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ORIGINAL ARTICLE

Summer pruning improves the branch growth and tea quality of tea trees (*Camellia sinensis*)

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Abstract

At present, twice-a-year pruning is usually adopted for the tea garden, in which only spring tea is picked. Effects of different times and heights of summer pruning on tea trees are unknown. Here the effects of summer pruning on 'Jin Guan Yin' oolong mature tea trees were studied. The results showed that pruning at 30 cm (summer pruning at 30 cm above the cut surface of spring pruning) on Jul 25 could benefit to the growth of lateral branches, whereas two pruning treatments in August reduced their growth vigor. Pruning at 30 cm could also provide a benefit by decreasing the ratio of total tea polyphenols to free amino acids (TP/AA), an important factor in tea quality. Pruning at 30 cm and two pruning in August all significantly enhanced the contents of ester catechins ['(−)-epigallocatechin gallate (EGCG) + (−)-epicatechin gallate (ECG)'] as compared to conventional summer pruning. Pruning on Aug 15 (summer pruning at 20 cm on Aug 15) had the lowest (EGCG + ECG)%. In addition, enhanced AA content induced by pruning at 30 cm was significantly associated with enhanced transcription of *glutamine synthetase* (*CsGS*) or *glutamine:2-oxoglutarate aminotransferase* (*CsGOGAT*). In a word, the summer pruning at 30 cm on Jul 25 was best to benefit the growth of lateral branches and spring tea yield and quality, whereas pruning on Aug 15 had the worst comprehensive effects.

Keywords Tea trees (*Camellia sinensis*) · Summer pruning · Lateral branch · Yield · Spring tea

Abbreviations

AA	Amino acids
C	(+)-Catechin
C4H	Cinnamate 4-hydroxylase
CG	Catechin gallate
4CL	P-coumarate: CoA ligase
EC	(–)-Epicatechin
ECG	(–)-Epicatechin gallate
EGC	(–)-Epigallocatechin
EGCG	(–)-Epigallocatechin gallate
ECG	(–)-Epicatechin gallate
FL	Flavonoid
GA	Gallic acid
GC	(–)-Gallocatechin
GCG	Gallocatechin gallate
GS	Glutamine synthetase
GOGAT	Glutamine: 2-oxoglutarate aminotransferase
PAL	Phenylalanine ammonialyase
PP	Phenylpropanoid
TP/AA	Total tea polyphenols/free amino acids

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Introduction

The perennial tea plant [*Camellia sinensis* (L.) O. Kuntze] is an economically important woody crop, with bud leaves in the lateral branches as its product organ (Yao and Wu 1990; Tounekti et al. 2013; Wu et al. 2015). Several active health-promoting ingredients, such as theanine, polyphenols, caffeine and catechin, are found in tea leaves (Chen et al. 2011; Kumar et al. 2015; Sun et al. 2018). Catechins, which belong to flavonoids, are the main constituents of polyphenols in tea (Sun et al. 2018; Wan et al. 2015). Oolong tea is one of the most popular beverages owning to its significant amount of catechins (Chen et al. 2009, 2011). A previous study showed that six kinds of catechins, namely, (+)-catechin (C), (−)-gallocatechin (GC), (−)-epicatechin (EC),

(−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin gallate (EGCG), are the major phenolic compounds in oolong tea (Chen et al. 2009). Furthermore, previous study reported that the contents of EGCG and catechin gallate (CG) in fresh tea leaf were significantly correlated with oolong tea quality (Chen et al. 2010, 2011).

Pruning is an essential agronomic practice in tea cultivation (Kumar et al. 2015; Sun et al. 2018).

Reasonable pruning can promote the growth of branches and shoots, maintain tea bushes under manageable conditions for picking, and improve the production and quality of fresh tea leaf (Kumar et al. 2015; Sun et al. 2018; Chen et al. 2009; Ravichandran 2004). The biological and physiological effects of pruning have been previously reported (Kumar et al. 2015; Sun et al. 2018). Firstly, the pruning degree could affect the growth, yield and quality of spring tea. For example, Jiang et al. (2018) reported that selective pruning in summer could improve spring tea yield and quality in comparison with deep pruning, which refers to pruning 55–60 cm above the ground. The polyphenols contents decreased with increasing pruning height (Satyanarayana et al. 1994; Wang et al. 2015). Secondly, the pruning time affects spring tea yield and quality. For example, Kumar et al. (2015) reported that the maximum yield was obtained when the trees were pruned in December. Pruning prior to picking considerably improves the productivity of tea trees (Kumar et al. 2015; Ravichandran 2004). Chen (1996) reported that pruning after spring could improve spring tea yield in comparison with pruning before spring. Finally, the combination of pruning degree and time could also affect spring tea yield and quality. For example, light-pruning after summer increased the yield and polyphenol content but slightly decreased the contents of theanine and caffeine (Xu et al. 2014a). In addition, Xu et al. (2014b) reported that deep-pruning after spring could improve the yield and quality of spring tea in the following year.

In tea production, tea trees are pruned once every 4 or 5 years in some countries, such as Turkey and India (Kumar et al. 2015; Ravichandran 2004; Yilmaz et al. 2004; Thomas et al. 2005). Previous studies suggested that the yield and quality of fresh tea leaf varied with time after pruning. For example, in a 5-year pruning cycle, the maximum yield and number of shoots were observed during the third to fourth year and then reduced (Kumar et al. 2015; Yilmaz et al. 2004). The polyphenol content in fresh tea leaf almost linearly decreased with time from pruning (Ravichandran 2004; Yilmaz et al. 2004). Thomas et al. (2005) reported that 1–5 years after pruning, the synthesis of caffeine steady increased up to the fourth year, while the content of gallic acid (GA), a kind of catechin fraction, both increased up to the third year. However, Thomas et al. (2005) suggested that the biosynthesis of GA did not follow any specific trend in terms of plant age after pruning. Phenylalanine ammonialyase (PAL), which catalyzes the first step of the phenylpropanoid (PP) pathway, is one of the most important synthases for the biosynthesis of flavonoid and catechins (Sun et al. 2018; Thomas et al. 2005). Thomas et al. (2005) found that the activity of PAL increased steadily until the third year after pruning but declined rapidly in the fourth year. In addition, in comparison with unpruned tea trees, there are increased contents of caffeine and EGCG as well as increased ratio of ester catechins [(EGCG + ECG)%] in fresh tea leaves of long-term pruned tea trees (Sun et al. 2018). In comparison with unpruned tea trees, there are upregulated expression of biosynthetic genes which encode EGCG, leucoanthocyanidin reductase, and serine carboxypeptidases, respectively, in the leaves of long-term pruned tea tree (Sun et al. 2018).

