



### Article Hormone Signals Involved in the Regulation of Cucumber Seedling Lateral Branch Elongation by Far-Red Light

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**Abstract:** Cucumber (*Cucumis sativus* L.) lateral branch elongation is influenced by a variety of environmental signals, including light [e.g., far-red (FR) light] and hormones. In this experiment, the effect of FR light on the lateral branch elongation of cucumber ('Zhongnong No. 26') seedlings was investigated. The results showed that FR light significantly inhibited the lateral branch elongation of cucumber seedlings. In addition, FR light significantly increased the auxin (indole-3-acetic acid, IAA) content, decreased the cytokinin (CTK; Zeatin) content, and suppressed the expression of most CTK synthetic-related genes, such as *IPTs*, in cucumber seedlings. The lateral branch elongation of cucumber seedlings was assessed in response to decapitation and exogenous 6-BA treatment to further investigate the relationship between IAA and CTK on the lateral branch elongation of cucumber seedlings under FR light. Both decapitation and exogenous 6-BA treatment eliminated the inhibitory effect of FR light on the lateral branch elongation of cucumber seedlings. In conclusion, these results indicated that IAA and CTK were involved in the regulatory effects of FR light on cucumber seedling lateral branch elongation.

Keywords: cucumber; lateral branch; far-red light; hormones; auxin; cytokinin



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### 1. Introduction

As an important agronomic trait, plant type affects light energy capture efficiency, photosynthesis and nutrient distribution [1,2] and is one of the determinants of fruit yield and quality [3,4]. The architectural characteristics of the plant, such as the length of internodes, the angle of leaves and the number of lateral branches, demonstrate a significant degree of phenotypic plasticity [5]. Cucumber (*Cucumis sativus* L.) is an important facility vegetable crop worldwide. Excessive numbers of lateral branches are harmful to the development of flowers and fruits, so producers often need to manually remove excess lateral branches to achieve high yields [6,7]. However, this process is time-consuming and labour-intensive, and producers are attempting to reduce production costs (especially labour costs) as competition increases among producing regions.

Lateral branch formation involves axillary bud formation, sprouting, growth and stem elongation through a complex network of intrinsic genetic factors (genes) and extrinsic environmental factors (e.g., light, hormones) [8]. In addition, integrative physiology, genetics and molecular tools have found that the formation and elongation of lateral branches is controlled by diverse phytohormones through their biosynthesis, transport and signalling and is coordinated with multiple environmental signals [9]. Fortunately, molecular-marker-assisted breeding and several agronomic regulatory strategies (e.g., light and plant growth regulators) have made it possible to breed cucumbers with fewer lateral branching traits, which reduces the labour cost. Light (including light intensity, light quality and photoperiod) regulates the growth and development of plant lateral branches [10–13]. Far-red (FR) light is invisible light that regulates plant growth and photomorphogenesis [14–16]. The red/far-red (R/FR) ratio is considered an important

light signal that induces adaptive responses in plants. A low R/FR ratio triggers shade avoidance syndrome (SAS), which is characterized mainly by a reduction in the number and length of lateral branches [17,18]. Low R/FR ratios regulate lateral branch growth and development mainly via phytochrome (phy, especially phyB), phytochrome interacting factors (PIFs) and endogenous hormones [e.g., auxin (indole-3-acetic acid, IAA), gibberellin (GA), abscisic acid (ABA) and cytokinin (CTK)] [6,19,20]. The phyB deficiency mutants exhibited a lower branching capacity than the wild-type plants, whereas increasing the R/FR ratio induced phyB accumulation, which caused lateral branch elongation [21].

Phytohormone regulatory networks play major roles in lateral branch growth and development in response to FR light, and reciprocal networks of upstream and downstream signals from different phytohormones can be generated. When the light signal (increased FR light) is altered, phyB is inactivated and PIFs are promoted, which can initiate downstream hormone (e.g., IAA) signalling to control lateral branch sprouting or dormancy [22]. IAA serves as a major hormone for maintaining apical dominance in plants, and a large amount of IAA, produced in the apical part of the main stem, moves downwards through polar transport and indirectly regulates lateral branch elongation [23]. CTK has an antagonistic effect on IAA, which acts as the second messenger of IAA to regulate the formation of lateral branches [24]. These two hormones are considered to be the most important regulators of branch formation. In addition, a low R/FR ratio can promote the accumulation of ABA and the expression of ABA biosynthesis-related genes and inhibit the expression of cell cycle-related genes to hinder the formation of lateral branches [25,26].

Although many studies have reported the regulation of lateral branch growth in higher plants (*Arabidopsis thaliana*, tomato, etc.) by FR light, there is still much research space in cucumber seedlings and the value of regulating the light environment in facility horticulture. To analyse the mechanism of hormone signalling involved in the FR light regulation of lateral branch elongation in cucumber seedlings, we observed and explored the changes in the morphology of lateral branches, endogenous hormones and related gene expression levels in cucumber seedlings under light signals (FR light) and/or hormone signals (IAA, CTK). The aim of this study was to increase the understanding of the interaction between FR light and/or hormone signals in regulating cucumber lateral branch elongation and to provide a theoretical basis for the use of light quality (FR light) to regulate cucumber lateral branch elongation.

#### 2. Materials and Methods

#### 2.1. Plant Materials and Treatments

The experiment was carried out at the Horticulture Science and Technology Building, Fujian Agriculture and Forestry University, China (119.23° E, 26.08° N). The cucumber variety for testing was selected as 'Zhongnong No. 26'; the seeds were soaked for 6 h and then germinated overnight and sown in a 50-hole tray. Upon the flattening of first true leaves, the seedlings were chosen and placed in plastic containers (containing 3.5 L of Yamazaki cucumber nutrient solution, pH = 6.8–7.0, EC = 2.2–2.5 mS·cm<sup>-1</sup>), with 5 plants per container. These containers were kept in a plant cultivation room at a temperature of  $27 \pm 1 \text{ °C}/23 \pm 1 \text{ °C}$  (day/night), and with a 12 h photoperiod using a full-spectrum LED white light (WL,  $\lambda$ max = 450 and 660 nm, JIUPO, Fuzhou, China, R/FR ratio = 14.82, PPFD = 157.16  $\pm$  9.64 µmol·m<sup>-2</sup>·s<sup>-1</sup>) [27], as determined by an MK350D portable spectrometer (UPRtek, Taiwan, China) except where specified. The spectrum graph is shown in Figure 1. Every 4 days, the nutrition solution was changed.

First, we investigated the effect of FR light on the elongating lateral branches of cucumber plants. Two treatments were used: (1) WL and (2) WL combined with FR light ( $\lambda$ max = 730 nm, SANANBIO, Quanzhou, China, 58.11 ± 6.95 µmol·m<sup>-2</sup>·s<sup>-1</sup>) (WL + FR, R/FR ratio = 1.45, PPFD = 153.21 ± 11.98 µmol·m<sup>-2</sup>·s<sup>-1</sup>).



Figure 1. The spectrum graph.

The seedlings were treated following the flattening of second true leaves (after approximately 7 days of hydroponics). After 8 days of FR light treatment, the lateral branch length was determined using a Vernier calliper (precision 0.01 mm). After 48 h of FR light treatment, the apical and basal internodes were excised. Subsequently, the samples were promptly frozen in liquid nitrogen and then preserved at -80 °C. Each experiment was conducted a minimum of three times.

Second, we studied the effect of decapitation on the elongation of the lateral branches of cucumber seedlings. Upon the flattening of second true leaves (after 7 days of hydroponics), the seedlings were treated by FR light. Moreover, the seedlings were subjected to FR light treatment for 4 days, after which, the plants were decapitated. There were four treatments in the experiment: (1) WL, (2) WL + decapitation, (3) WL + FR and (4) WL + FR + decapitation. After 4 days of decapitation, the lateral branch length was determined using a Vernier calliper (precision 0.01 mm). After 6 h of decapitation treatment, the first and second internodes (from the bottom) were excised. Subsequently, the samples were promptly frozen in liquid nitrogen and then preserved at -80 °C. Each experiment was conducted a minimum of three times.

Finally, we studied the effect of CTK on the elongation of the lateral branches of cucumber plants. Upon the flattening of second true leaves (after 7 days of hydroponics), the seedlings were treated. There were four treatments in the experiment: (1) WL, (2) WL + 6-BA, (3) WL + FR and (4) WL + FR + 6-BA. The concentration of 6-BA (Sangon Biotech, Shanghai, China) was 50  $\mu$ mol·L<sup>-1</sup> in the morning every 2 days. [28]. After 8 days of treatment, the lateral branch length was determined using a Vernier calliper (precision 0.01 mm). After 6 h of 6-BA treatment, the first internode (from the bottom) was excised. Subsequently, the samples were promptly frozen in liquid nitrogen and then preserved at -80 °C. Each experiment was conducted a minimum of three times.

