EFFECTS OF GIBBERELLINS AND FORCHLORFENURON ON RAPID CHLOROPHYLL FLUORESCENCE KINETICS CHARACTERISTICS IN BLUEBERRY LEAVES

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Abstract. Research on photosynthesis is critically important, as it is the primary means by which plants obtain energy and carbon sources, and it also forms the foundation of plant growth and development. However, studies on photosynthesis in blueberries adjusted with plant regulators require further exploration. This study examines the impact of Gibberellins (GA3) and forchlorfenuron (CPPU) on 8-year-old 'Northland' and 'Bonnie' blueberry varieties. The regulators were applied foliarly at concentrations 50+5, 50+10, 50+20, 100+5, 100+10, 100+20, 150+5, 150+10, 150+20 mg/L during flowering and fruit expansion, using water as a control. Results showed that 100+5 mg/L GA3+CPPU applied during fruit expansion significantly increased chlorophyll content in both varieties. 'Northland' and 'Bonnie' exhibited increases in chlorophyll a by 15.1% and 26.3%, chlorophyll b by 41.7% and 21.0%, and total chlorophyll by 16.0% and 43.4% over the control. This concentration also enhanced photochemical efficiency, decreasing relative variable fluorescence at the J (2 ms) and I (30 ms) steps and raising the quantum yield for electron transport by around 22%. The treatment amplified the number of active photosynthetic reaction centers, promoting photosynthetic efficiency. This study offers valuable insights into optimizing blueberry cultivation through targeted use of plant growth regulators.
Keywords: *blueberry, hormones, chlorophyll, photosynthetic performance index*

Introduction

Blueberries belong to the genus *Vaccinium corymbosum* of the family Ericaceae (Pritts and Hancock, 1985) are small berry producing perennial shrubs, native to North America. Blueberries are known for their juicy flesh, unique taste, and rich nutritional content, offering high nutritional value, as well as significant economic value (Stote et al., 2020; Delpino et al., 2022). The cultivation of blueberries in China began in 1983 when Professor Hao at Jilin Agricultural University introduced blueberry seedlings from abroad, completing the cultivation of blueberry varieties in China (Wu, 2016). Over the years, extensive research has been conducted on blueberry variety selection, soil types required for cultivation, irrigation and fertilization methods, post-harvest storage and transportation, and processing methods of blueberry fruits (Wu et al., 2003, 2022; Dong and Jiang, 2015). Changbai Mountain is the birthplace of blueberry cultivation in China, holding a crucial position in the country's blueberry industry (Liu, 2023).

Plant hormones play a crucial role in plant growth and development. Studies have shown that key endogenous hormones such as Indole-3-acetic acid (IAA), Ethylene (ETH), Abscisic acid (ABA), Gibberellins (GA), forchlorfenuron (CPPU) and cytokinin (CTK) are essential in fruit growth and development, and there is a consensus on this

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(Yang et al., 2000; Li et al., 2024). As the fruit grows and develops, these hormones affect the fruit quality and their levels change (Wu et al., 2025). GA can promote cell division and elongation, participating in various growth and development stages of fruit trees. Specifically, GA3 is often used as a plant growth regulator in fruit tree cultivation. Studies have shown that GA3 can not only prevent chlorophyll degradation but also inhibit the expression of genes related to plant aging, thus delaying senescence (Rosenvasser et al., 2006). The use of CPPU in plants promotes chlorophyll synthesis and extends the plant's growth period. Research by Sun et al. (2021). found that spraying CPPU at the initial enlargement stage of sweet potatoes significantly affects chlorophyll content in the leaves. Chen et al. (2001) discovered that CPPU can inhibit chlorophyll degradation and delay plant senescence. Our previous study had concluded that GA and CPPU could improve blueberry quality (Ma et al., 2023).

Therefore, this study focuses on the main production area of blueberries in Jingyu, Changbai Mountain region, using 8-year-old half-highbush blueberry 'Northland' and highbush blueberry 'Bonnie' as experimental materials. By applying exogenous hormones, this research investigates the effects of GA3 and CPPU combination treatment on blueberry leaf chlorophyll content and rapid chlorophyll fluorescence induction kinetics curves. It is expected to achieve high-quality cultivation of blueberries, thus providing a reference for the further development of blueberry production and cultivation techniques.

Materials and methods

Experimental materials

This experiment selected 8-year-old half-highbush blueberry 'Northland' and highbush blueberry 'Bonnie' as experimental materials. These plants were chosen for their similar tree size, shape, and growth conditions, planted at a spacing of 2 x 2 meters, with consistent management levels and vigorous growth in the Jingyu area. The experiment was conducted from May to August in 2021 and 2022 at a blueberry farm located at the northwest gate entrance of Bai Mountain Lake Renyi Scenic Area in Jingyu County, Baishan City, Jilin Province. The geographical coordinates are between 126°30′E to 127°16′E longitude and 42°06′N to 42°48′N latitude, with an altitude of 597 meters. The annual average precipitation is 776.4 mm, the annual average temperature is 3.7℃, and the frost-free period is approximately 108 days. The soil is dark brown forest soil, classified as slightly acidic, with a pH value of 5.5 to 6.5 and a relatively high organic matter content (higher than 80 g/kg).