Taken together, previous reports listed above have mainly studied the effects of pruning degree, pruning time, periodic pruning and long-term pruning on the growth, yield and quality of fresh tea leaves. For shrub-type mature tea garden in which only spring tea was picked up, tea plants usually need pruning twice a year. For the first time, tea trees were heavily pruned after spring tea in late April, and all the branch leaves that are approximately 50 cm above the ground are pruned. The second one is summer pruning. For the 2nd pruning, the heigh is increased by 20 cm from the last cut surface around Jul 20, and the trees are allowed to grow naturally. However, studies on the effects of times and heights of summer pruning are few. The mature tea trees of the 'Jin Guan Yin' oolong tea were used in this study. The effects of different times and heights of summer pruning on the growth of lateral branches, as well as the yield and quality of fresh tea leaf in the next spring, were investigated. The results of this study may serve as theoretical basis and technical support for establishing a model for summer pruning to economically benefit tea cultivation.

Material and methods

Plant material and growth conditions

For the summer pruning experiment, 'Jin Guan Yin' oolong tea trees (*C. sinensis* L.), which were heavily pruned in late April, were chosen. Tea trees have been planted for 4 years and are under the uniform soil and agronomic management conditions. The 2 year study (2018–2019) was conducted in the tea garden of Shengzhou experiment base, Tea Research Institute, Chinese Academy of Agricultural Sciences, located in Shaoxing, Zhejiang Province, China (longitude 120°48′E and latitude 29°44′N, 70 m above sea level). Shengzhou city belongs to northern subtropical zone humid climatic region of China. The climate is mild and humid with four clear seasons. The annual mean temperature is 16.5 °C. The mean temperature in January and July is 4.2–28.6 °C, respectively. The mean annual precipitation is about 1300 mm. The annual sunshine hours is close to 2000 h. The frost-free period is 235 days. The weather of four seasons in Shengzhou city is showen in Table 1 (China weather news).

Summer pruning treatment

The experiment was laid out in a randomized complete-block design, and each treatment comprises three replications. Each replication spans an area of 20 m^2 consisting of 40 tea bushes. After heavy pruning in late April, control tea bushes were not pruned again the same year. The conventional summer pruning was conducted at a height that was 20 cm higher than the last cut surface around Jul 20 every year, and it was conducted on Jul 25 here. For the three pruning heights on Jul 25, the other two heights were higher by 10 and 30 cm from the last cut surface, respectively. For the three pruning times at 20 cm, the other two pruning time was on Aug 5 and Aug 15, respectively. Six pruning treatments were performed in this study, as follows: (1) control, pruning once a year; (2) summer pruning at 10 cm on Jul 25 (pruning at 10 cm); (3) summer pruning at 20 cm on Jul 25 (conventional summer pruning); (4) summer pruning at 30 cm on Jul 25 (pruning at 30 cm); (5) summer pruning at 20 cm on Aug 5 (pruning on Aug 5); and (6) summer pruning at 20 cm on Aug 15 (pruning on Aug 15). The effects of different heights and times of summer pruning were examined as compared to conventional summer pruning.

Index measurement and methods

The leaf number per lateral shoot, length of lateral branch, bud number per new shoot and number of lateral branch growing out after pruning were measured after the trees dormancy in December. For each pruning treatment, 15 – 30 new shoots or lateral buds/branches were measured. The new shoots refer to the new branches sprouting from the main shoots after summer pruning.

The branch number per m^2, weight of 100 buds, density of bud and yield of spring tea was measured in the next spring. A square frame with 0.1 m^2 area was made using a hard iron wire. The number of leaf buds in the range of the square frame was counted, and the density of bud was calculated. The leaf buds in the range of the square frame were weighted, and the yield was calculated.

On a warm and cloudy day, April 1, 2019, young shoots with two leaves and a bud of spring tea were picked. The young shoots used for PAL activity analysis and RNA extraction were immediately frozen in liquid nitrogen, and subsequently stored at – 80 °C. Young shoots for analyzing of TP, AA, catechins and caffeine were dried to constant weight and stored in sealed bags.

Table 1 The weather of four seasons in Shengzhou city, Shaoxing, Zhejiang Province, China

	Spring	Summer	Autumn	Winter
Mean maximum temperature/°C	22	32	23	10
Mean minimum temperature/°C	12	24	15	3
Mean rainfall/mm	384	583	275	206

Quantification of TP and total free AA

TP content was determined in accordance with the method described by Li et al. (2016). Briefly, the diluted sample extract (1.0 mL) was transferred to tubes in duplicate, where each tube contained 5.0 mL of a 1/10 dilution of Folin–Ciocalteu's reagent in water. Then, 4.0 mL of sodium carbonate solution (7.5% w/v) was added into each tube. The tubes were kept at room temperature for 60 min, and the absorbance at 765 nm was monitored. Total AA from tea leaf sample were extracted in 80% ethanol at 80 °C. Following evaporation, dried samples were dissolved in 0.02 N HCl. AA was subjected to postcolumn reaction with ninhydrin reagent and detected spectrophotometrically.

Quantitative determination of catechins and caffeine

The standards of caffeine, GC, gallocatechin gallate (GCG), EGC, C, gallic acid (GA), EC and ECG were purchased from Biopurify Phytochemicals, Ltd. (Chengdu, China). The standards of EGCG and CG were purchased from Sigma-Aldrich (Shanghai, China). Caffeine and catechins were measured as follows: the plant samples were ultrasonically extracted with 75% methanol for 15 min. Their concentrations were determined via HPLC (Waters 2695, Waters Corp. USA). 10 μL of extract was injected into C18 reverse-phase column (250 × 4.6 mm i.d., Phenomenex, Torrance, CA, USA) maintained at 35 ℃ and eluted with solutions A and B with gradients running at 1 mL/min. Solution A and B were 2% formic acid and acetonitrile, respectively (Zhang et al. 2017).

PAL activity assay

A tea leaf sample (0.3 g) was homogenized in 3 mL of 50 mM potassium phosphate buffer (pH 8.8, containing 2 mM EDTA, 2% PVPP, and 0.1% mercaptoethanol). The homogenate was centrifuged at 15,000 rpm for 20 min at 4 °C and the crude enzyme extract was obtained as the supernatant. PAL activity was estimated with L-phenylalanine as the substrate (Li et al. 2016).

