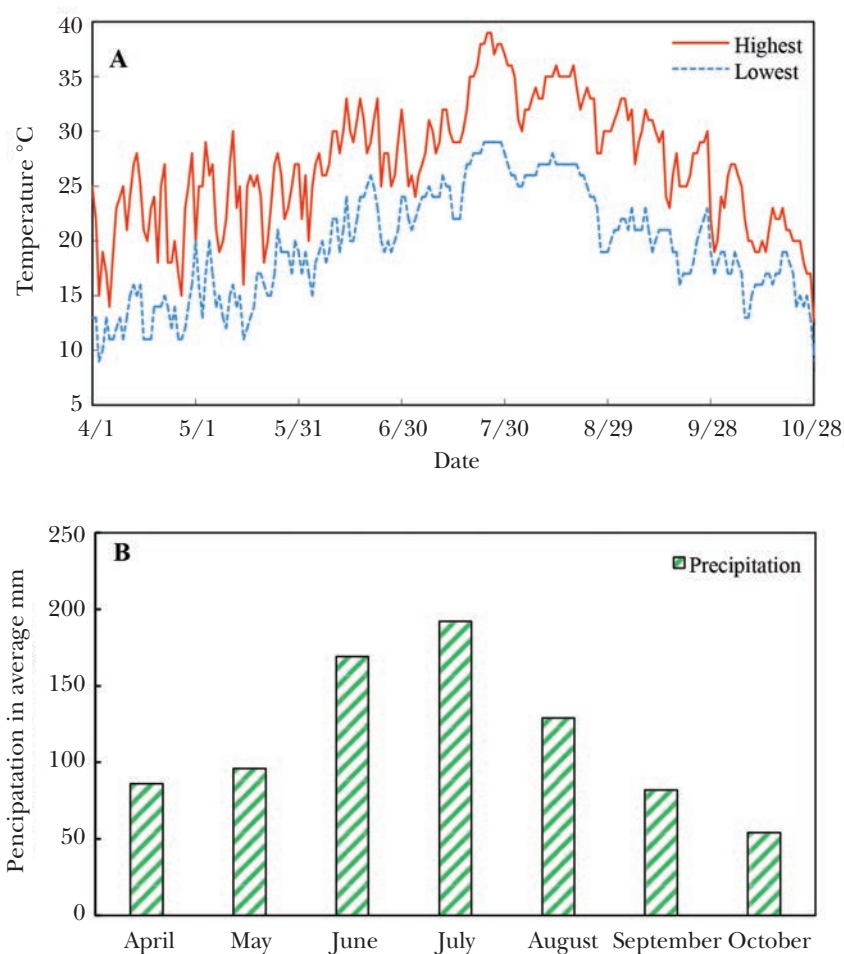
Figure S1 Meteorological data recorded at Luhe District, Nanjing, China from April to October, 2016; A = highest and lowest temperature daily, B = average precipitation monthly; data acquired from the public service website of Chinese Meteorological Administration (http://www.weather.com.cn/)
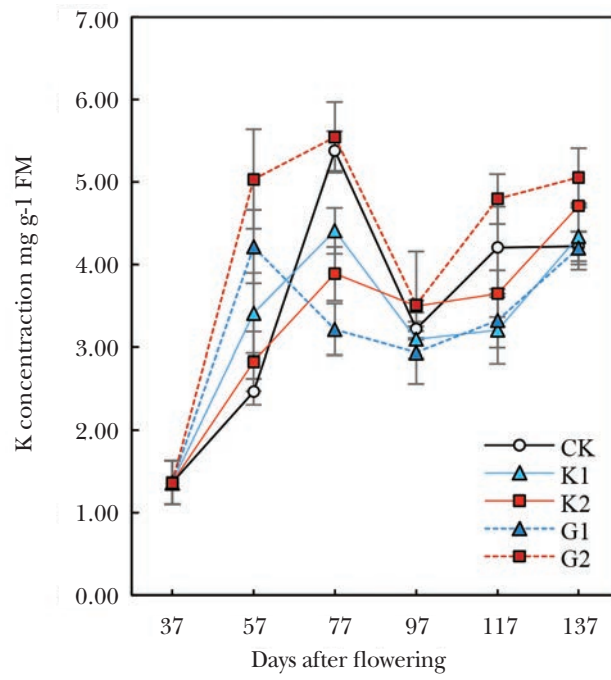
Figure S2 Potassium concentration dynamics in developing *Styrax tonkinensis* kernels after foliar spray of K1, K2, G1, G2 and CK