Experimental treatments

The GA3 treatment concentrations were 50, 100, and 150 mg/L, and the CPPU (purchased from Shanghai Ruiyong Biotechnology Co., Ltd., with a forchlorfenuron active ingredient content of 99%) treatment concentrations were 5, 10, and 20 mg/L. Using a completely randomized design, nine combination treatments of these two hormones were formed: GA3+CPPU combinations at concentrations of 50+5, 50+10, 50+20, 100+5, 100+10, 100+20, 150+5, 150+10, 150+20 mg/L (denoted as A1 to A9), with a clear water spray as the control (denoted as CK). During the fruit enlargement stage, the above combinations and clear water were sprayed on the entire plant until thoroughly wet and dripping, each plant requires 5 liters of solution. This was done at 7-

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day intervals for a total of two applications. Each treatment involved spraying 5 plants, and each plant's treatment concentration was marked with tags and marker pens. Ten days after spraying, three healthy, mature leaves from the 4th to 6th nodes on new shoots and fruiting branches were collected for measurements of related indicators. Five blueberry plants per treatment were used, with three replicates set up.

Chlorophyll Measurement Method: This method is an improved version combining the techniques of Gao et al. (2016). Remove the petiole and midrib of the leaves, cut the leaves into small pieces, and weigh 0.2 g. Then, place the leaves in a 1:1 mixture of anhydrous ethanol and acetone (10 mL) and perform dark treatment. Extract until the leaves turn white, and use a spectrophotometer to measure the absorbance values at wavelengths of 645 nm and 663 nm. Substitute these values into the formula to calculate the chlorophyll content. A663 means the absorbance in 663 nm, A645 means the absorbance in 645 nm.

Ca = 12.72 A663－2.59 A645

Cb = 22.88 A645－4.67 A663

CT = Ca + Cb = 20.29 A645 + 8.05 A663

Rapid chlorophyll fluorescence kinetic curve measurement method: Conduct the test immediately after taking the leaves. First, subject all treated blueberry leaves to dark adaptation for 30 minutes. Then measure the rapid chlorophyll fluorescence induction kinetics curve (OJIP curve) of the blueberry leaves using a portable plant efficiency analyzer, Pocket PEA (Hansatech, UK). Calculate the relevant fluorescence parameters based on the method of Strasser et al. (2000). The specific parameter meanings and calculation methods can be found in the attachment A1.

Chlorophyll measurement method

In this study, the chlorophyll content was determined using an improved method based on the techniques of Gao et al. (2016). The petioles and midribs were removed from the blueberry leaves, and the leaves were cut into small pieces and weighed (0.2 g). The leaf samples were then placed in a 1:1 mixture of anhydrous ethanol and acetone (10 mL) for dark adaptation. Extracts were obtained until the leaves turned completely white, and absorbance values were measured using a spectrophotometer at wavelengths of 645 nm and 663 nm. The chlorophyll content was calculated using the following formulas:

Ca = 12.72 A663 － 2.59 A645

Cb = 22.88 A645 － 4.67 A663

CT = Ca + Cb = 20.29 A645 + 8.05 A663

Note: A663 represents the absorbance at 663 nm, and A645 represents the absorbance at 645 nm.

Rapid chlorophyll fluorescence kinetic curve measurement method

In this study, the rapid chlorophyll fluorescence induction kinetic curve (OJIP curve) of blueberry leaves was measured using a portable plant efficiency analyzer, Pocket PEA (Hansatech, UK). The test was conducted immediately after leaf collection. First, all treated blueberry leaves were subjected to dark adaptation for 30 minutes. Subsequently, the OJIP curves were measured, and the relevant fluorescence parameters were calculated according to the method of Strasser et al. (2000). The specific meanings and calculation methods of these parameters are detailed in Appendix A1. The analysis of variance (ANOVA) with SPSS 21 was used to analyze the differences among different treatments.