Real-time quantitative PCR (qPCR) analysis

Total RNA was extracted using an RNA extraction kit (Tiangen Biotech, Beijing, China) and reverse transcribed using a ReverTra Ace qPCR RT kit (Toyobo, Osaka, Japan) following the manufacturer's instructions. The gene-specific primer sequences are listed in Table 2. qPCR analysis was carried out using the StepOnePlus Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) with Power SYBR Green PCR Master Mix (Applied Biosystems). The qPCR conditions were as follows: 95 ℃ for 30 s, followed by 40 cycles of 95 ℃ for 5 s, and 60 ℃ for 34 s. Relative gene expression was calculated in accordance with a $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Statistical analysis

Data were analyzed using Statistica (SAS Institute, Inc., Cary, NC, USA, http://www.statsoft.com/). In each table and figure, the differences in each index among different pruning treatment were analyzed using one-way ANOVA, and significant ($P < 0.05$) levels were further analyzed via Duncan's multiple range test.

Results

Effects of summer pruning on the growth of tea trees and the yield of spring tea

The results showed that in comparison with the control (pruning once a year), pruning at 10 cm significantly enhanced the weight of 100 buds (Table 4), however, it significantly decreased the bud number per new shoot (Table 3) and bud density (Table 4). Pruning on Aug 5 significantly enhanced the weight of 100 buds; however, it significantly decreased bud density. Pruning on Aug 15 significantly decreased bud density (Table 4). In comparison with the control, pruning on Aug 15 significantly decreased spring tea yield, whereas the other pruning treatments did not affect the yield. In comparison with the control, summer pruning

Table 2 Primer sequences used for real-time quantitative PCR (qPCR)

Gene	Functional annotation		Forward primer 5′–3′	Reverse primer 5′–3′
CsPTB	POLYPYRIMIDINE TRACT-BINDING PROTEIN	GAAC01052498.1	TGACCAAGCACACTCCACACTATCG	TGCCCCCTTATCATCATCCACAA
CsPAL	PHENYLALANINE AMMONIA-LYASE	D26596	GAATGCCGGTCTTATCCACT	CGGTGAACACCTTGTCAAAC
CsC4H	CINNAMATE 4-HYDROXY-LASE	AY641731	CGAGAGGTTCTTGGAAGAGG	AGAATTGGCAGAGCAAGGAT
Cs4CL	P-COUMARATE:COA LIGASE	DQ194356	GGAGGTTATCCTGGACCTCA	GGCAAGCCTTGTAGTGTGAA
CsGS	GLUTAMINE SYNTHETASE	EU284131	CCTCAGAAGCAAAGCAAGGACT	AACATCAGGGTGGCTGAAAATC
CsGOGAT	GLUTAMINE: 2-OXOGLUTARATE	JN602371	TGCTTCAGGACGTTTTGGTGT	CATGATGTGGAGGTGGGGATAT

Table 3 Effects of different heights and times of summer pruning on the number of lateral branch growing out after pruning, leaf number per lateral branch, length and diameter of lateral branch as well as bud number per new shoot

Pruning heights and times		Number of lateral branch growing out after pruning	Leaf number per lateral branch	Length of lateral branch (cm)	Diameter of lateral branch (cm)	Bud number per new shoot
Control		–	22.75 ± 0.66e	63.02 ± 1.82d	–	22.83 ± 0.58f
Jul 25	10 cm	3.13 ± 0.31b	11.22 ± 0.48bc	28.52 ± 1.61ab	3.12 ± 0.04a	10.27 ± 0.15c
	20 cm	3.93 ± 0.23d	10.96 ± 0.22bc	31.16 ± 2.24b	3.34 ± 0.10ab	11.30 ± 0.10d
	30 cm	3.53 ± 0.46c	12.84 ± 0.93d	38.32 ± 5.30c	3.63 ± 0.18b	12.30 ± 0.10e
Aug 5 20 cm		3.87 ± 0.42d	10.17 ± 0.13b	26.40 ± 1.81ab	3.39 ± 0.54ab	9.47 ± 0.31b
Aug 15 20 cm		2.81 ± 0.23a	9.15 ± 0.26a	23.98 ± 0.65a	3.00 ± 0.05a	7.53 ± 0.25a

Control (pruning once a year) tea bushes were heavily pruned in late April and were not pruned once again this year. The results are expressed as the mean values ± SE ($n = 15 - 30$). Letters indicate significant differences in each index among different pruning treatments ($P < 0.05$, Duncan's multiple range test)

Table 4 Effects of different heights and times of summer pruning on the growth and yield of spring tea

Pruning heights and times		Branch number per m^2	Weight of 100 buds (g)	Density of bud (bud/m^2)	Yield (g/m^2)
Control		32.00 ± 1.80a	6.56 ± 3.53a	580.00 ± 30.41c	37.93 ± 3.05bc
Jul 25	10 cm	41.33 ± 5.51b	8.68 ± 2.52bc	475.00 ± 21.79b	41.43 ± 4.60c
	20 cm	43.78 ± 0.58b	7.75 ± 2.95abc	520.00 ± 52.20bc	39.93 ± 4.72bc
	30 cm	44.46 ± 2.42b	6.95 ± 2.52ab	561.67 ± 56.20c	39.44 ± 3.30bc
Aug 5 20 cm		54.33 ± 3.14c	9.18 ± 1.00c	391.67 ± 15.28a	35.73 ± 1.89b
Aug 15 20 cm		51.48 ± 3.71c	6.53 ± 1.48a	465.00 ± 36.06b	30.07 ± 1.38a

Control (pruning once a year) tea bushes were heavily pruned in late April and were not pruned again this year. The results are expressed as the mean values ± SE ($n = 15-30$). Letters indicate significant differences in each index among different pruning treatments ($P < 0.05$, Duncan's multiple range test)

significantly increased the branch number per m^2 and two pruning treatments in August both resulted in excess amount of branch per m^2 (Table 4).

In comparison with the conventional summer pruning of 20 cm around Jul 20 (Jul 25 here), pruning at 10 cm significantly decreased the number of lateral branch and bud number per new shoot. Pruning at 30 cm markedly increased the leaf number per lateral branch, the length of lateral branch and the bud number per new shoot. However, it significantly decreased the number of lateral branch growing out after pruning (Table 3). Pruning on Aug 5 significantly decreased bud density (Table 4). Pruning on Aug 15 markedly decreased the number of lateral branch growing out after pruning, the bud number per new shoot, the leaf number per lateral branch, the length of lateral branch (Table 3) and the yield of spring tea (Table 4). Two pruning treatments in August both decreased the bud number per new shoot (Table 3).