#### 2.2. Measurement of Endogenous Hormone Contents

The contents of IAA and Zeatin were determined by an Elisa Kit (mlbio, Shanghai, China), and the methods were described by He et al. and Tsago et al. [29,30]. Briefly, a total amount of 0.3 g of frozen stem samples was homogenized in 3 mL of cold 50 mmol·L<sup>-1</sup> phosphate-buffered saline (PBS, pH 7.2–7.4). The resulting homogenates were centrifuged at 2000 rpm·min<sup>-1</sup> (H1750R, Cence, Changsha, China) at 4 °C for 10 min. According to the manufacturer's instructions, 40  $\mu$ L of sample dilution buffer and 10  $\mu$ L of supernatant were added to the bottom of the sample wells. And 100  $\mu$ L of horseradish peroxidase-conjugated reagent was added to each well except the blank control. The solution was sealed with the closure plate membrane and incubated at 37 °C for 60 min. Then, the closure plate membrane

was peeled off and the solution was removed. The wells were refilled with the wash solution, left to stand for 30 s and then drained, and the process was repeated 5 times. After washing, 50  $\mu$ L of chromogen solution A and 50  $\mu$ L of chromogen solution B were added to the wells and evaded light preservation at 37 °C for 15 min. After colouring, 50  $\mu$ L of stop solution was added to stop the reaction. The absorbance was carried out at 450 nm with a multimode microplate reader (SpectraMax iD3, Molecular Devices, San Jose, CA, USA), within 15 min after the termination of the reaction. A standard curve was established for each micro-titre plate to calculate the IAA and Zeatin content.

#### 2.3. Real-Time Quantitative PCR (RT-qPCR) Analysis

The extraction of total RNA was performed using 0.2 g internode samples using a FastPure Plant Total RNA Isolation Kit (Polysaccharides & Polyphenolics-rich) (Vazyme Nanjing, China). The RNA was converted into complementary DNA (cDNA) using FastK-ing gDNA Dispelling RT SuperMix (Tiangen, Beijing, China) and 1 µg of total RNA in a 20 µL reaction volume. For qPCR in a 20 µL reaction volume containing a 1 µL cDNA template, 10 µL or 2 × RealStar Fast SYBR qPCR Mix (GenStar, Beijing, China) and 0.5 µL of primer were used. The RT-qPCR analysis was conducted utilizing a LightCycler<sup>®</sup> 96 real-time PCR system (Roche, Basel, Switzerland) at 95 °C for 2 min, while cycling 40 times at 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s. The  $2^{-\Delta\Delta CT}$  method was used to calculate the relative expression levels of the genes. The primers designed specifically for the *LOG* genes were obtained from Mandal et al. [32]. The primers designed specifically for the *LOG* genes were obtained from Zhu et al. [33]. Tua: F 5'-CTCTCAACCCATTCTCTCTTGG-3', R 5'-CGGTTGAGGTTCGAGTAGTTAG-3'. The gene-specific primers used here are shown in Tables 1 and 2.

Table 1. YUC and LOG genes' primer sequences for RT-qPCR.

Genes	Accession Number	Primer Sequences (Forward/Reverse)
YUC2	Csa020745	F 5'-TGCAAGGCAAAAGACTTCACG-3'
		R 5'-GCTATACATTCGGCTCTTTCAAGGA-3'
NIIC4	Csa006121	F 5'-TGGCTAAAGCAGGGGTGTGA-3'
1404		R 5'-CGTTGGCGATTTTCATAGCG-3'
VIIC6	Csa021077	F 5'-GAAGTATTTGGAGGATTACGCTG-3'
IUCO		R 5'-TGTTTCCTCAGAACGACCGC -3'
VIIC7	Csa000192	F 5'-GGTGAGGCTTACCGTGGGAAAC-3'
YUC/		R 5'-CTTGGCATCATGGTTACAAAGA-3'
YUC8	Csa008610	F 5'-CATACGCCAAGCATTTTGAGAT-3'
		R 5'-ATGTATTCGACCTCGTTACGGG-3'
VIICO	Csa001400	F 5'-CCGAGTCTTCCGTTTCTGGT-3'
1403		R 5'-GGCATGACACACTCTGCATTTTC-3'
VLIC10a	Csa017301	F 5'-GTCCTTCTGGCTTGGCTACCT-3'
1 action		R 5'-TGGCTAAGTGAAGGCATAAACG-3'
VLIC10h	Csa008786	F 5'-CCTTCTGGTCTTGCCACTGC-3'
100100		R 5'-CAAAACCGATTGGGGAGG-3'
VLIC10c	Csa019194	F 5'-CTATCCACCGCCGCATGTTTA-3'
Tuciot		R 5'-CGCCAGCTCCGATGATTTCTT-3'
VIIC11	Csa016452	F 5'-CAATGCACCGACGTATATTTCG-3'
TUCII		R 5'-CCTCTCTTGCTCACCACTACTTGT-3'
LOC1	Csa6G127300	F 5'-CAACTCGGGTCATCGAAAAG-3'
LUGI		R 5'-CCATCAACCCAACACTTCCT-3'
LOG2	Csa4G646190	F 5'-CGGAGGAGGTAGTGTTGGA-3'
		R 5'-ACTGCCTTCACTTCTCCCACT-3'