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Results

Effect of GA3 and CPPU treatments on chlorophyll content in blueberry leaves

Photosynthesis in plants refers to the process by which green plants absorb light energy to convert carbon dioxide and water into organic matter while releasing oxygen. Chlorophyll plays a crucial role in this process, and can reflect the health status of the plant. According to *Table 1*, spraying different concentrations of GA3 + CPPU during the fruit expansion period has a certain impact on the chlorophyll content (Chl a, Chl b, and Chl a+b) in the leaves of 'Northland'. In treatments A1 and A4, the contents of Chl a, Chl b, and Chl a+b in blueberry leaves increased significantly. Compared to the control, treatment A1 increased these contents by 11.9%, 37.5%, and 11.5%, respectively, while treatment A4 increased them by 15.1%, 41.7%, and 16.0%. During the fruit expansion period, the GA3 + CPPU combination treatments resulted in significant differences in Chl b content in 'Northland' leaves compared to the control. When the GA3 concentration was 100mg/L (A4, A5 and A6), the Chl b content significantly increased compared to the control. However, as the CPPU concentration increased, the Chl b content gradually decreased. With the increase in GA3 concentration, the Chl a content in 'Northland' leaves first increased and then decreased, and the Chl a content significantly reduced when high concentrations of GA3 + CPPU (A9) were used. In treatment A9, the contents of Chl a and Chl a+b in 'Northland' leaves significantly decreased by 20.6% and 22.4%, respectively, compared to the control.

Table 1. *Effect of different concentration combination treatments on chlorophyll content of 'Northland' leaves during fruit expansion*

Treatment	Chlorophyll a $mg \cdot g^{-1}$	Chlorophyll b $mg \cdot g^{-1}$	Chlorophyll a+b $mg \cdot g^{-1}$
CK	1.26±0.04b	0.24±0.00e	1.56±0.06b
A1	1.41±0.02a	0.33±0.00ab	1.74±0.01a
A2	1.27±0.02b	0.31±0.01bc	1.60±0.02b
A3	1.31±0.01b	0.24±0.02d	1.62±0.01b
A4	1.45±0.03a	0.34±0.01a	1.81±0.08a
A5	1.28±0.02b	0.33±0.00ab	1.71±0.02a
A6	1.03±0.01c	0.30±0.00bc	1.65±0.03b
A7	1.33±0.04b	0.29±0.00c	1.29±0.04c
A8	1.25±0.04b	0.27±0.01d	1.61±0.00b

Note: Different lowercase letters indicated that the differences were significant with different treatments at the same time ($p<0.05$),as determined by Spearman's rank correlation coefficients

According to *Table 2*, spraying different concentrations of GA3 + CPPU during the fruit expansion period has a certain impact on the chlorophyll content (Chl a, Chl b, and Chl a+b) in the leaves of 'Bonnie'. Treatment A4 significantly increased the chlorophyll content (Chl a, Chl b, and Chl a+b) in blueberry leaves, with increases of 26.3%, 21.0%, and 43.4%, respectively, compared to the control. Under different concentrations of GA3 + CPPU combinations, the Chl a content in blueberry leaves showed significant differences compared to the control. When the CPPU concentration was higher than 10 mg/L, the Chl a content in blueberry leaves gradually decreased with an increase in CPPU concentration. With the GA3 concentration was higher than 100 mg/L, the Chl a content in blueberry leaves increased initially and then decreased as the GA3 concentration

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increased, and the Chl a content was higher in leaves treated with low CPPU concentrations than in those treated with high CPPU concentrations. The Chl b content in blueberry leaves decreased with increasing CPPU concentration, and when the CPPU reached a certain concentration in the treatment combinations, the Chl b content in blueberry leaves showed no significant difference compared to the control. In treatment A9, the Chl a+b content in blueberry leaves showed no significant difference compared to the control, while all other treatments were significantly higher than the control. In treatment A4, the Chl a+b content in blueberry leaves reached the highest level.

Table 2. *Effect of different concentration combination treatments on chlorophyll content of 'Bonnie' leaves during fruit expansion*

Treatment	Chlorophyll a $mg \cdot g^{-1}$	Chlorophyll b $mg \cdot g^{-1}$	Chlorophyll a+b $mg \cdot g^{-1}$
CK	1.33±0.00e	0.19±0.05d	1.52±0.02e
A1	1.44±0.00c	0.39±0.01b	1.75±0.00c
A2	1.42±0.02cd	0.37±0.01b	1.87±0.02b
A3	1.39±0.00d	0.33±0.02c	1.88±0.02b
A4	1.68±0.02a	0.50±0.01a	2.18±0.03a
A5	1.50±0.01b	0.40±0.02b	1.89±0.00b
A6	1.48±0.02b	0.39±0.01b	1.86±0.003b
A7	1.43±0.01c	0.31±0.00c	1.69±0.01d
A8	1.41±0.03cd	0.27±0.03d	1.73±0.02c

Note: Different lowercase letters indicated that the differences were significant with different treatments at the same time ($p<0.05$), as determined by Spearman's rank correlation coefficients

Effects of GA3 and CPPU treatments on the rapid chlorophyll fluorescence induction kinetics curve in blueberry leaves