Based on the above results, it can be concluded that among all the treatments, pruning on Aug 15 resulted in the lowest yield of spring tea, whereas no marked differences in terms of yield were observed among other treatments. Thus, pruning at 30 cm could significantly benefit the growth of lateral branches in terms of inducing the thickest and longest lateral branch, the largest quantity of leaves per lateral branch and buds per new shoot, and the highest bud density. Among all of the pruning treatments, pruning on Aug 15 negatively affected the growth of lateral branch, because it resulted in the fewest number of lateral branch growing out after pruning and leaf per lateral branch, shortest lateral branch, thinnest lateral branch, smallest quantity of bud number per new shoot, the lowest weight of 100 buds, and excessive branch number per unit area. Thus, pruning on Aug 15 significantly decreased the yield of spring tea.

Effects of summer pruning on TP and AA

TP, AA and TP-to-AA ratio (TP/AA) are the major determinants of tea quality. We analyzed the concentrations of TP and AA as well as the TP/AA of the fresh tea leaves in the next spring. The results showed that in comparison with the control, pruning at 30 cm did not affect TP concentration, whereas other pruning treatments significantly increased TP concentration. In comparison with conventional summer pruning, pruning at 30 cm significantly decreased TP content, whereas other treatments significantly enhanced TP content (Fig. 1a).

In comparison with the control, none of the pruning treatments significantly affected AA accumulation. In comparison with conventional summer pruning, pruning at 30 cm significantly enhanced AA content, whereas other pruning did not significantly affect AA content. Moreover, the concentrations of AA induced by pruning at 30 cm were significantly heigher than that induced by the two pruning treatments in August (Fig. 1b).

In comparison with conventional summer pruning, the above alterations in the concentrations of TP and AA induced by pruning at 30 cm decreased TP/AA, whereas other pruning did not significantly affect TP/AA. In comparison with the control, pruning on Aug 15 significantly increased TP/AA, whereas other pruning did not significantly affect TP/AA (Fig. 1c). Pruning at 10 cm resulted in the lowest caffeine content, whereas the differences in caffeine content among other pruning were not significant (Fig. 1d).

Effects of summer pruning on PAL activity and transcript levels of biosynthetic genes of TP and theanine

PAL is a key enzyme for TP biosynthesis, and *CsPAL* is the key gene that encodes PAL protein in tea. We analyzed PAL activity and the transcript levels of *CsPAL* to confirm whether the change in tea composition is attributed to the change in the biosynthesis of secondary metabolites. The result showed that all of the pruning did not significantly affect PAL activity as compared to the control. In comparison with conventional summer pruning, pruning at 10–30 cm

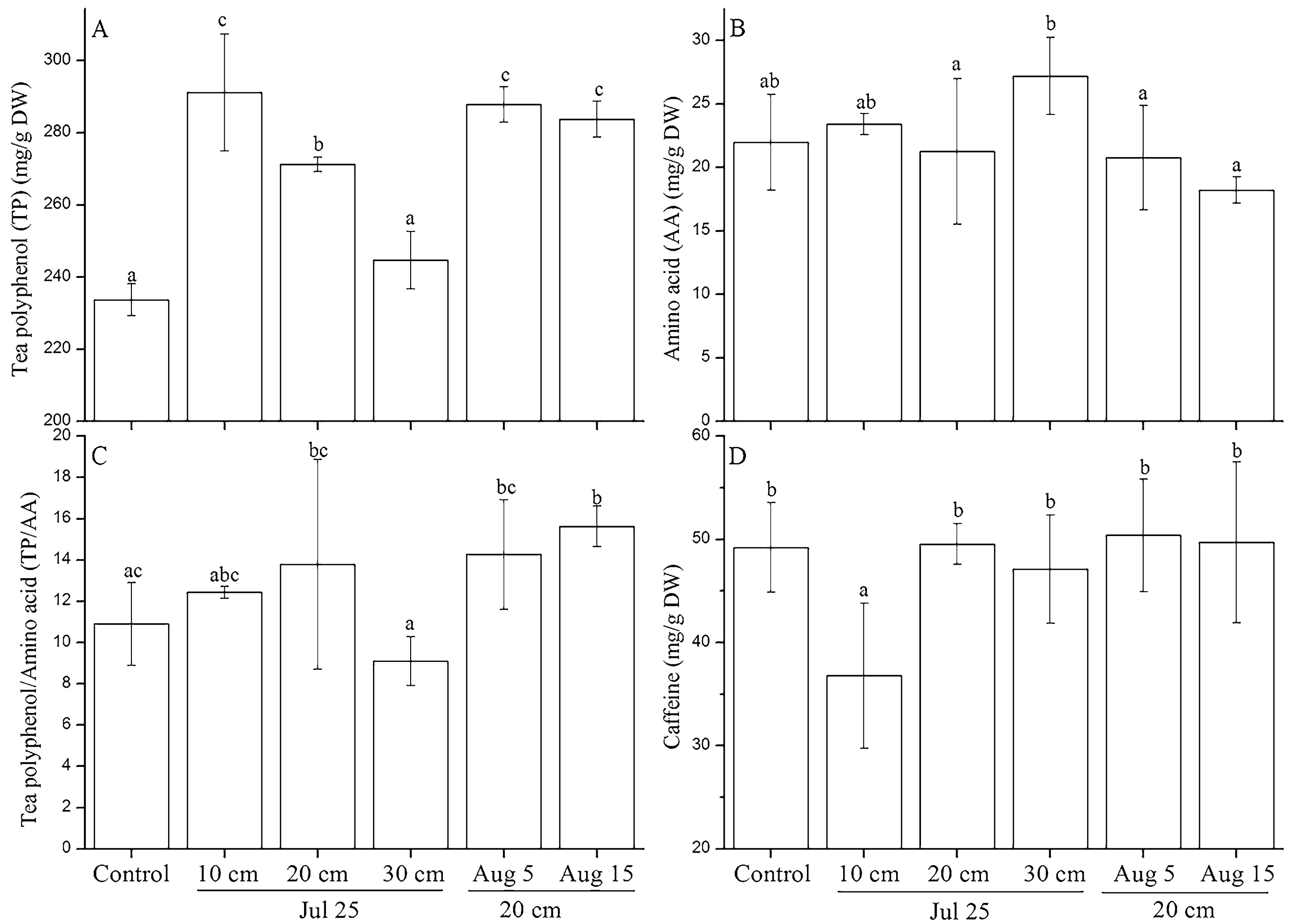

Fig. 1 Effects of different heights and times of summer pruning on total tea polyphenols (TP), free amino acids (AA), and caffeine concentrations in fresh tea leaves. Error bars indicate SD ($n=5$). For each figure, the letters indicate significant differences among treatments ($P<0.05$, Duncan's multiple range test)