$ \begin{array}{c} C_{sa} 3M150100 & F5'-CGGCGGAAAGATAAAGTGG-3' \\ R5'-CGACATCAAGTCCTTCATTAAACCTG-3' \\ R5'-CGACATCAAGTCCTTCCATTATCGCGC-3' \\ R5'-CGACAGTAACGAAGAGTTGAGG-3' \\ R5'-CGACATCAAGTCCTTCCCTTGCTG-3' \\ R5'-CGACATGAACGACTTCTGCCTGCTG-3' \\ R5'-CGACATCAATCCCTTGCCTGCC-3' \\ R5'-CGACATCAATCCCTTGCCTGCC-3' \\ R5'-CGACACTCAATCCCTTCACC-3' \\ R5'-CGACACTCAATCCCTTCACC-3' \\ R5'-CGACACTCACTCGCTGG-3' \\ R5'-AATGGCAAACTCAACGG-3' \\ R5'-CAGACACACACGACTCACGG-3' \\ Csa4M083690 & F5'-TGCTCCGTGGG-3' \\ Csa4M083690.2 & F5'-TTCCATTCGCTGGG-3' \\ Csa4M083690.2 & F5'-ACTCAATCTTGGTGGG-3' \\ Csa7M392940 & R5'-CACAAGAGACGACAATGCTGG-3' \\ R5'-ACTCAGCACAATGTTCTGGTGGG-3' \\ R5'-CAGATGAAACGACAAGAGCAGATCACGACG-3' \\ R5'-CAGATGACAAGAGCAAGAGCAGAGC-3' \\ R5'-CAGATGACAAGAACGACAAGGC-3' \\ R5'-CACTCAGCACCAATGTTCTGGC-3' \\ Csa1M589560 & F5'-CACTCAGCACCAATGCTGGC-3' \\ Csa1M589070 & F5'-GCTCAGGAGCAAGAAGCAGCAGC-3' \\ Csa1M589070 & F5'-GCTCAGGAGCACGAAGCAGCAGC-3' \\ Csa1M589070 & F5'-GTCCGTGGGGGATTATTGC-3' \\ Csa1M589070 & F5'-GTCCGTGGGGGAGATACC-3' \\ Csa2M000440 & R5'-CGTAACCAACACACACAAAACA-3' \\ Csa2M000440 & R5'-CCATCAACAACACACACAAACA-3' \\ Csa2M000440 & R5'-CCATCAACCAACACACAACA-3' \\ Csa2M000440 & R5'-CCATCAACCAACACACACAACA-3' \\ Csa2M000440 & R5'-CCATCAACCAACACACACACA-3' \\ Csa2M000440 & R5'-CCATCAACCAACACACACACA-3' \\ Csa3M82303 & F5'-CCATCAACCAACACACACACA-3' \\ Csa3M82303 & F5'-CCATCAACCAACCAACCA-3' \\ Csa4M43359 & F5'-CCATCAACCAACCAACCA-3' \\ Csa4M647490 & R5'-CCATCAACCAACCAACCA-3' \\ Csa5M175820 & F5'-CCATCAACCAACCAACCA-3' \\ Csa5M175820 & F5'-CCATCAACCAACCAACACA-3' \\ Csa5M175820 & F5'-CCATCAACCAACCAACCA-3' \\ Csa5M175820 & F5'-CCATCAACCAACCAACCAACAC-3' \\ Csa5M175820 & F5'-CCATCAACCAACCAACCAACCA-3' \\ Csa5M175820 & F5'-CCATCAACCAACCAACCAACCA-3' \\ Csa5M175820 & F5'-CCATCAACCAACCAACCAACCA-3' \\ Csa5M175820 & F5'-CCATCAACCATGGGATTCCTCCTCACACCCC-3' \\ Csa5M175820 & F5'-CCATCAACCAGTGCACCCCAACGTCTCCCCCACCC-3' \\ Csa5M175820 & F5'-CCATCAACCAGTGCACACCCCAACGCACCCCCCCCCCCC$	Genes	Accession Number	Primer Sequences (Forward/Reverse)
CsabM150100         R5'-CAACATCAAGTCCTTCATTAAACCTG-3'           F5'-AAACGATGTCTCCCTATTCTGCC-3'         R5'-CCGACACGAACGAGTTGAGG-3'           R5'-CCGACACGAACGAGTTGACG-3'         R5'-CCGACACGAACGAGTTGACG-3'           CsafM030440         R5'-CCGACTCACTCACCCACACCCCACC-3'           F5'-GCGACGCACTCTTCCCCTGC-3'         R5'-CCGACTCCTTCCCCTCC-3'           CsafM030440         R5'-CTCAAACGCTCACCACCCCGC-3'           F5'-GCTCCAACTCCTTCATTCACC-3'         R5'-CAGAGACTCAACGCACTCAACCG-3'           Csa1M152000         R5'-ATGGGAAACGCACTCAACCGG-3'           Csa1M152000         R5'-CTCCAATACTTTGGGTGG-3'           Csa4M083690.2         R5'-CAGAAGAACGCACTAACAACGGCAT-3'           Csa4M083690.2         R5'-ACTTCACCAGCAAAGGAGCAGG-3'           Csa7M392940         R5'-CTCCGATAGTTTGGGGGC-3'           Csa1M588560         F5'-GGCATGGATCATACAAGGAGCGAGC-3'           Csa1M5889060         R5'-GATCGATCAGCGAAGCGAAGCG-3'           Csa1M589070         R5'-GACGACCGAACCGAACGCA3/           CKXs         Csa2M00440         F5'-CCCTCAAACCGAAACCGAACCGAACG-3'           CKXs         Csa2M362450         R5'-CAACTCACCACGAAACCGAACCG-3'           Csa3M823030         R5'-CCACTCATTTCCATCACCCC-3'           Csa4M647490         F5'-CCCTCAATTCCACTCGC-3'           R5'-CCAGTAACCAACACCAACACCAACACCA'         R5'-CAATTCCCCCTCTGGAACCTACC-3'		Csa3M150100	F 5'-CGGCGGAAAGATAAAGTGG-3'
$ \begin{array}{c} Csa6M237640 & F s^{S} AAACGATGTTCCCCTATTCGGC-3' \\ R^{S} C CGAACGCAACGAACGAGTTGAG-3' \\ F^{S} C CGAACTTCTTCCCTTGCCTGTTC-3' \\ R^{S} C CGAACTTCTTCCACTAACCCTAAC-3' \\ R^{S} C CGAACTTCTTCCACTAACCCTAAC-3' \\ R^{S} C CGACTCCTTCACCTTCCCCTGC-3' \\ R^{S} C CTCAAATCCCTTCACCTCACG-3' \\ R^{S} C CGAACTCCTTCACCTCACCG-3' \\ R^{S} C CGAACACCCTTCATCCACG-3' \\ Csa1M152000 & R^{S} C CTCAAATCCCAACGCCTAACACG-3' \\ Csa4M083690 & R^{S} C CTTCCATTCCCGCAAGTCTTGGCGG-3' \\ Csa4M083690.2 & R^{S} C CTTCCATTCCCGCAAGTCTG-3' \\ Csa4M083690.2 & R^{S} C CTTCCATTCCCGCAAGTCTGGGG-3' \\ Csa7M392940 & R^{S} CTTCCATTCCGCAAGTCTGCG-3' \\ R^{S} C CSa1AGTTTGTGGGCAACTTTTGTGGCGG-3' \\ R^{S} CGATTGAACCAATCCACCAGCAAAGCGCG-3' \\ R^{S} CGATTGAACTATTTGGGCATGCGG-3' \\ C Sa1M589060 & R^{S} CGATTGAACTATTCGCGAGGAAGCGGC-3' \\ R^{S} CGACTGAAGTGAAGCAGCGAAGCAGCG-3' \\ C Sa1M589060 & R^{S} CGACTGAAGTGAGCGGAATGACGAGATC-3' \\ C Sa1M589060 & R^{S} CGACTGAAGTGAGCGGAACCGAAACG-3' \\ C Sa2M000440 & R^{S} CGCGAAGCAACCAAACAAC-3' \\ C Sa2M000440 & R^{S} CGAACCGAACCAACAACA-3' \\ C Sa2M000440 & R^{S} CCCCTCAAACCAACAACA-3' \\ C Sa2M823030 & R^{S} CCAACCCAACAACA-3' \\ C Sa3M823030 & R^{S} CCATCAACCACACAACCAACA-3' \\ C Sa4M647490 & R^{S} CCATAACCCTTTCCACACACACC-3' \\ C Sa4M647490 & R^{S} CCACTCAAACCCTTCCCCCAGAACCCAGTC-3' \\ \mathsf{R^{S} CCAAACCAACCACCCCCC-3' \\ R^{S} CCATAACCCCTTTCCCCCCCCC-3' \\ \mathsf{C Sa4M647490 & R^{S} CCACTCAACCCCTCTCACACACGCC-3' \\ \mathsf{R^{S} CCATAACCCCTCTCCCCCACCCC-3' \\ \mathsf{R^{S} CCATAACCCCTCTCCCCCCCCCC-3' \\ \mathsf{C Sa5M175820 & R^{S} CAACCCACACCCCCCCCCCCCCCCCCCCCCCCCCCCC$			R 5'- CAACATCAAGTCCTTCATAAACCTG-3'
$ \begin{array}{c} \mbox{Csabble57640} & \mbox{R}^{5-2}\mbox{CGAACGACGACGACGAGTGAGGA3} \\ \mbox{F}^{5-2}\mbox{CGAACTTCTTCACCAGGA3} \\ \mbox{F}^{5-2}\mbox{CGAACTTCTTCCATAACCCCTATC-3} \\ \mbox{R}^{5-2}\mbox{CGAACTTCTTCCATAACCCCTATC-3} \\ \mbox{R}^{5-2}\mbox{CGAACTTCTTCATACC3} \\ \mbox{R}^{5-2}\mbox{CGAACTTCTTCACG-3} \\ \mbox{R}^{5-2}\mbox{CGAACTCCTCATCTCACC-3} \\ \mbox{R}^{5-2}\mbox{CGAACACGACTAACTTCTCGCG-3} \\ \mbox{Csa1M152000} & \mbox{R}^{5-2}\mbox{CGACACGACTAACTAACGACTAACCA3} \\ \mbox{Csa4M083690} & \mbox{R}^{5-2}\mbox{CTCATTCTCCGCAACTCTCG-3} \\ \mbox{Csa4M083690.2} & \mbox{R}^{5-2}\mbox{CTCATTCTCCGCAACTCTG-3} \\ \mbox{Csa4M083690.2} & \mbox{R}^{5-2}\mbox{CTCATTCTCGCCAATCTCTG-3} \\ \mbox{Csa4M083690.2} & \mbox{R}^{5-2}\mbox{CTCATGCACAAGAACCAACAAC-3} \\ \mbox{R}^{5-2}\mbox{CaCAACTCACGCAAACAACAACAACA-3} \\ \mbox{R}^{5-2}\mbox{CaCAACTCTCAGCACCAATCTCTG-3} \\ \mbox{Csa1M588560} & \mbox{R}^{5-2}\mbox{CaTCGATGTGTAGGAACTATTACCC-3} \\ \mbox{Csa1M589070} & \mbox{R}^{5-2}\mbox{CGCAACTCACGAACC-3} \\ \mbox{Csa2M000440} & \mbox{R}^{5-2}\mbox{CGCAACCCAACC-3} \\ \mbox{Csa2M000440} & \mbox{R}^{5-2}\mbox{CGCAACCCAAACC-3} \\ \mbox{Csa2M32030} & \mbox{R}^{5-2}\mbox{CACCCAACAACCAAACC-3} \\ \mbox{R}^{5-2}\mbox{CACCCAACCCAACC-3} \\ \mbox{R}^{5-2}\mbox{CACCCAACCCCCCC-3} \\ \mbox{Csa4M343590} & \mbox{R}^{5-2}\mbox{CACCCAACCCCCCC-3} \\ \mbox{Csa4M647490} & \mbox{R}^{5-2}\mbox{CCTCACACCCCCCCCCC} \\ \mbox{R}^{5-2}CCACACACCAACCCCCCCCCCCCCCCCCCCCCCCCCC$		Csa6M237640	F 5'-AAACGATGTCTCCCTATTCTGGC-3'