As shown in *Figures 1* and *2*, spraying GA3 + CPPU during the fruit expansion period does not cause significant changes in the shape of the OJIP curves in the leaves of 'Northland' and 'Bonnie'. In treatments A4 and A5, the Fo in 'Northland' leaves decreased, while in treatments A4 and A6, the Fm value increased, though the differences were not significant compared to the control. Different concentrations of GA3 + CPPU combinations had no significant effect on the Fo of 'Bonnie' leaves, but the Fm value increased under treatment A4. Fo represents the fluorescence intensity when the PSII reaction center is fully open, so Fo increases when the photosystem reaction center is damaged. This indicates that spraying a certain concentration of GA3 + CPPU during the fruit expansion period has a promoting effect on chlorophyll fluorescence in 'Northland' leaves.

Effects of GA3 and CPPU treatments on Fv/Fm in blueberry leaves

As shown in *Figures 3* and *4*, during the fruit expansion period, treatments A1, A2, A3, A4, A5, and A6 resulted in a significant increase in Fv/Fm in 'Northland' leaves compared to the control. With a fixed GA3 concentration, the Fv/Fm of blueberry leaves showed a decreasing trend as the CPPU concentration increased. During the fruit expansion period, treatments A4, A5, and A6 resulted in a significant increase in Fv/Fm in 'Bonnie' leaves compared to the control, while other treatments showed no significant difference from the control. Fv/Fm represents the maximum quantum yield of PSII

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reaction centers under dark adaptation and can reflect the light energy conversion efficiency of plant leaves. This indicates that different concentrations of GA3 and CPPU combinations can effectively promote the light energy conversion and electron transfer efficiency of PSII reaction centers, thereby improving the photosynthetic performance of blueberry leaves.

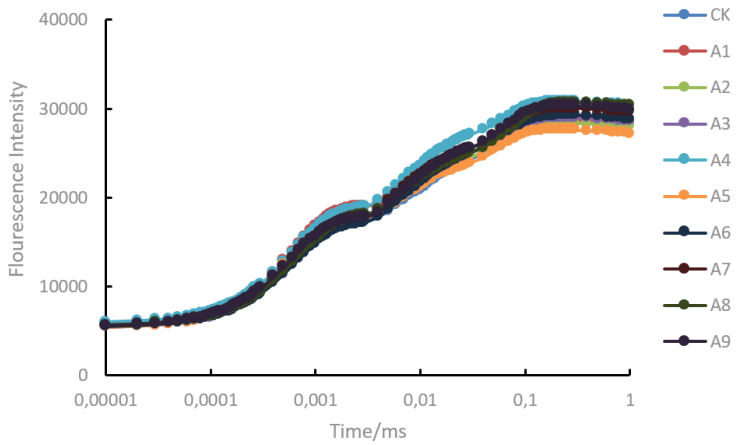

Figure 1. *Effect of different concentration combination treatment on the rapid chlorophyll fluorescence induction kinetic curve of 'Northland' leaves*

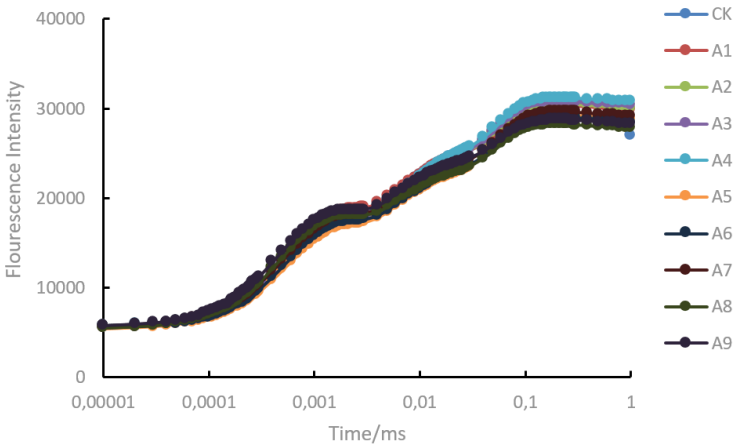

Figure 2. *Effect of different concentration combination treatment on the rapid chlorophyll fluorescence induction kinetic curve of 'Bonnie' leaves*

Effects of GA3 and CPPU treatments on Vj, Vi, and quantum yield in blueberry leaves

According to *Table 3*, spraying GA3 + CPPU during the fruit expansion period significantly reduced the Vj value in 'Northland' leaves under treatments A4, A5, and A6, with reductions of 22.0%, 16.9%, and 16.9%, respectively, compared to the control. The Vi value in 'Northland' leaves also significantly decreased under treatments A1, A3, A4, A5, and A6, with reductions of 4.8%, 6.0%, 7.1%, 7.1%, and 6.0%, respectively, compared to the control. The probability (Ψo) that electrons are transferred to electron acceptors beyond QA in the electron chain was significantly higher than the control under treatments A4, A5, and A6, with increases of 26.6%, 20.9%, and 18.6%, respectively.