both did not markedly affect PAL activity, whereas two pruning treatments in August both significantly decreased PAL activity (Fig. 2a). In addition, compared with the control, pruning at 10 cm and pruning on Aug 15 both significantly decreased *CsPAL* expression, but the differences in the expression levels of *CsPAL* between other pruning treatments and the control were not significant. Compared with conventional summer pruning, other pruning all did not significantly affect *CsPAL* expression. Furthermore, *CsPAL* expression levels induced by pruning at 30 cm were significantly higher than that inducd by pruning at 10 cm and pruning on Aug 15 (Fig. 2b). The above results showed that the expression of *CsPAL* induced by pruning was more or less consistent with PAL activity, but they are not significantly related to TP content.

We analyzed two key genes in the theanine synthesis pathway, namely, *glutamine synthetase* (*CsGS*) and *glutamine:2-oxoglutarate aminotransferase* (*CsGOGAT*) to assess whether increased amino acid content after pruning was attributed to theanine biosynthesis. The result showed that compared with the control or conventional summer pruning, all of the other pruning treatments did not significantly affect *CsGS* expression. In addition, the expression levels of *CsGS* induced by pruning at 30 cm were significantly higher than those induced by pruning at 10 cm and the two pruning treatments in August (Fig. 2c). Compared with the control or conventional summer pruning, the other pruning treeatments all did not affect the transcript levels of *CsGOGAT*. The expression levels of *CsGOGAT* induced by pruning at 30 cm were significantly higher than those induced by pruning at 10 cm and pruning on Aug 5 (Fig. 2d).

Effects of summer pruning on catechins content

The result showed that compared with the control, pruning at 10 cm significantly increased GCG content but significantly decreased the contents of ester catechins (EGCG + ECG) and GC. Pruning at 20 cm significantly increased C content

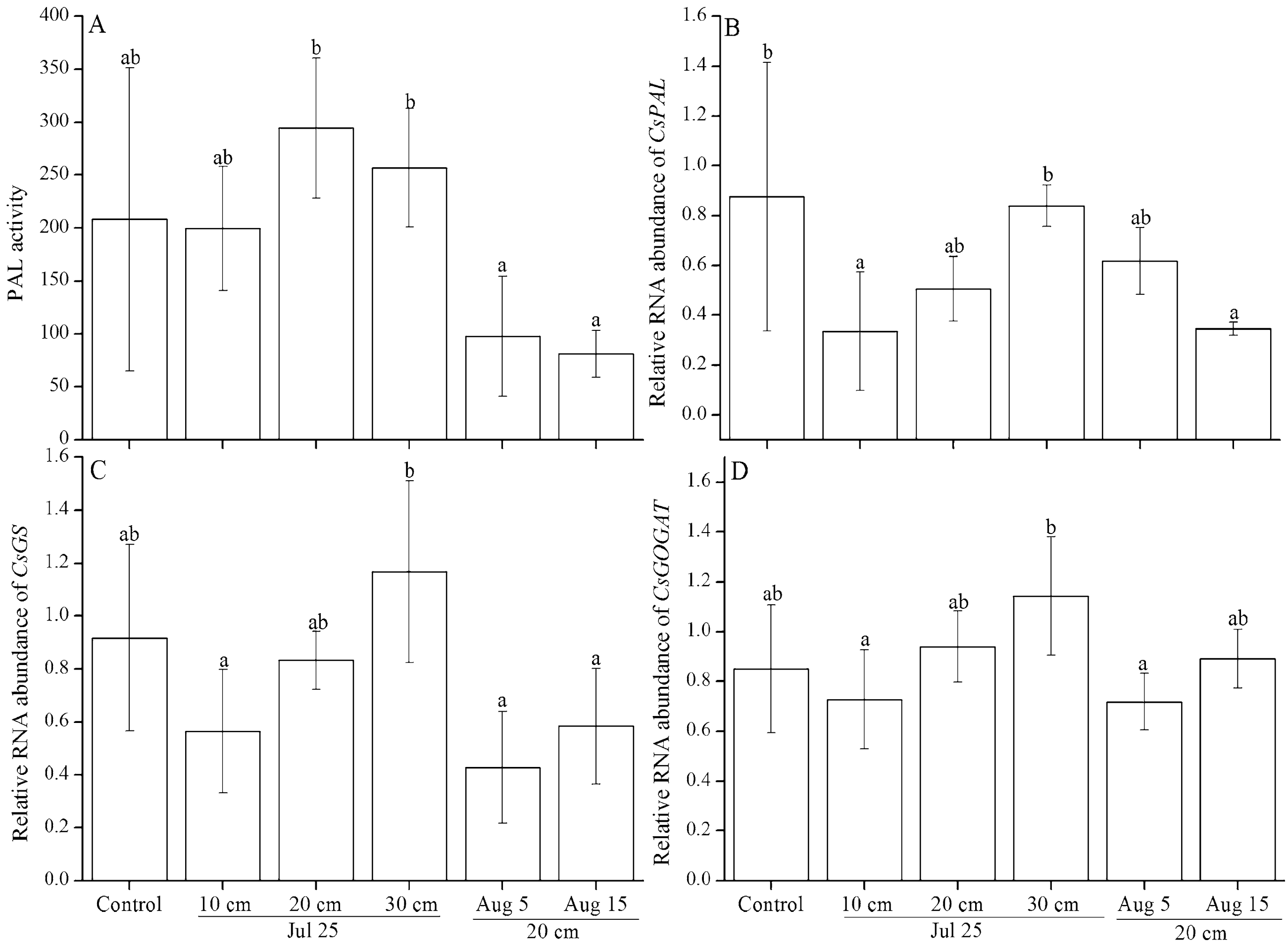

Fig. 2 Effects of different heights and times of summer pruning on PAL activity and on the transcript levels of *CsPAL*, *CsGS*, and *CsGOGAT* in fresh tea leaves. Control (pruning once a year) tea bushes were heavily pruned in late April and not pruned again this year. Error bars indicate SD ($n = 5$). For each figure, letters indicate significant differences among treatments ($P < 0.05$, Duncan's multiple range test)

but significantly decreased the contents of ester catechins (EGCG + ECG) and GC. Pruning at 30 cm significantly increased the contents of EGC, C and CG, but it significantly decreased (EGCG + ECG)%. Pruning on Aug 5 significantly increased the contents of GA, EGC, C and CG. Pruning on Aug 15 significantly increased the contents of EGC, C, ECG and GC, while it significantly decreased (EGCG + ECG)%. In addition, compared with the control, all of the pruning significantly decreased EC content but resulted in no significant differences in EGCG content (Table 5).