$\begin{array}{c} \mbox{Csa7M253720} & F.5'-CGATGTTGCTCTGCCTGTTC-3'\\ R.5'-CGAACTTCTTCCACTAAC-3'\\ F.5'-GCGGGTGGATGACAACTAAC-3'\\ R.5'-CTTCAAATGCTCTTGCCTGC-3'\\ R.5'-CTTCAAATGCTCTTGCCTGC-3'\\ Csa5M609740 & R.5'-CTTCAATGCTCATCACGC-3'\\ Csa1M152000 & R.5'-CTTCAATGCTCAAGAGC-3'\\ Csa4M083690 & R.5'-CTTCATTGCGGAACTGCTGG-3'\\ Csa4M083690.2 & R.5'-CTTCCGTAGTTGTGGTGG-3'\\ Csa4M083690.2 & R.5'-CTTCCGTGGG-3'\\ Csa7M392940 & R.5'-CTTCGATGGACTACAAGAGCAGTG-3'\\ R.5'-ACTTCAGCAGCAACTTGTGGGC-3'\\ Csa1M589560 & R.5'-CTTCGATGGACTATGCTGG-3'\\ Csa1M589060 & R.5'-CTTCGATGGACTACGAAGAGCAGATG-3'\\ R.5'-CAGCAAGGGAAAGAAGCAGATG-3'\\ Csa1M589070 & R.5'-CGATGACTATTAGAGGGTTGC-3'\\ Csa1M589070 & R.5'-CGTCAGGACTAACCAACGAAGGC-3'\\ Csa2M000440 & R.5'-CGTCAGGCGACGAACCGAAACG-3'\\ Csa2M000440 & R.5'-CGTAATCCGACGACGAAACG-3'\\ Csa2M362450 & R.5'-CGTCATGCGACGAACCAAACA-3'\\ Csa2M362450 & R.5'-CGTAATCCGACGAGCCGACCGAACCG-3'\\ R.5'-CGTAATCCGACGAGCCGAACCGAAACG-3'\\ R.5'-CGTAATCCGACGACCGAACCGAAACC-3'\\ Csa3M823030 & R.5'-CCTCATTCCAACCACCC-3'\\ Csa4M343590 & R.5'-CCATAAACCAACCAACCAACCA-3'\\ R.5'-CGCAAGAGTCCCACTGCGGTGGACTTACCC-3'\\ Csa4M647490 & R.5'-CCGCAAGGGAAGGAAGGCG-3'\\ R.5'-CTTGCACCACCCCTGTGCG-3'\\ R.5'-CGCGAAGGGAAGGAAGGAGGC-3'\\ R.5'-CTTGCACCCACCTTTCCAATCCGACGGCG-3'\\ R.5'-CGCCACAACCTTTCCAATCCCACCG-3'\\ R.5'-CGCCACAACCTTTCCAATCCCACCCC-3'\\ R.5'-CGCAAGTCCCACCCCCC-3'\\ R.5'-CGCAAGTCCCACCCCCC-3'\\ R.5'-CGCAAGTCCCACCCCCC-3'\\ R.5'-CGCAAGTCCCACCCCCC-3'\\ R.5'-CGCAAGTCCCACCCCCCC-3'\\ R.5'-CGCAAGTCCCACCCCCCC-3'\\ R.5'-CGCAAGTCCCACCCCCCC-3'\\ R.5'-CGCAAGTCCCACCCCCCC-3'\\ R.5'-CGCAAGTCCCACCCCCCCCC-3'\\ R.5'-CGCAAGTCCCACCCCCCCCC-3'\\ R.5'-CGCCAAGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC$			R 5'-CCGACACGAACGAGTTGAGG-3'
IPTsCsa6M030440R5'-GGACTTCTTCCACTAACCCTAAC-3' F5'-GGCGGGGAGGATGGGATGGC3' R5'-CTTCAAATGCTCTTGCCTGC-3' F5'-TTCGATGGCAACTCCAACGTCTAACG-3' Csa1M152000F5'-GGTCCAACGCCTCAATCCGTCG-3' F5'-TGTTCCGATAGTTGTCGGTGG-3' Csa4M083690Csa4M083690F5'-TGTTCCGATAGTTTGTCGGGG-3' Csa4M083690.2R5'-CTCATTTCTCCGCAAGTCG-3' F5'-TTCCGATAGTTGGTGGG-3' Csa4M083690.2Csa1M152000R5'-CAGAAGAAACGGACTAACAAGAGC-3' F5'-TTCCGATAGTTTGTGGTGGG-3' Csa4M083690.2R5'-CTCCATTTCTCCCGAAGTCGG-3' R5'-CTCCATTGTGCGGACTTTGTGGCG-3' R5'-TTCGTTGGGAACTTAGCGC-3'Csa1M588560F5'-GACGCAAGGAAGAAGAGAGGAGGAGGAGGAGGAGGAGGAG		Csa7M253720	F 5'-CGATGTTGCTCTGCCTGTTC-3'
$\begin{tabular}{lllllllllllllllllllllllllllllllllll$			R 5'-CGAACTTCTTCCACTAACCCTAAC-3'
IPTsCsa5M609740R 5'-CTTCAAATGCTCTTGCCTGC-3' F 5'-GGTCCAACTCTCATTCACG-3' R 5'-AATGGGAAACTCAACGTCTTAACC-3' F 5'-TTGATGGCATAACTACACGTCTTACC-3' R 5'-AATGGCAAACCGAAGAGC-3' Csa4M083690R 5'-CAGAGGAAACCGAAGAGC-3' R 5'-TTGCGTTGGTGGG-3' R 5'-TTCCGATAGTTTGGTGGG-3' Csa4M083690.2R 5'-TTGCGATAGTTTGGTGGG-3' R 5'-TTCCGATAGTTTGGTGGG-3' R 5'-AACTTCAGCAGCAAAGAGCAGATG-3' R 5'-TTCGTTGGGAAACTGATGCTGG-3' R 5'-TTCGTTGGGAAACTGATGCTGG-3' R 5'-GGCATGGATTGTAGGGAAAGAACGAAGCGAATG-3' R 5'-GGATTGAACTATTTAGAGGGTTTGC-3' R 5'-GGCATGAGTGAGAAGAAGCAGATG-3' C Sa1M589060F 5'-GGCATGGATGAGAAGAAGCAGATG-3' R 5'-GATCGAGTGAGTGAGAAGAACCAACCAACCAAACCAACCA		Csa6M030440	F 5'- GCGGGTGGATGAGATGTTG-3'
$\begin{array}{c c c c c c c c c c c c c c c c c c c $			R 5'-CTTCAAATGCTCTTGCCTGC-3'
$\begin{array}{c c} CsalM00740 & R 5'-AATGGGAAACTCAACGTCTAACACG' \\ F 5'-TTGATGGCATAATTTCTCGTGG-3' \\ R 5'-CAGAAGAAACGGACTAACAAGAGC-3' \\ Csa4M083690 & R 5'-CTTCCATTTCTCCGCAAGTCTG-3' \\ Csa4M083690.2 & R 5'-TTCCGATAGTTTGTGGTGGG-3' \\ Csa4M083690.2 & R 5'-ACTTCAGCAGCAATGTCTGG-3' \\ Csa7M392940 & R 5'-ACTTCAGCAGCAATGTCTGG-3' \\ Csa7M392940 & R 5'-ACCTAGGAAACGAAGCAGATG-3' \\ R 5'-GGATTGATGTGGGAACTTTTAGAGGGTTTGC-3' \\ Csa1M589660 & R 5'-GATGATTGTAGAGGGTTGC-3' \\ Csa1M589060 & R 5'-GATCGATTGTAGCAGCGAGGAGGAGGG-3' \\ Csa1M589070 & R 5'-GATCGATTGTAGCGAGTGAGAGGGG-3' \\ Csa2M000440 & F 5'-GCTCGTGGGGGGAGTACCGAACGAACG-3' \\ Csa2M000440 & R 5'-CCAACCAACAACCAAACA-3' \\ Csa2M000440 & R 5'-CCATATCCAACCAACAACAACA-3' \\ Csa2M000440 & R 5'-CCATATCCAACCACACAACA-3' \\ Csa3M823030 & F 5'-GGCATAGTTATCAACCACGCC-3' \\ Csa4M343590 & R 5'-CAACTCATTTCAAACATGGAGTCC-3' \\ Csa4M647490 & R 5'-CCATAAACCATGGCAGGG-3' \\ F 5'-GCTCAGGCAGAAGGAAGGAGG-3' \\ Csa5M175820 & F 5'-CCATCAGCAAGCAAGGAGGG-3' \\ CsaCAGCAGCAGAGGAAGGAAGCGTG-3' \\ R 5'-CCATAAACCTTTCCAACACGCTG-3' \\ R 5'-CCATCAACCTTTCCAACATGGAGTCC-3' \\ R 5'-CCATAAACCTTCCAACTGGAGTC-3' \\ R 5'-CCATAAACCTTCCACACGCC-3' \\ R 5'-CCATAAACCTTCCACACGCCG-3' \\ R 5'-CCATAAACCTTCCACACCCC-3' \\ R 5'-CCATAAACCTTCCACACGGAGTG-3' \\ R 5'-CCATAAACCTTCCACACCCC-3' \\ R 5'-CCATAAACCTTCCACACCCC-3' \\ R 5'-CCATAAACCTTCCACACCCC-3' \\ R 5'-CCATAAACCTTCCACACCCC-3' \\ R 5'-CCATAAACCTTCCACACGCCG-3' \\ R 5'-CCATAAACCTTCCACACCCC-3' \\ R 5'-CCATAAACCTTCCACACCCCC-3' \\ R 5'-CCATAAACCTTCCACACCCCC-3' \\ R 5'-CCATAAACCTTCCCACAGGAGGAAGCAGTG-3' \\ R 5'-CCATAAACCTTCCCCACAGGAGGAAGCAAGGAAGCAGTC-3' \\ R 5'-CCATAAACCTTCCCCACACGTC-3' \\ R 5'-CCATAAACCTTCCCCACAGTCTCCTACAACACCCCCCCCC$	IPTs	Csa5M609740	F 5'-GGTCCAACTCCTTCATTCACG-3'
$ \begin{array}{llllllllllllllllllllllllllllllllllll$		CSa51V1009740	R 5'-AATGGGAAACTCAACGTCTAACC-3'