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The quantum yield for electron transport (φEo) in the leaves was significantly higher than the control under treatments A4, A5, and A8, with increases of 22.2%, 16.7%, and 16.7%, respectively. However, spraying different concentrations of GA3 + CPPU during the fruit expansion period had no significant effect on the quantum ratio for heat dissipation (φDo) in 'Northland'.

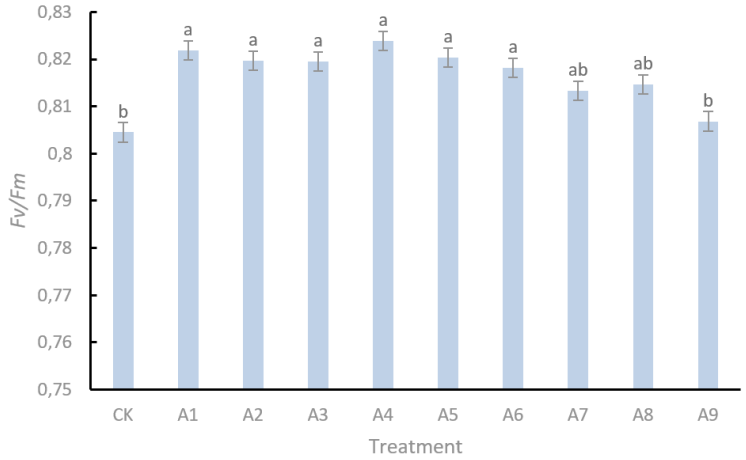

Figure 3. *Effect of different concentration combinations on Fv/Fm of leaves of 'Northland'*

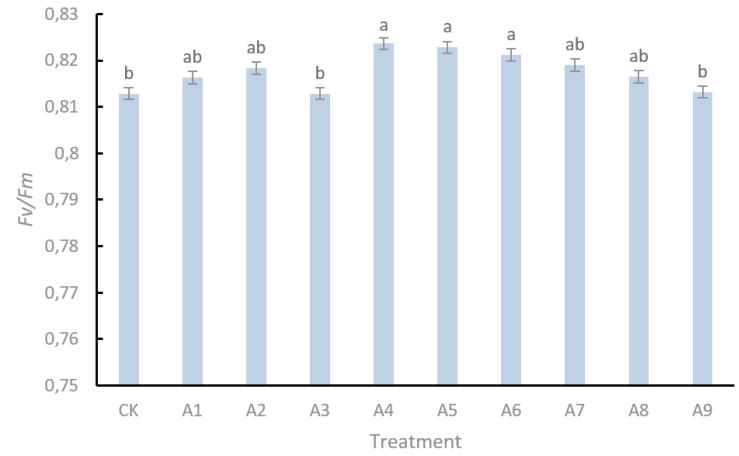

Figure 4. *Effect of different concentration combinations on Fv/Fm of leaves of 'Bonnie'*

Spraying GA3 + CPPU during the fruit expansion period significantly reduced the Vj value in 'Bonnie' leaves under treatments A4 and A6, with reductions of 16.9%, and 13.2%, respectively, compared to the control. The Vi value in 'Bonnie' leaves was lowest under treatments A1 and A4, with reductions of 6.2% and 7.4%, respectively, compared to the control. Other treatments showed slight decreases or increases, but none were significantly different from the control. After spraying different concentrations of GA3 + CPPU during the fruit expansion period on 'Bonnie', the φEo value in 'Bonnie' leaves increased, reaching the highest under treatment A4, with a significant increase of 21.1% compared to the control. However, spraying different concentrations of GA3 + CPPU during this period had no significant effect on the φDo value in 'Bonnie' except A1 and A5 (*Table 4*).

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Table 3. *Effect of different concentration combinations on Vj, Vi and quantum yield of 'Northland' leaves during fruit expansion*

Treatment	Vj	Vi	Ψo	φEo	φDo
CK	0.59±0.00a	0.84±0.0.1a	0.43±0.05b	0.36±0.03b	0.18±0.01a
A1	0.51±0.01ab	0.80±0.01b	0.48±0.03ab	0.40±0.02ab	0.17±0.02a
A2	0.51±0.01ab	0.83±0.01ab	0.50±0.02ab	0.41±0.02ab	0.17±0.02a
A3	0.53±0.02ab	0.79±0.01b	0.50±0.02ab	0.41±0.02ab	0.18±0.01a
A4	0.46±0.06b	0.78±0.02b	0.54±0.03a	0.44±0.02a	0.18±0.01a
A5	0.49±0.04b	0.78±0.02b	0.52±0.01a	0.42±0.01a	0.17±0.01a
A6	0.49±0.04b	0.79±0.01b	0.51±0.01a	0.41±0.02ab	0.18±0.00a
A7	0.55±0.03ab	0.83±0.01ab	0.50±0.02ab	0.41±0.02ab	0.18±0.01a
A8	0.50±0.02ab	0.83±0.01ab	0.50±0.02ab	0.42±0.01a	0.18±0.00a
A9	0.50±0.02ab	0.82±0.02ab	0.50±0.02ab	0.41±0.02ab	0.18±0.00a