Compared with conventional summer pruning, pruning at 10 cm significantly decreased EC content; however, it did not significantly affect the contents of GA, EGCG, CG and (EGCG + ECG)%. Pruning at 30 cm significantly increased the contents of four kinds of catechins (GC, EGC, C, ECG), as well as the total amount of ester catechins ('EGCG + ECG'), while it significantly decreased the contents of EC and GCG and did not significantly affect the contents of GA, EGCG, CG and (EGCG + ECG)% present in fresh tea leaf. Furthermore, compared with conventional summer pruning, two pruning in August both significantly enhanced the contents of five kinds of catechins (GC, EGC, C, EGCG, and ECG) and ester catechins ('EGCG + ECG'), while they significantly decreased the contents of EC and GCG and did not significantly affect CG content. In addition, pruning on Aug 5 significantly increased GA contents, whereas pruning on Aug 15 significantly decreased the contents of (EGCG + ECG)% (Table 5).

Effects of summer pruning on transcript levels of catechins biosynthetic genes

Cinnamate 4-hydroxylase (*CsC4H*) and *p-coumarate:CoA ligase* (*Cs4CL*) are key regulatory genes in the catechins biosynthetic pathway (Wang et al. 2015). This study showed that compared with the control, pruning at 30 cm

Table 5 Effects of heights and times of summer pruning on catechins contents of spring tea

	Control	Jul 25			Aug 5 20 cm	Aug 15 20 cm
		10 cm	20 cm	30 cm		
GA	3.64 ± 0.87a	4.29 ± 0.28a	3.59 ± 0.90a	3.98 ± 0.71a	7.23 ± 0.89b	4.53 ± 1.04a
GC	100.86 ± 13.00b	69.74 ± 4.82a	76.28 ± 3.59a	120.94 ± 14.70b	114.07 ± 17.07b	190.11 ± 28.81c
EGC	35.90 ± 2.85a	32.19 ± 2.29a	37.09 ± 2.85a	43.15 ± 4.34b	51.80 ± 6.43c	131.06 ± 5.49d
C	30.80 ± 4.18a	32.91 ± 2.99ab	36.52 ± 1.91b	43.08 ± 2.96c	53.16 ± 5.37d	55.28 ± 6.34d
EC	13.11 ± 0.82c	7.00 ± 1.14a	10.06 ± 1.04b	7.59 ± 1.03a	8.50 ± 1.26a	8.12 ± 1.52a
EGCG	85.96 ± 7.42ab	71.35 ± 13.12a	72.71 ± 8.52a	85.35 ± 5.97ab	94.96 ± 13.80b	88.75 ± 15.24b
GCG	4.60 ± 2.18ab	7.47 ± 1.28c	6.45 ± 2.13bc	2.38 ± 1.01a	2.37 ± 0.35a	3.06 ± 0.85a
ECG	34.60 ± 1.60ab	33.08 ± 2.64a	33.70 ± 4.87a	40.03 ± 3.41bc	43.27 ± 7.62bc	40.35 ± 4.25c
CG	2.33 ± 0.47a	2.55 ± 0.39ab	3.14 ± 0.41ab	3.65 ± 0.86b	3.60 ± 0.70b	3.29 ± 1.03ab
EGCG + ECG	123.46 ± 9.91b	100.67 ± 11.63a	103.98 ± 6.36a	124.23 ± 11.17b	139.85 ± 14.19b	129.10 ± 18.59b
(EGCG + ECG)%	39.46 ± 2.06c	39.48 ± 1.52c	37.52 ± 1.87bc	35.50 ± 2.40b	36.71 ± 2.48bc	24.78 ± 2.24a

Control (pruning once a year) tea bushes were heavily pruned in late April and not pruned again this year

The results are expressed as the mean values ± SD ($n = 6$). Letters indicate significant differences in each index among different treatment ($P < 0.05$, Duncan's multiple range test). (−)-gallocatechin (GC), gallocatechin gallate (GCG), (−)-epigallocatechin (EGC), (+)-catechin (C), gallic acid (GA), (−)-epicatechin (EC), (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), catechin gallate (CG)

significantly enhanced the expression of *CsC4H* gene, whereas no marked differences in *CsC4H* expression were observed between other pruning and the control. Moreover, there were no significant differences in *CsC4H* expression between all of the other pruning treatment and conventional summer pruning. In addition, the expression levels of *CsC4H* induced by pruning at 30 cm were significantly heigher than those induced by pruning at 10 cm and pruning on Aug 15 (Fig. 3a). Compared with the control, all of the pruning did not affect the transcript levels of *Cs4CL*. Moreover, the expression levels of *Cs4CL* induced by pruning at 30 cm were significant heigher than those induced by all of the other pruning (Fig. 3b).

Discussion

Previous studies have reported the effects of pruning on the growth of lateral branches as well as the yield and quality of fresh tea leaf (Sun et al. 2018; Ravichandran 2004; Thomas et al. 2005; Chen et al. 2017). However, these reports have mainly focused on the biological and physiological effects of long-term pruning (Sun et al.