CsalMin52000       R 5'-CAGAAGAAACGGACTAACAACAGGC-3'         Csa4M083690       R 5'-CTTCATTTCTCCGCAAGTCTG-3'         R 5'-CTTCATTTCTCCCGATAGTTTGTGGTGGG-3'       R 5'-CTTCATTTCTCCCGAAGTCTG-3'         Csa4M083690.2       R 5'-ACTTCAGCAGCAAGTGCTGG-3'         Csa7M392940       F 5'-AGCAAGGAAAGAAGCAGATG-3'         R 5'-CTTCGTTGGGAACTTTGTGGCG-3'       R 5'-TTCGTTGGGAACTTTGTGGC-3'         Csa1M588560       F 5'-GGATTGAACTATTTAGAGGGTTTGC-3'         Csa1M589060       F 5'-GATCGAGGTGAGATTACCGACGAGGG-3'         Csa1M589070       F 5'-GATCGAGGTGGAGATTACCG-3'         Csa2M000440       F 5'-CCTCGTTGGACGAACCGAAACG-3'         Csa2M362450       R 5'-TCTATTCCAACAACCAAACAACA-3'         Csa3M823030       F 5'-GGCATGAATTACCGACGAGCCCG-3'         Csa4M343590       R 5'-CCATCAATTCCAACAACCAACCA-3'         R 5'-CCATCAACCTCTTCAATCAACATGGAGTCC-3'       Csa4M343590         R 5'-CCATCAACCCTCTTCCAATCAG-3'       R 5'-CCATCAACCCTCTTCCAATCAG-3'         Csa4M647490       F 5'-CCATAAACCAACAAGGAAGCGAATG-3'         R 5'-CCATAAACCAACGGAAGCGG-3'       S 5'-CCATAAACCATCATCAACATGGAGTC-3'         Csa4M647490       F 5'-CCATCAACCCTCTTCCAACA-3'         R 5'-CCATAAACCAACAAGGGAAGCGATG-3'       S 5'-CCATAAACCATCATTAGACCTTCGCGG-3'         Csa5M175820       R 5'-CCATAAACCAAGGGAAGCGATTCTTCCAACA-3'		Csa1M152000	F 5'-TTGATGGCATAATTTCTCGTGG-3'
$\begin{array}{c} \label{eq:csa4M083690} & F5'-IGTTCGATAGTTIGTGGTGG-3' \\ R5'-TTCCGATAGTTIGTGGTGGG-3' \\ R5'-AACTTCAGCAGCAAGTGTGGGG-3' \\ R5'-AACTTCAGCAGCAAGTGTGGGG-3' \\ R5'-AACTTCAGCAGCAAGAGGGAAGAGCAGATG-3' \\ R5'-GACGCAGCAGAGGAAAGAAGCAGATG-3' \\ R5'-GGATGGACTATTTAGAGGGTTIGC-3' \\ R5'-GATGGATGAACATTTTAGAGGGTTIGC-3' \\ R5'-GATGGATGAACAGTGAGAGAGGG-3' \\ R5'-GATGGATGAACGATGAGAGAGGG-3' \\ R5'-GACGCCAGAAGTGAGAAGGGG-3' \\ Csa1M589070 & F5'- GTCTCGTGGGGTGGATTATTTGC-3' \\ R5'-GCTCGTGGGGGGGGATATTTGC-3' \\ Csa2M000440 & R5'-CGTAACCGAACGAACGA-3' \\ R5'-CGTAACCGAACAACAACA-3' \\ R5'-CGTAACCGACGCAGAGCGAACGA-3' \\ Csa2M362450 & F5'-TCTTATGACCGAGAGCCAACA-3' \\ R5'-GGCATGCTATTCAACCAGCAGCC-3' \\ Csa3M823030 & F5'-GGCATGATTCAACATGGAGTCC-3' \\ R5'-AGATTCCCACTCTGAACCACCA-3' \\ R5'-AGATTCCCACTCTGAACCTTCC-3' \\ R5'-AGATTCCCACTCTGAACCTTC-3' \\ R5'-CGTAAACCCATCTTCAACATGGAGTC-3' \\ R5'-CGATAACCCATCTTCAACATGGAGTC-3' \\ R5'-CGATAACCCACTCTGAACCTTC-3' \\ R5'-CGATAACCCACTCTCGAACCACC-3' \\ R5'-CCATCAACCCTTCCACATCACA-3' \\ R5'-CCATCAACCCTTCCACTTGAACCTTC-3' \\ R5'-CCATCAACCCTCTTCAACATGGAGTCC-3' \\ R5'-CCATCAACCCTTCCACTTGAACCTTC-3' \\ R5'-CCATCAACCCTTCCGACCTCG-3' \\ R5'-CCATCAACCCTTCCGACCTTC-3' \\ R5'-CCATCAACCCTTCCGACCACGT-3' \\ R5'-CCATCAACCCTTCCGACCACTTC-3' \\ R5'-CCATCAACCCTTCCGACCACTTC-3' \\ R5'-CCATCAACCCTTCCACTCGACCACTTC-3' \\ R5'-CCATCAACCCTTCCACTTC-3' \\ R5'-CCATCAACCCTTCCGACCACGT-3' \\ R5'-CCATCAACCCTTCCGACCACTTC-3' \\ R5'-CCATCAACCCTTCCGACCACTTC-3' \\ R5'-CCATCAACCCTTCCCACCACTC-3' \\ R5'-CCATCAACCCTTCCCACCACTC-3' \\ R5'-CCATCAACCCTTCCCACCACTC-3' \\ R5'-CCATCAACCCTCTCCCACACCC-3' \\ R5'-CCATCAACCCTTCCCACCACTC-3' \\ R5'-CCATCAACCCTTCCCACCTCTCCACAC-3' \\ R5'-CCATCAACCCTCTCCCACCC-3' \\ R5'-CCATCAACCCTTCCCACACCC-3' \\ R5'-CCATCAACCCTTCCCACACCC-3' \\ R5'-CCATCAACCCTTCCCACCACGTTC-3' \\ R5'-CCATCACCCCCCCCCCCC-3' \\ R5'-CCATCACCCCCCCCCCCCC-3' \\ R5'-CCATCACCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC$		C5a1101102000	R 5'-CAGAAGAAACGGACTAACAAGAGC-3'
CsaintiooodR 5'-CTTCATTTCCCGCAAGTCTG-3' F 5'-TTCCGTAGTGTGTGGGG-3' R 5'-AACTTCAGCAGCAATGTCTGG-3' R 5'-AACTTCAGCAGCAATGTCTGG-3' R 5'-ACCTAGCGAAAGCAGCAGATG-3' R 5'-GATGGACTGTGGGGGAAGCAGGAGGAGGAGGGAGGGAGGG		Csa4M083690	F 5'-TGTTCCGATAGTTTGTGGTGG-3'
$ \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c} \begin{array}{c}$		CS44101005050	R 5'-CTTCATTTCTCCGCAAGTCTG-3'
$\begin{array}{c} R 5'-AACTTCAGCAGCAGTGTCTGG-3' \\ F 5'-AGCAAGGGAAAGAGCAGATG-3' \\ R 5'-TTCGTTGGGAAACTTTTAGAGGGTTGC-3' \\ R 5'-GATGAACTATTTAGAGGGTTGC-3' \\ R 5'-GATGAACTATTTAGAGGGTTGC-3' \\ R 5'-GATCGAGTTGAAGTGAGAGAGAGAGGG-3' \\ F 5'-GATCGAGTTCATACCGACGAGGGAGGACGAGGG-3' \\ Csa1M589070 \\ R 5'-GATCGAGGTGGATGAGAGTGAGGATTACGC-3' \\ Csa2M000440 \\ R 5'-CGTAAACCAACAAACAACA-3' \\ Csa2M362450 \\ Csa2M362450 \\ Csa3M823030 \\ R 5'-GGCATAGTTACCGACGAGGCCGA-3' \\ Csa3M823030 \\ R 5'-GGCATAGTTATCAACCAGCGC-3' \\ Csa4M343590 \\ R 5'-CCATCAACCAACCAACCAACC-3' \\ Csa4M647490 \\ R 5'-CCATCAACCTTCGCTCGGG-3' \\ Csa5M175820 \\ \end{array}$		Csa4M083690 2	F 5'-TTCCGATAGTTTGTGGTGGG-3'
Csa7M392940F 5'-AGCAAGGGAAAGAAGCAGATG-3' R 5'-TTCGTTGGGAACTTTGTGGC-3'Csa1M588560F 5'-GGATTGAACTATTTAGAGGGTTTGC-3' R 5'-GATCGAGTTGATAGAGAGGGAAGCAGGAG'Csa1M589060R 5'-GATCGAGTTCATACCGACGAGGAG' R 5'-GACGCCAGAAGTGAGAGATTACCG-3' R 5'-GCGTAGGACCGAACCGAAACG-3' R 5'-TGAGGACCGAACCGAAACG-3'Csa1M589070R 5'-GTCTCGTGGGTGGATTATTTGC-3' R 5'-TGAGGACCGAACCGAAACG-3' R 5'-CGTAATCCGACGAGACCGAAACG-3' R 5'-CCGTAAACCAACAACAACA-3' R 5'-CCGTAAACCAACAACCAACAACA-3' Csa2M362450CKXsCsa2M362450Csa3M823030F 5'-GCCATAGTTATCAACATGGAGTCC-3' R 5'-CCATCAATCCCACTGTGAACCTGC-3' R 5'-CCATCAACCCTCTTCACATCAG-3' R 5'-CCATCAACCCTCTTCCACTCAG-3' R 5'-CCATCAACCCTCTTCCACTCAG-3' R 5'-CCATCAACCCTGCG-3'Csa4M647490R 5'-CCATCAACCCTCTTCCACACG-3' R 5'-CCTTGGAACCAGAGGGG-3'Csa5M175820F 5'-TGCCTCAGCACAGTTCTTCCTACAA-3'		C3a4101003070.2	R 5'-AACTTCAGCAGCAATGTCTGG-3'
CsailM52516R 5'-TTCGTTGGGAACTTTGTGGC-3'CsailM588560F 5'-GATGAATGAAGAGTGATGAGAAGGTG-3'CsailM589060F 5'-GATCGAGTTCATACCGACGAGG-3'CsailM589070F 5'-GTCTCGTGGGGTGGATTATTGC-3'Csa2M000440R 5'-TGAGGACCGAACCGAAACG-3'Csa2M362450F 5'-CCGTAAACCAACAACCAACAACC-3'Csa3M823030F 5'-GCCATAGTTATCACACGAGGTCC-3'Csa4M343590R 5'-CCATCAACCTTTCAATCACACACCG-3'Csa4M647490R 5'-CCATCAACCTTTCGC-3'Csa5M175820F 5'-CCAGCAGAGGAAGCAGG-3'R 5'-TGCCTCAGCACGAAGCGA-3'R 5'-CCACTCATTTCAATCACACAACA-3'		Csa7M392940	F 5'-AGCAAGGGAAAGAAGCAGATG-3'
Csa1M588560         F 5'-GGATTGAACTATTTAGAGGGTTTGC-3' R 5'-GATGATTGTAGAGTGATGAGAAGGTG-3'           Csa1M589060         F 5'-GATCGAGTTCATACCGACGAGG-3'           R 5'-GACGCCAGAAGTGAGATTACGC-3'         F 5'-GTCTCGTGGGTGGATTATTTGC-3'           Csa1M589070         R 5'-TGAGGACCGAACCGAAACG-3'           Csa2M000440         F 5'-CCGTAAACCAACAACCAAACA-3'           Csa2M362450         F 5'-CCATAACCAACAACAACCAACA-3'           Csa3M823030         R 5'-CCATCATTCAATCACCACCC-3'           Csa4M343590         R 5'-CCATCAACTGTGGAGGAGCTGGAGCCGG-3'           R 5'-CCATAAACCATGTGGAACCTGC-3'         R 5'-CCATAAACCATGGAAGCAGTG-3'           Csa4M647490         R 5'-CCATAAACCAAGAGGAAGCAGTG-3'           R 5'-CCATGGAACGCAGAAGAGGAAGCAGTTG-3'         R 5'-CCATGAAACCAACCAACA-3'           R 5'-CCATAAACCTTTCGCTCGG-3'         R 5'-CCATCAACCTGTGGAACCTGC-3'           R 5'-CCATCAACCTGTGGAACCTGC-3'         R 5'-CCATCAACCTTTCGCTCGG-3'           R 5'-CCATCAACCTTTCGCTCGG-3'         R 5'-CCATCAACCTTTCGCTCGG-3'           R 5'-CCATCAACCTTTCGCTCGG-3'         R 5'-CCATGAACGCAGAAGAGGA-3'           R 5'-CCATCAACCTTTCGCTCGG-3'         R 5'-CCATCAACCTTTCGCTCGG-3'           R 5'-CCATCAACCTTTCGCTCGG-3'         R 5'-CCATCAACCTTTCCCTACAA-3'           R 5'-CCATCAACCCTCTTCCCTCAGCACGTTG-3'         R 5'-CCATCAACCCCCTTTCCCTACAA-3'			R 5'-TTCGTTGGGAACTTTGTGGC-3'