Note: Different lowercase letters indicated that the differences were significant with different treatments at the same time ($p<0.05$), as determined by Spearman's rank correlation coefficients

Table 4. *Effect of different concentration combinations on Vj, Vi and quantum yield of 'Bonnie' leaves during fruit expansion*

Treatment	Vj	Vi	Ψo	φEo	φDo
CK	0.53±0.02ab	0.81±0.02ab	0.48±0.01cd	0.38±0.02c	0.18±0.01a
A1	0.51±0.01ab	0.76±0.03b	0.54±0.01ab	0.39±0.01c	0.17±0.01b
A2	0.51±0.01ab	0.80±0.01ab	0.49±0.00cd	0.41±0.01bc	0.18±0.00a
A3	0.49±0.01bc	0.79±0.02ab	0.50±0.01bc	0.41±0.01bc	0.18±0.01a
A4	0.44±0.03c	0.75±0.04b	0.56±0.05a	0.46±0.01a	0.18±0.02a
A5	0.49±0.01ab	0.79±0.02ab	0.47±0.02d	0.44±0.01ab	0.17±0.01b
A6	0.46±0.01c	0.79±0.02ab	0.54±0.01ab	0.44±0.01ab	0.17±0.01a
A7	0.55±0.05a	0.79±0.02ab	0.52±0.01ab	0.42±0.03ab	0.18±0.00a
A8	0.51±0.01ab	0.82±0.01a	0.52±0.01ab	0.43±0.02ab	0.18±0.01a
A9	0.49±0.01ab	0.82±0.01a	0.50±0.01bc	0.41±0.01bc	0.18±0.00a

Note: Different lowercase letters indicated that the differences were significant with different treatments at the same time ($p<0.05$), as determined by Spearman's rank correlation coefficients

Effects of GA3 and CPPU treatments on the energy flow specific activity parameters of PSII reaction centers in blueberry leaves

Through chlorophyll fluorescence induction kinetics curves, we can further analyze the specific activity of the PSII reaction centers in blueberry leaves. As shown in the table, during the fruit expansion period, changes in 'Northland' under the A4 treatment were significant. Compared to the control, the energy absorbed per reaction center (ABS/RC), the energy used for QA reduction per reaction center (TRo/RC), and the energy dissipated per reaction center (DIo/RC) in 'Northland' leaves were significantly reduced, while the

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number of active reaction centers per unit area (RC/CSo) significantly increased. The energy used for electron transport per reaction center (ETo/RC) did not show a significant difference compared to the control. Therefore, during the fruit expansion period, when 100+5 mg/L GA3+CPPU was applied, the energy dissipated per reaction center in blueberry leaves was significantly reduced, the number of active reaction centers increased, and the energy during electron transport increased, thereby enhancing the photosynthetic capacity of blueberry plants (*Table 5*).

Table 5. *Effect of different concentration combinations on the specific activity parameters of energy flow of PSII reaction center in 'Northland' leaves during fruit expansion*

Treatment	ABS/RC	TRo/RC	ETo/RC	DIo/RC	RC/CSo
CK	1.77±0.08a	1.45±0.05a	0.69±0.00a	0.31±0.00a	2992.53±238.35b
A1	1.73±0.04a	1.43±0.04a	0.72±0.03a	0.29±0.01abc	3053.64±187.28b
A2	1.65±0.05ab	1.38±0.00ab	0.69±0.0a	0.27±0.01bc	3431.39±196.56ab
A3	1.66±0.03ab	1.43±0.03a	0.68±0.01a	0.29±0.01abc	3462.57±236.87ab
A4	1.59±0.03b	1.22±0.1b	0.65±0.03a	0.25±0.01c	3798.73±252.63a
A5	1.77±0.08a	1.46±0.06a	0.67±0.02a	0.29±0.01abc	3003.48±196.56b
A6	1.69±0.01a	1.38±0.09ab	0.69±0.01a	0.30±0.00a	3146.92±309.66b
A7	1.73±0.04a	1.41±0.01a	0.66±0.04a	0.31±0.01a	3027.29±168.07b
A8	1.74±0.03a	1.43±0.03a	0.67±0.02a	0.31±0.01a	3221.47±212.61b
A9	1.68±0.01a	1.36±0.05ab	0.67±0.01a	0.30±0.01a	3216.21±243.54b