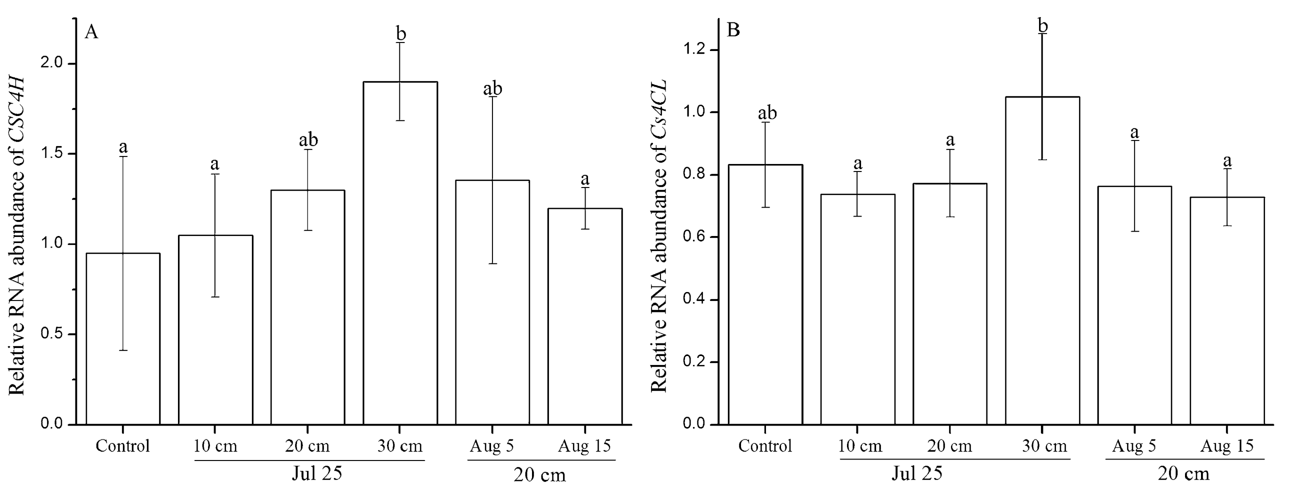

Fig. 3 Effects of different heights and times of summer pruning on the transcript levels of *CsC4H* and *Cs4CL* in fresh tea leaves. Control (pruning once a year) tea bushes were heavily pruned in late April and not pruned once again this year. Error bars indicate SD ($n = 5$). For each figure, the letters indicate significant differences among treatments ($P < 0.05$, Duncan's multiple range test)

2018), periodic pruning (Kumar et al. 2015; Ravichandran 2004; Yilmaz et al. 2004; Thomas et al. 2005), pruning time (Kumar et al. 2015; Ravichandran 2004; Chen 1996; Xu et al. 2014a, b) and pruning degree on tea trees (Jiang et al. 2018; Satyanarayana et al. 1994; Wang et al. 2015). In tea production, pruning twice a year is widely used for shrub-type mature tea garden in which only spring tea is collected. In this study, using the mature tea trees of the 'Jin Guan Yin' oolong tea, the branch growth as well as the yield and quality of spring tea after different pruning treatment were studied.

Compared with conventional summer pruning, firstly, pruning at 30 cm could significantly benefit to the growth of lateral branches by inducing the thickest and longest lateral branch, largest quantity of leaves and buds, and the highest bud density. Secondly, compared with conventional summer pruning, the growth of lateral branch after pruning on Aug 15 was the poorest and characterized by markedly decreased leaf number, bud number, and weight of 100 buds as well as the number, diameter, and length of lateral branch (Table 3). On the other hand, the branch number per unit area was the largest after two pruning in August (Table 4), and excessive latreal branches may have led to the thin diameter of lateral branch, decreased bud density, and low yield of spring tea.

Pruning increases the production of fresh tea leaf due to the enhanced branching and increased number of tender leaf (Kumar et al. 2015; Ravichandran 2004; Satyanarayana et al. 1994). This study showed that compared with the control, pruning at 10 cm and on Aug 5 both significantly decreased bud density, whereas they significantly enhanced the weight of 100 buds, which may be the reason why the yield did not change significantly. On the other hand, pruning on Aug 15 resulted in the lowest yield of spring tea, whereas there were no significant differences among all the other pruning treatments in terms of spring tea yield. It can be presumed that performing of the summer pruning too late might decrease the accumulation of carbohydrates in the next spring, thereby decreasing the yield of spring tea. Therefore, the summer pruning of tea tree should be not later than Aug 15 in tea production.

The native levels of the characteristic compounds in the fresh leaf largely determine the quality of made tea (Chen et al. 2009; Thomas et al. 2005). Pruning causes changes in the new distribution and balance of metabolites (Sun et al. 2018; Ravichandran 2004). Next, we detected quality indices of fresh tea leaves. Theanine is the major tea amino acids (AA) accounting for more than 50% of total free amino acid in tea (Li et al. 2019). Tea polyphenol is a class of main secondary metabolites accounting for 18–36% dry weight of fresh leaf and tender shoot of tea trees, and catechins account for 70% of the total amount of tea polyphenol (Xia and Gao 2009). This study showed that compared with conventional summer pruning, pruning at 30 cm is beneficial for decreasing TP content and enhancing AA content, thereby decreasing TP/AA. In addition, two pruning treatments in August both significantly increased TP content but did not change TP/AA as compared to conventional summer pruning. Moreover, AA content and TP/AA induced by pruning at 30 cm was significantly heigher and lower than that induced by two pruning in August, respectively.

TP are synthesized through the PP and flavonoid (FL) pathways, where PAL is the key enzyme in the first step of the PP pathway (Li et al. 2016). This study showed that two pruning treatments in August both significantly decreased the activity of PAL enzyme as compared to conventional summer pruning. Moreover, pruning at 10 cm and pruning on Aug 15 both significantly decreased the expression levels of *CsPAL* as compared to control trees. From the above results, it can be concluded that TP content was not related to PAL activity and the expression of *CsPAL*. Moreover, decreased PAL activity was closely related to the decreased expression of *CsPAL* induced by pruning on Aug 15 as compared to those induced by pruning at 30 cm.

GS and GOGAT are the two key determinants of theanine biosynthesis, which catalyzes the initial steps of NH_3 assimilation (Li et al. 2016). In this study, we analyzed the transcript levels of *CsGS* and *CsGOGAT* to elucidate the mechanism underlying summer pruning-induced AA accumulation. Here, the results showed that the expression levels of *CsGS* and *CsGOGAT* induced by pruning at 30 cm both were significantly higher than those induced by pruning at 10 cm. In addition, the expression levels of *CsGS* induced by pruning at 30 cm were significantly higher than those induced by two pruning treatments in August. Moreover, the expression levels of *CsGOGAT* induced by pruning at 30 cm were significantly higher than those induced by pruning at 10 cm and pruning on Aug 5.

Altogether, the above results showed that enhanced AA content induced by pruning at 30 cm is significantly associated with the enhanced transcript levels of *CsGS* or *CsGOGAT*, respectively, as compared to those induced by pruning at 10 cm or two pruning in August. Similar results were found in our previous study, which showed that increased theanine concentration induced by elevated CO_2 is related to the upregulated transcription of *CsGS* in tea leaves (Li et al. 2019). Therefore, it is quite plausible that summer pruning might directly or indirectly upregulate the transcription of two key theanine biosynthetic genes *CsGS* and *CsGOGAT*, thereby resulting in the increased levels of theanine and total free amino acids in fresh leaves.