Csa1Mi588960R 5'-GATGATTGTAGAGTGATGAGAAGGTG-3' F 5'-GATCGAGTTCATACCGACGAGGG-3' R 5'-GACGCCAGAAGTGAGATTACGC-3' R 5'-GTCTCGTGGGTGGATTATTTGC-3' R 5'-TGAGGACCGAACCGAAACG-3' 		Csa1M588560	F 5'-GGATTGAACTATTTAGAGGGTTTGC-3'
Csa1M589060F 5'-GATCGAGTTCATACCGACGAGG-3' R 5'-GACGCCAGAAGTGAGATTACGC-3'Csa1M589070F 5'- GTCTCGTGGGTGGATTATTTGC-3' R 5'-TGAGGACCGAACCGAAACG-3'Csa2M000440F 5'-CCGTAAACCAACAACCAAACA-3' R 5'-CGTAATCCGACGAGGCTATG-3'CKXsCsa2M362450F 5'-TCTTATGACCCGAGAGCCGC-3' R 5'-CCACTCATTTCAATCACCACCC-3'Csa3M823030F 5'-GGCATAGTTATCAACATGGAGTCC-3' R 5'-CCATCAACCTTCGCACGCAGACCCGC-3'Csa4M343590F 5'-CCATCAACCCTCTTCACATCAG-3' R 5'-CCATAAACCTTCGCTCGG-3'Csa4M647490F 5'-GCGGAAGAGGAAGCAGTTG-3' R 5'-CTTGGAACGCAGAAGAGGG-3'Csa5M175820F 5'-TGCCTCAGCACAGTTCTTTCTA-3' R 5'-CAAGCAGTGGATTTCCCTACAA-3'			R 5'-GATGATTGTAGAGTGATGAGAAGGTG-3'
Csa1M389000R 5'-GACGCCAGAAGTGAGATTACGC-3'Csa1M589070F 5'- GTCTCGTGGGTGGATTATTTGC-3'R 5'-TGAGGACCGAACCGAAACG-3'R 5'-CGTAAACCAACCAAACA-3'Csa2M000440F 5'-CCGTAAACCAACCAAACA-3'R 5'- CGTAATCCGACGAGGCTATG-3'R 5'- CGTAATCCGACGAGGCCTATG-3'CKXsCsa2M362450F 5'-TCTTATGACCCGAGACCCGC-3'Csa3M823030R 5'-CCACTCATTTCAATCACCACCC-3'Csa4M343590F 5'-CCATCAACCTTCTCACATCAG-3'Csa4M647490F 5'-GCGGAAGAGGAAGCAGTTG-3'Csa5M175820F 5'-TGCCTCAGCACAGTTGTTTCTA-3'R 5'-CAAGCAGTGGATTTCCCTACAA-3'		Csa1M589060	F 5'-GATCGAGTTCATACCGACGAGG-3'
Csa1M589070F 5'- GTCTCGTGGGTGGATTATTTGC-3' R 5'-TGAGGACCGAACCGAAACG-3'Csa2M000440F 5'-CCGTAAACCAACAACAACA-3' R 5'- CGTAATCCGACGAGGCTATG-3' R 5'-CCACTCATTTCAATCACCGAGACCCGC-3'CkXsCsa2M362450F 5'-TCTTATGACCCGAGACCCGC-3' R 5'-CCACTCATTTCAATCACCACCC-3'Csa3M823030F 5'-GGCATAGTTATCAACATGGAGTCC-3' R 5'-AGATTCCCACTGTGAACCTGC-3' R 5'-CCATCAACCTTTCACATCAG-3' R 5'-CCATAAACCTTTCGCTCGG-3' R 5'-CCATAAACCTTTCGCTCGG-3' Csa4M647490Csa4M647490F 5'-CCATCAACCTTTCGCTCGG-3' R 5'-CTTGGAACGCAGAAGAGGAAGCAGTTG-3' R 5'-CTTGGAACGCAGAAGAGGGA3' F 5'-TGCCTCAGCACAGTTCTTTCTA-3' R 5'-CCAAGCAGTGGATTTCCCTACAA-3'			R 5'-GACGCCAGAAGTGAGATTACGC-3'
CsaIM339070R 5'-TGAGGACCGAACCGAAACG-3'Csa2M000440F 5'-CCGTAAACCAACAACCAAACA-3'R 5'- CGTAATCCGACGAGGCTATG-3'CKXsCsa2M362450Csa3M823030F 5'-TCTTATGACCCGAGACCCGC-3'Csa4M343590F 5'-GGCATAGTTATCAACATGGAGTCC-3'Csa4M343590F 5'-CCATCAACCTTCTCACATCAG-3'Csa4M647490F 5'-GCGGAAGAGGAAGCAGTTG-3'Csa5M175820F 5'-TGCCTCAGCACAGTTCTTTCTA-3'R 5'-CAAGCAGTGGATTTCCCTACAA-3'	CKXs	Csa1M589070	F 5'- GTCTCGTGGGTGGATTATTTGC-3'
Csa2M000440F 5'-CCGTAAACCAAACAACCAAACA-3' R 5'- CGTAATCCGACGAGGCTATG-3'CKXsCsa2M362450F 5'-TCTTATGACCCGAGACCCGC-3' R 5'-CCACTCATTTCAATCACCACCC-3'Csa3M823030F 5'-GGCATAGTTATCAACATGGAGTCC-3' R 5'-AGATTCCCACTGTGAACCTGC-3'Csa4M343590F 5'-CCATCAACCCTCTTCACATCAG-3' R 5'-CCATAAACCTTTCGCTCGG-3'Csa4M647490F 5'-GCGGAAGAGGAAGCAGTTG-3' R 5'-CTTGGAACGCAGAAGAGGG-3'Csa5M175820F 5'-TGCCTCAGCACAGTTCTTTCA-3' R 5'-CAAGCAGTGGATTTCCCTACAA-3'			R 5'-TGAGGACCGAACCGAAACG-3'
Csa2M000440R 5'- CGTAATCCGACGAGGCTATG-3'CKXsCsa2M362450F 5'-TCTTATGACCCGAGACCCGC-3'Csa3M823030F 5'-GGCATAGTTATCAACATGGAGTCC-3'Csa4M343590F 5'-CCATCAACCTCTTCACATCAG-3'Csa4M647490F 5'-CCATCAAACCTTTCGCTCGG-3'Csa5M175820F 5'-TGCCTCAGCACAGTGGATTCCTTCACAA-3'		Csa2M000440	F 5'-CCGTAAACCAACAACCAACA-3'
CKXsCsa2M362450F 5'-TCTTATGACCCGAGACCCGC-3' R 5'-CCACTCATTTCAATCACCACCC-3' R 5'-GGCATAGTTATCAACATGGAGTCC-3' R 5'-AGATTCCCACTGTGAACCTGC-3' R 5'-CCATCAACCCTCTTCACATCAG-3' Csa4M343590F 5'-GGCATAGTTATCAACATGGAGTCC-3' R 5'-CCATCAACCCTCTTCACATCAG-3' R 5'-CCATCAACCTTTCGCTCGGC-3' R 5'-CCATGAACCAGAGGAAGCAGTTG-3' R 5'-CTTGGAACGCAGAAGAGGG-3' F 5'-TGCCTCAGCACAGTTCTTTCTA-3' R 5'-CAAGCAGTGGATTTCCCTACAA-3'			R 5'- CGTAATCCGACGAGGCTATG-3'
Csa2M302430R 5'-CCACTCATTTCAATCACCACCC-3'Csa3M823030F 5'-GGCATAGTTATCAACATGGAGTCC-3'R 5'-AGATTCCCACTGTGAACCTGC-3'R 5'-AGATTCCCACTGTGAACCTGC-3'Csa4M343590F 5'-CCATCAACCCTCTTCACATCAG-3'Csa4M647490F 5'-GCGGAAGAGGAAGCAGTTG-3'Csa5M175820F 5'-TGCCTCAGCACAGTTCTTTCTA-3'R 5'-CAAGCAGTGGATTTCCCTACAA-3'		Csa2M362450	F 5'-TCTTATGACCCGAGACCCGC-3'
Csa3M823030F 5'-GGCATAGTTATCAACATGGAGTCC-3' R 5'-AGATTCCCACTGTGAACCTGC-3' F 5'-CCATCAACCCTCTTCACATCAG-3' R 5'-CCATAAACCTTTCGCTCGG-3'Csa4M343590F 5'-CCATCAACCCTCTTCACATCAG-3' R 5'-CCATAAACCTTTCGCTCGG-3' R 5'-CTTGGAACGCAGAAGAGGG-3' F 5'-TGCCTCAGCACAGTTCTTTCTA-3' R 5'-CAAGCAGTGGATTTCCCTACAA-3'			R 5'-CCACTCATTTCAATCACCACCC-3'
CsabMb20000R 5'-AGATTCCCACTGTGAACCTGC-3'Csa4M343590F 5'-CCATCAACCCTCTTCACATCAG-3'Csa4M647490F 5'-CCATAAACCTTTCGCTCGG-3'Csa5M175820F 5'-TGCCTCAGCACAGTGCATTCTTCA-3'Csa5M175820R 5'-CAAGCAGTGGATTTCCCTACAA-3'		Csa3M823030	F 5'-GGCATAGTTATCAACATGGAGTCC-3'
Csa4M343590F 5'-CCATCAACCCTCTTCACATCAG-3' R 5'-CCATAAACCTTTCGCTCGG-3'Csa4M647490F 5'-GCGGAAGAGGAAGCAGTTG-3' R 5'-CTTGGAACGCAGAAGAGGG-3'Csa5M175820F 5'-TGCCTCAGCACAGTTCTTTCTA-3' R 5'-CAAGCAGTGGATTTCCCTACAA-3'			R 5'-AGATTCCCACTGTGAACCTGC-3'
Csa4Mi343330R 5'-CCATAAACCTTTCGCTCGG-3'Csa4Mi647490F 5'-GCGGAAGAGGAAGCAGTTG-3'Csa5M175820F 5'-TGCCTCAGCACAGTTCTTTCTA-3'R 5'-CAAGCAGTGGATTTCCCTACAA-3'		Csa4M343590	F 5'-CCATCAACCCTCTTCACATCAG-3'
Csa4M647490F 5'-GCGGAAGAGGAAGCAGTTG-3' R 5'-CTTGGAACGCAGAAGAGGG-3'Csa5M175820F 5'-TGCCTCAGCACAGTTCTTTCTA-3' R 5'-CAAGCAGTGGATTTCCCTACAA-3'			R 5'-CCATAAACCTTTCGCTCGG-3'
CsafM04/420R 5'-CTTGGAACGCAGAAGAGGG-3'Csa5M175820F 5'-TGCCTCAGCACAGTTCTTTCTA-3'R 5'-CAAGCAGTGGATTTCCCTACAA-3'		Csa4M647490	F 5'-GCGGAAGAGGAAGCAGTTG-3'
Csa5M175820 F 5'-TGCCTCAGCACAGTTCTTTCTA-3' R 5'-CAAGCAGTGGATTTCCCTACAA-3'			R 5'-CTTGGAACGCAGAAGAGGG-3'
R 5'-CAAGCAGTGGATTTCCCTACAA-3'		Csa5M175820	F 5'-TGCCTCAGCACAGTTCTTTCTA-3'
			R 5'-CAAGCAGTGGATTTCCCTACAA-3'