Note: Different lowercase letters indicated that the differences were significant with different treatments at the same time ($p<0.05$),as determined by Spearman's rank correlation coefficients

As shown in *Table 6*, under treatment A1, the ABS/RC, TRo/RC, and DIo/RC values in 'Bonnie' leaves significantly decreased by 15.1%, 9.6%, and 17.2%, respectively, compared to the control. The RC/CSo value significantly increased, reaching the highest level and increasing by 25.6 compared to the control. Under treatment A4, the ABS/RC, TRo/RC, and DIo/RC values in blueberry leaves significantly decreased by 9.0%, 12.5%, and 10.3%, respectively, compared to the control, and the TRo/RC value reached its lowest under A4. Other treatments did not show significant changes compared to the control. This indicates that during the fruit expansion period, spraying 50+5 mg/L and 100+5 mg/L GA3+CPPU significantly improved the distribution of energy per active center in 'Bonnie' leaves, increased electron transport efficiency, and thus enhanced the photosynthetic capacity of blueberry leaves.

Table 6. *Effect of different concentration combinations on the specific activity parameters of energy flow of PSII reaction center in 'Bonnie' leaves during fruit expansion*

Treatment	ABS/RC	TRo/RC	ETo/RC	DIo/RC	RC/CSo
CK	1.66±0.01a	1.36±0.07a	0.64±0.04ab	0.29±0.01a	3169.42±125.89c
A1	1.41±0.25b	1.23±0.01b	0.65±0.04ab	0.24±0.01c	3980.73±280.26a
A2	1.69±0.11a	1.34±0.06a	0.67±0.02ab	0.29±0.01a	3354.39±256.24bc
A3	1.66±0.10a	1.36±0.07a	0.69±0.01a	0.30±0.02a	3062.37±106.23c
A4	1.45±0.20b	1.19±0.04b	0.65±0.03ab	0.26±0.01bc	3532.84±177.18ab
A5	1.69±0.09a	1.41±0.08a	0.62±0.03b	0.27±0.01ab	3213.29±96.75c
A6	1.59±0.06a	1.30±0.05a	0.71±0.03a	0.28±0.01a	3246.21±89.42c
A7	1.64±0.01a	1.33±0.01a	0.65±0.03ab	0.28±0.00a	3301.27±26.77c

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A8	1.68±0.11a	1.45±0.10a	0.70±0.02a	0.30±0.02a	3011.69±125.46c
A9	1.69±0.11a	1.42±0.09a	0.70±0.02a	0.31±0.03a	3145.39±88.93c

Note: Different lowercase letters indicated that the differences were significant with different treatments at the same time ($p<0.05$), as determined by Spearman's rank correlation coefficients

Discussion

This experiment shows that spraying different concentrations of GA3+CPPU has varying effects on the chlorophyll in blueberry leaves. When 100+5 mg/L GA3+CPPU was applied, the chlorophyll content in blueberry leaves significantly increased, enhancing the photosynthetic efficiency of the plants. This is similar to the study by Zhu et al. (2014) on the effects of GA3 and CPPU on the yield and fruit quality of 'Guifei' mangoes in Hainan. Chlorophyll, when excited by light, exhibits fluorescence phenomena, which are closely related to the process of photosynthesis. In recent years, the chlorophyll fluorescence kinetics method has been widely applied in the physiological and ecological studies of plant photosynthesis. It allows for quick, sensitive, and non-destructive detection of plant photosynthesis (Feng et al., 2002), helping to identify the location and degree of damage to photosynthetic machinery under stress conditions (Yu and Qiao, 2021). Studies have shown that gibberellins can increase the maximum fluorescence (Fm) in millet while reducing the initial fluorescence (Fo). Li. (2018) demonstrated that exogenous gibberellins can increase leaf chlorophyll content and reduce the initial fluorescence (Fo) value, thereby enhancing the photosynthetic efficiency of melon leaves. This experiment found that spraying 50+5 mg/L and 100+5 mg/L GA3+CPPU during the fruit expansion period can reduce the Fo value and increase the Fm value in blueberry leaves. This indicates that exogenous GA3+CPPU can promote energy flow towards the photochemical part of the PSII reaction center, improving the photosynthetic performance of blueberry leaves.