Thomas et al. (2005) found that a steady increase in caffeine synthesis was found in all tea clones up to the fourth year after pruning. The long-term pruning induced the accumulation of caffeine in tea leaves compared with those of the unpruned tea trees (Sun et al. 2018). However, the current study showed that summer pruning at 10 cm resulted in the lowest caffeine content, whereas there were no significant differences in caffeine content among other treatments (Fig. 1d).

The amount of catechins in oolong tea is considerably higher than that in green tea cultivars (Chen et al. 2009, 2011). Agricultural practices, especially pruning, often affect the compositions and contents of catechin in tea leaves (Chen et al. 2009, 2011; Thomas et al. 2005). We detected the contents of nine kinds of catechins in the fresh leaves. The results showed that the contents of four nonester catechins (EC, EGC, GC, and C) and two ester catechins (ECG and EGCG) were significantly heigher than those of the three other kinds of catechins (GA, GCG and CG). This results confirmed the findings of previous reports, which suggested that the above mentioned six kinds of catechins (four nonester and two ester catechins) are the major phenolic compounds present in oolong tea (Sun et al. 2018; Chen et al. 2009,2010).

The results also showed that compared with the control, pruning at 10 cm and conventional summer pruning both significantly decreased GC content, whereas pruning on Aug 15 significantly increased GC content. Pruning at 30 cm and two pruning treatments in August both significantly increased EGC content as compared to the control. All of the summer pruning significantly decreased EC content, whereas all of the other pruning all significantly increased C content except for pruning at 10 cm as compared to the control. Furthermore, compared with conventional summer pruning, pruning at 10 cm significantly decreased EC content. Compared with conventional summer pruning, pruning at 30 cm significantly decreased the contents of EC and GCG, while it significantly increased the levels of GC and EGC. Pruning on Aug 5 significantly increased the GA content as compared to conventional summer pruning. Compared with conventional summer pruning, two pruning treatments in August both significantly enhanced the contents of GC, EGC and C, whereas they significantly decreased the contents of EC and GCG.

Previous study reported that ester catechins (ECG and EGCG) comprise the majority of total catechins in oolong tea (Katalinic et al. 2006). The content of EGCG and the ratio of ester catechins [(EGCG + ECG)%] were all increased in the leaves of the pruned tea trees compared with those of the unpruned tea trees (Sun et al. 2018). The current study showed that compared with the control, pruning at 30 cm significantly decreased (EGCG + ECG)%. Moreover, (EGCG + ECG)% is the lowest after pruning on Aug 15. Compared with the control, pruning at 10 cm and 20 cm both decreased the contents of ester catechins (EGCG + ECG), and pruning on Aug 15 significantly enhanced the content of ECG. All of the summer pruning did not affect EGCG content as compared to the control trees. In addition, compared with conventional summer pruning, pruning at 30 cm significantly enhanced the content of ECG and the total amount of ester catechins ('EGCG + ECG'), whereas pruning on Aug 15 significantly decreased (EGCG + ECG)%. Compared with conventional summer pruning, two pruning treatments in August both significantly enhanced the contents of EGCG and ECG, and thus enhanced the contents of ester catechins ('EGCG + ECG').

Previous studies showed that the contents of CG and EGCG in fresh leaf were significantly correlated with the quality of oolong tea (Chen et al. 2010, 2011). The patterns of EGCG accumulation have been analyzed in the above paragraph. Pruning at 30 cm and pruning on Aug 5 significantly increased CG content as compared to the control. Catechins belong to flavonoids and PAL is the key enzyme responsible for catechins biosynthesis (Sun et al. 2018; Wan et al. 2015). Increased PAL activity resulted in an increased concentration of flavonoids in tomato roots and *Vitis vinifera* grape berry (Li et al. 2016). The expression levels of *CsPAL* is correlated with the endogenous concentration of catechins in albino tea plants (Xiong et al. 2013).

C4H and 4CL are two key enzymes for catechin biosynthesis in the PP pathway of tea trees (Sun et al. 2018; Wan et al. 2015). In this study, *CsC4H* expression induced by pruning at 30 cm were significantly heigher than those of induced by control trees, pruning at 10 cm and pruning on Aug 15. The expression of *Cs4CL* induced by pruning at 30 cm were significantly heigher than those of induced by the other pruning treatments.

Altogether, pruning at 30 cm could benefit the growth of lateral branches, moreover, it had the best effect on spring tea quality, including it could benefit to decreasing TP/AA and enhanced the contents of ester catechins ('EGCG + ECG'). However, two pruning in August induced a excessive mumber of latreal branches and led to thinnest lateral branches and decreased bud density. Pruning on Aug 15 had the lowest spring tea yield. Two pruning in August both also significantly enhanced the contents of ester catechins ('EGCG + ECG') as compared to conventional summer pruning. Pruning on Aug 15 had the lowest (EGCG + ECG)%. In addition, TP content was not related to PAL activity and *CsPAL* expression. Enhanced AA content induced by pruning at 30 cm was significantly associated with enhanced transcription of *CsGS* or *CsGOGAT*.

Conclusion

In conclusion, for shrub-type mature tea garden in which only spring tea was picked up, tea plants usually need pruning twice a year. In addition, pruning once a year and three times a year is required for the cultivar with weaker growth vigor and stronger growth vigor, respectively. This study demonstrates that for the shrub-type 'Jin Guan Yin' oolong mature tea garden in which only spring tea was plucked, summer pruning at 30 cm above the cut surface of spring pruning on Jul 25 was best to benefit the growth of lateral branches as well as the yield and quality of spring tea, whereas pruning at 20 cm on Aug 15 had the worst comprehensive effects. For summer pruning, it is suggested to increase the tree height by 30 cm from the last cut surface around Jul 20 in tea production. Furthermore, in order to not sharply reduce the production of fresh leaf, the summer pruning of tea tree should not be later than Aug 15.

The results of this study may serve as a theoretical basis for providing a novel strategy for tea cultivation management. Although this technique has only been examined 'Jin Guan Yin' adult tea garden in which only spring tea was picked, the developed technique may also be applicable to other tea varieties with similar branching characteristics when grown in regions with similar conditions.

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Author contribution statement Wenyan Han conceived and designed the research. ML, XL, PY and LZ performed the experiment. LZ and ML analyzed the experimental results. LZ wrote the main manuscript text. All authors reviewed the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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