Table 2. IPT and CKX genes' primer sequences for RT-qPCR.

#### 2.4. Statistical Analyses

DPS software (v17.10, Zhejiang University, Hangzhou, China) was used to analyse whether there were any significant differences between the treatments, and then, the results were subjected to an LSD test with a significance level of 5% (p < 0.05).

### 3. Results

#### 3.1. FR Light Inhibits Lateral Branch Elongation in Cucumber Seedlings

In the present study, FR light was applied to cucumber seedlings in the two-leaf stage. The results showed that FR light treatment significantly inhibited the elongation of the lateral branches of cucumber seedlings after 8 days (Figure 2), and the lengths of the first, second, third and total lateral branches (from the bottom to the top of the stems) were reduced by 41.22%, 93.96%, 43.27% and 81.88%, respectively, compared with those of the control (full-spectrum LED white light).



**Figure 2.** Effects of far-red (FR) light on the elongating lateral bud of cucumber seedlings (**A**). The data are the mean  $\pm$  SD (n = 5) (**B**). Different lowercase letters indicate significant differences at among treatments (p < 0.05).

## 3.2. Effect of FR Light on the Endogenous Hormone Contents and Related Gene Expression in Cucumber Seedlings

The results showed that FR light significantly promoted IAA levels in the apical and basal internodes of cucumber seedlings by as much as 36.98% and 41.96%, respectively, and significantly reduced Zeatin levels by 19.69% and 8.24%, respectively (Figure 3A,B). In addition, we observed the response of the IAA synthetic genes—*YUCs*—to FR light and found that the expression levels of *YUC4*, *YUC6*, *YUC9*, *YUC10b* and *YUC11* were significantly downregulated, while only the expression level of *YUC8* was significantly upregulated (Figure 3C,D). Moreover, the expression levels of most of the CTK synthetic genes, *IPTs*, also showed significant downregulation (Figure 3G,H), which was consistent with the Zeatin content; moreover, the expression levels of the CTK synthetic gene, *LOG1*, showed significant downregulation only in the basal internodes (Figure 3F). Interestingly, the expression of most of the CTK hydrolytic genes, such as *CKXs*, was also significantly downregulated, while that of only one gene (Csa4G343590) was significantly upregulated (Figure 3I,J), which may be related to feedback regulation.

# 3.3. The Inhibitory Effect of FR Light on the Branch Elongation of Cucumber Seedlings Was Related to IAA

To investigate the relationship between IAA and lateral branch elongation in cucumber seedlings under FR light, we investigated the effect of decapitation on lateral branch elongation in cucumber seedlings under FR light. Consistent with the expected results, decapitation eliminated the inhibitory effect of FR light on lateral branch elongation in cucumber seedlings (Figure 4). Additionally, decapitation significantly downregulated the expression of IAA synthetic genes, *YUCs (YUC7, YUC9* and *YUC10b*), but an increase in IAA levels was observed (Figure 5A,B). In addition, decapitation significantly upregulated the expression of most of the CTK synthetic genes, *IPTs* (Figure 5D); interestingly, the CTK synthetic gene, *LOG2*, and most of the CTK hydrolytic genes, such as *CKXs*, were significantly downregulated (Figure 5C,E). The trend of the changes in Zeatin content was also consistent with that of the synthetic genes, which increased by 17.46% after decapitation compared with that in the FR light group (Figure 5A). These results suggest that FR light enhances apical dominance through IAA signalling, thereby inhibiting lateral branch elongation.



**Figure 3.** Effects of FR light on the endogenous hormone contents (**A**,**B**) and related gene expression (**C**–**J**) in cucumber seedlings. The data are the mean  $\pm$  SD (n = 3). Different lowercase letters indicate significant differences among treatments (p < 0.05).



**Figure 4.** Effects of FR light and/or decapitation on the elongating lateral bud of cucumber seedlings (**A**). The data are the mean  $\pm$  SD (n = 5) (**B**). Different lowercase letters indicate significant differences among treatments (p < 0.05).