Using the JIP-test for further analysis of the chlorophyll fluorescence transient process and quantitative analysis of photosynthetic parameters, it was found that during the fruit expansion period, treatment with 100+5 mg/L GA3+CPPU significantly reduced Vi and Vj in blueberry leaves. This significantly decreased the accumulation of QA and improved the ability of plastoquinone (PQ) to accept electrons, promoting electron transfer from QA to QB and allowing more electrons to be transported in the electron transport chain. This indicates that spraying GA3+CPPU can increase the number of electron acceptors on the PSII acceptor side, thereby enhancing electron transport capacity. The induction effect of cytokinins on genes involved in photosynthesis is often discussed (Kentaro et al., 2002). Li et al. (2014) results that spraying GA3 can significantly increase the Fv/Fm value in litchi leaves, enhancing the photochemical activity of chlorophyll fluorescence in the leaves. And Luo (2011) also indicates that applying CPPU can increase Fv/Fm in tomatoes, thereby improving the photosynthetic performance of crops. This study found that spraying different concentrations of GA3+CPPU during the fruit expansion period can enhance the Fv/Fm of blueberry leaves, similar to previous research findings. The ABS/RC parameter is an indicator of specific energy absorption flux and can be used to measure the effective antenna pigment size of active reaction centers (Oukarroum et al., 2015). Research has found that when ABS/CS and TRo/RC increase, it indicates an increase in antenna pigment size (Brestic et al., 2012). Peng et al. (2013) demonstrated that plant growth regulators can increase the proportion of open PSII reaction centers, improving the photosynthetic performance of

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tea shoot leaves. Other studies have shown that different CO_2 concentrations affect the ability of PSII reaction centers to either dissipate or capture light energy (Chang et al., 2021). This experiment revealed that spraying 100+5 mg/L GA3+CPPU during the fruit expansion period can significantly reduce the light energy absorbed per reaction center (ABS/RC), the energy captured per reaction center for QA reduction (TRo/RC), and the energy dissipated per reaction center (DIo/RC), while increasing the number of active reaction centers per unit area (RC/CSo). This improves the photosynthetic performance of blueberry leaves and promotes plant growth and development.

Conclusion

During the fruit expansion period, foliar application of 100+5 mg/L GA3+CPPU on 'Northland' and 'Bonnie' blueberry leaves resulted in a significant comprehensive improvement in chlorophyll content and chlorophyll fluorescence compared to other treatments. Compared to the control, chlorophyll a increased by 15.1% and 26.3%, chlorophyll b by 41.7% and 21.0%, and total chlorophyll (a+b) by 16.0% and 43.4%, respectively. The relative variable fluorescence at the J point decreased by 22.0% and 16.9%, while at the I point, it decreased by 7.1% and 7.4%, respectively. The quantum yield for electron transport increased by 22.2% and 21.1%, respectively. Additionally, the application of 100+5 mg/L GA3+CPPU increased the number of active reaction centers and the energy available for electron transport in blueberry leaves, thereby enhancing the photosynthetic capacity of the blueberry plants.

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APPENDIX

Table A1. *JIP- formula used for parameter determination and its meaning*

Parameters	Meanings
F_0	Minimum fluorescence intensity after dark adaptation
F_j	Fluorescence intensity at point J (2 ms)
F_i	Fluorescence intensity at point I (30 ms)
F_m	Maximum fluorescence intensity after dark adaptation
$F_v/F_m=(F_m-F_o)/F_m$	Maximum quantum yield of PSII after dark adaptation
$V_t\equiv(F_t-F_o)/(F_m-F_o)$	Relative variable fluorescence intensity at time t
$V_i\equiv(F_i-F_o)/(F_m-F_o)$	Relative variable fluorescence intensity at time i
$V_j\equiv(F_j-F_o)/(F_m-F_o)$	Relative variable fluorescence intensity at time j
$M_o\equiv4(F_{300\mu s}-F_o)/(F_m-F_o)$	Initial slope of the OJIP fluorescence induction curve
$\psi_o\equiv ET_o/TR_o=(1-V_j)$	Probability that an exciton captured by a reaction center will transfer an electron to electron acceptors beyond the quinone acceptor (QA) in the electron transport chain
$\varphi_{Eo}\equiv ET_o/ABS=[1-(F_o/F_m)]\psi_o$	Quantum yield for electron transport
$\varphi_{Do}\equiv1-\varphi_{Po}=(F_o/F_m)$	Quantum ratio for heat dissipation
$ABS/RC=M_o(1/V_j)(1/\varphi_{Po})$	Light energy absorbed per reaction center
$TR_o/RC=M_o(1/V_j)$	Energy absorbed by a reaction center used to reduce QA
$ET_o/RC=M_o(1/V_j)\psi_o$	Energy absorbed by a reaction center used for electron transport

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$DI_o/RC = ABS/RC - TR_o/RC$	Energy dissipated by a reaction center
$RC/CS_o = \varphi_{Po}(V_j/M_o)F_o$	Number of active reaction centers per unit area
$PI_{abs} \equiv (RC/ABS)[\varphi_{Po}/(1-\varphi_{Po})][\psi_o/(1-\psi_o)]$	Performance index based on absorbed light energy

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