**Figure 5.** Effects of FR light and/or decapitation on the endogenous hormone contents (**A**) and related gene expression (**B**–**E**) in cucumber seedlings. The data are the mean  $\pm$  SD (n = 3). Different lowercase letters indicate significant differences among treatments (p < 0.05).

# 3.4. The Inhibitory Effect of FR Light on the Branch Elongation of Cucumber Seedlings Was Related to CTK

We investigated the effect of exogenous 6-BA on lateral branch elongation in cucumber seedlings under FR light to elucidate the relationship between CTK and lateral branch elongation in cucumber seedlings. Consistent with the expected results, exogenous 6-BA eliminated the inhibitory effect of FR light on lateral branch elongation in cucumber seedlings (Figure 6). Exogenous 6-BA upregulated the expression of the CTK synthetic gene, *LOG2*, to a certain extent and significantly upregulated the expression of *IPTs* (Csa7G253720 and Csa5G609740) (Figure 7B,C). Interestingly, compared with those in the FR light group, the expression of most of the CTK hydrolytic genes, such as *CKXs*, was significantly upregulated (Figure 7D), and the Zeatin content increased by 32.46% (Figure 7A). These results further indicated that FR light inhibited lateral branch elongation by regulating CTK levels.



**Figure 6.** Effects of FR light and/or 6-BA on the elongating lateral bud of cucumber seedlings (**A**). The data are the mean  $\pm$  SD (n = 5) (**B**). Different lowercase letters indicate significant differences among treatments (p < 0.05).



Figure 7. Cont.



**Figure 7.** Effects of FR light and/or 6-BA on the endogenous hormone contents (**A**) and related gene expression (**B**–**D**) in cucumber seedlings. The data are the mean  $\pm$  SD (n = 3). Different lowercase letters indicate significant differences among treatments (p < 0.05).

#### 4. Discussion

In recent years, light has been recognized as a signal to regulate lateral branch elongation. For instance, increasing photosynthetically active radiation (PAR) usually promotes an increase in the number of lateral branches [10], and the addition of red light induces lateral branch elongation [13]. Conversely, the addition of blue or FR light inhibits lateral branch elongation [34–36]. The regulation of lateral branch elongation by light involves multiple mechanisms, including the expression levels of photoreceptors, endogenous hormones and their related genes. The *Cryptochrome 1* (*CRY1*) gene mediates the response to blue light [34], and the *phyB* gene mediates the response to red/FR light [6] to regulate lateral branch elongation. At the same time, stem branch branching is antagonistically regulated by multiple hormones [including IAA, CTK, GA, ABA, strigolactone (SL) and brassinosteroid (BR)] that act through *branched1* (*BRC1*, a transcription repressor of bud outgrowth) [37,38].

The classical view is that IAA is considered to be the primary hormone controlling the elongation of lateral branches [39,40]. In the "polar transport of IAA" theory, IAA transport in the stem is a systematic signal, and the high flux of IAA in the main stem prevents the export of IAA from lateral shoots, resulting in the inhibition of lateral branch elongation [41]. Normally, PhyB attenuates IAA signalling to promote lateral branch elongation [42], whereas a decreased R/FR ratio inactivates phyB, which in turn enhances the inhibitory effect of IAA on lateral branches [35]. Correspondingly, PIFs can also bind to the promoters of IAA synthetic genes and response genes to regulate their expression directly [43–45]. Therefore, under low-R/FR conditions, the accumulation and signal transduction of IAA in plants increase rapidly [42]. Our results also showed that FR light promoted the accumulation of IAA in the top and basal internodes (Figure 3A,B); however, the expression levels of most IAA synthetic genes, such as YUCs, were significantly downregulated (Figure 3C,D), indicating that the response of IAA content and signal transduction in the main stem to FR light was weak and transient [46]. As expected, decapitation eliminated the inhibitory effect of FR light on lateral branch elongation (Figure 4), and the above results showed that the inhibition of IAA signalling affects the efficacy of FR light, thus suggesting that the inhibition of lateral branch elongation in cucumber seedlings by FR light is related to IAA. Interestingly, although decapitation suppressed the expression of YUCs (YUC7, YUC9 and YUC10b) in the basal internode (Figure 5B), the IAA levels increased (Figure 5A), which might be related to the output of IAA in the lateral branches after decapitation.

CTK is considered to be another important factor in the regulation of plant lateral branch development, which acts by decreasing the transcript levels of the *BRC1* gene and antagonizing the apical dominance of IAA and SL [10,47,48]. CTK levels are mainly related to its biosynthesis and hydrolysis [49]. In terms of synthesis, adenosine phosphate-

isopentenyltransferases (IPTs) are the catalytic enzymes involved in the first step of CTK biosynthesis and are also rate-limiting enzymes. The LONELY GUY enzyme (LOG) can directly convert inactive cytokinins (iPRMP and tZRMP) into biologically active free cytokinins (iP and tZ) [50]. In terms of hydrolysis, cytokinin oxidase (CKX) controls endogenous CTK levels (CKX catalyses the irreversible hydrolysis of CTK and plays an indicative role in monitoring CTK levels) [51]. The results showed that FR light reduced the accumulation of Zeatin in internodes (Figure 3A,B); accordingly, the relative expression levels of most of its synthesized genes, namely *IPTs*, were significantly downregulated (Figure 3G,H). However, most of the hydrolysed genes, such as CKXs, were downregulated, except for Csa4G343590, suggesting that the decrease in Zeatin levels was mainly related to the inhibition of synthesis. In addition, IAA in the polar transport stream also inhibits the biosynthesis of CTK by downregulating the expression of *IPTs* [52,53]. Undoubtedly, decapitation upregulated the expression of most IPTs and downregulated the expression of most CKXs (Figure 5D,E), thereby promoting the accumulation of Zeatin in basal internodes (Figure 5A). As stated in the second messenger model, CTK acts downstream of IAA to control lateral branch elongation; in this model, the localized synthesis of CTK in stem nodes is sufficient to promote lateral branch elongation. Interestingly, decapitation promoted Zeatin accumulation and subsequently promoted IAA accumulation in basal internodes (Figure 5A). Cao et al. [22] also reported that after decapitation, the rate of CTK accumulation in basal internodes was much higher than the rate of IAA depletion, suggesting that the depletion of IAA induced by decapitation was not the initial signal that triggered CTK accumulation. Next, we utilized exogenous 6-BA treatment to verify the interactions between FR light and CTK. Similar to what was observed with the decapitation treatment, in the FR light treatment, 6-BA promoted the elongation of lateral branches (Figure 6), which may be related to the application of exogenous 6-BA to promote the biosynthesis of endogenous CTK [54]. Our results also showed that exogenous 6-BA supply at the stem nodes directly promoted the accumulation of endogenous Zeatin in the internodes (Figure 7A), which subsequently induced the negative feedback regulation of CTK biosynthesis, leading to an increase in the expression level of the CTK hydrolytic genes, such as CKXs, (Figure 7D). In addition, CTK can activate the expression of the cytokinin receptor AHK4 [55], upregulate sucrose convertase [56] and promote the cell cycle [57]. In conclusion, high levels of CTK inhibit IAA activity, subsequently inducing lateral branch elongation.

In addition to IAA and CTK, other hormones may also play a role in regulating lateral branch elongation. SL functions as an inhibitor of lateral branch elongation by acting on BRC1 to promote its high expression, thereby enhancing the inhibitory effect of IAA on lateral branch elongation [58]. BR receives CTK signal and activates the BZR1 transcription factor to inhibit the transcript levels of the BRC1 gene, thereby promoting lateral branch elongation [28]. In addition to hormones, sugars have also been demonstrated to have a signalling functions in the growth and development of lateral branches. The provision of exogenous sucrose or its derivative hexose (glucose and fructose) has been found to inhibit the transcript of the BRC1 gene, which in turn promotes the elongation of lateral branches [56,59]. Furthermore, there exists a complex interplay between sugar and hormonal pathways. The growth of lateral branches is regulated by the antagonistic coupling of sugar and IAA levels. In vitro experiments have shown that the provision of exogenous sucrose partially counteracts the inhibitory effect of IAA and reduces endogenous IAA levels [59,60]. Additionally, the elongation of lateral branches induced by exogenous sucrose has been associated with the synthesis of CTK and the downregulation of the SL signalling gene (MAX2) [56]. These results show that other hormones and signalling molecules (including SL, BR and sugar), interact with IAA and CTK and regulate lateral elongation. Accordingly, it has been reported that SL, BR and ABA are involved in the regulation of lateral branch elongation by FR light [25,36]. Then, do they (SL, BR, ABA and sugar) also mediate the FR light-regulated lateral elongation of cucumber seedlings? If so, how they mediate this process and their interaction networks with IAA and CTK remain to be further studied.

#### 5. Conclusions

In summary, our study determined that FR light regulates lateral branch elongation in cucumber seedlings by promoting IAA signalling and inhibiting CTK biosynthesis (e.g., by reducing the transcriptional level of *IPTs*) to enhance apical dominance. However, other phytohormones (e.g., BR and SL) and signalling molecules (e.g., sugar) may also contribute to the response to FR light in this process. Furthermore, there could be further interactions. In the future, we will focus on these potential aspects to carry out more detailed mechanistic research.

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