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Effect of gibberellic acid application on plant growth attributes, return bloom, and fruit quality of rabbiteye blueberry

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1. Introduction

Synergistic interactions between plant biology and chemical biology highlights emerging frontiers in plant hormones that regulate plant growth and mediate plant interactions with their external environments. The first 'green revolution' for increased output of crops in Chinese agricultural production was to foster lodging resistant crop varieties using dwarf plant crops with irreversible defects in GA₃ synthesis and signal transduction. Since the early 1970s when China succeeded in the industrial production of gibberellin, GA₃ has been widely used in commercial horticultural cultivation as a growth regulator to improve plant growth. Exogenous application of GA₃ led to bigger shoots, leaves, stem and root by stimulating cell growth and division in many plants (Bose et al., 2013). As a result, stems and inter nodal lengths could be improved along with a better extensive root system in rabbiteye blueberry (*Vaccinium ashei*) by treating with GA₃.

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ABSTRACT

Gibberellic acid (GA₃) plays an important role in many plant growth and development processes. In order to evaluate the effect of GA₃ on plant growth, return bloom, and fruit quality of rabbiteye blueberry, three representative cultivars (Powder blue, Garden blue, Climax) were treated by 500 mg/L GA₃. Foliar application of GA₃ dramatically increased return bloom for three cultivars. Compared to the untreated control plants, 'Powder blue', 'Garden blue', and 'Climax' treated with GA3 increased inflorescences number by 54.3%, 69.7%, and 60.0% in the next year, respectively (p < 0.05). Foliar GA₃ application also increased leaf area, leaf fresh weight, leaf dry weight, chlorophyll content, the level of chlorophyll a and b, individual fruit weight, and number of fertile seeds. However fruit ripening and the harvest date of all three cultivars were delayed in the following year. GA₃ application did not significantly change length/diameter ratio of rabbiteye blueberry fruits. All the results suggest that GA₃ application is potentially promising for enhancing plant growth and fruit quality of rabbiteye blueberry with delayed ripening time.

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Rabbiteye blueberry (*V. ashei*) is a species of blueberry native to the southeastern United States. It is known to contain appreciable levels of phenolic compounds, which have high biological activity and may provide health benefits as dietary antioxidants (Castagnini et al., 2015; Lin et al., 2016; Shen et al., 2014). China recently became one of the leading countries in berry cultivation. Although consumption of wild berries was popular only in the northern region, berries are now grown in other large areas, especially in Zhejiang province in recognition of the well-known antioxidant properties.

Due to its good taste and health benefits, blueberry has been highly demanded by Chinese consumers, and its cultivation area in Zhejiang province has expanded to total 3333.3 ha in 2013. About ten rabbiteye blueberry cultivars are commercially grown in Zhejiang Province. Rabbiteye blueberry has lower winter chilling requirement, higher yield and easier storage, harvesting, shipping and handling than highbush blueberry (*Vaccinium corymbosum* L.) cultivars (Silva et al., 2005). To date rabbiteye blueberry has become an important economic crop in Zhejiang province of China.

The phenological pattern of rabbiteye blueberry has been described in the literature (Kim et al., 2011). Rabbiteye blueberry begins its phenological cycle at the end of winter or at the beginning of spring accompanied by the development of buds. It opens reproductive and vegetative buds almost simultaneously, and so







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development of dolichoblasts, flower bud formation and flowering occur in a short spring period during which there is a considerable overlapping stage of development. Flowering starts in the middle of March and the development of dolichoblasts usually takes place later, towards the end of flowering or when it has totally finished. Fruit development starts shortly after pollination and remains small for about 10–11 weeks, and most of the fruit growth is resumed approximately at the end of vegetative growth, reaching its final size in about 50 days. This phenophase sequencing has been widely observed in different individuals, populations and years in the study area.

Like other berry fruits, certain rabbiteye blueberries ripen in a close period of time. However, successful production and marketing of this crop necessitates use of appropriate certain techniques to disperse harvest date. Previous experiments have indicated that various applications of GA₃ delay harvest date because they put off bud break in grape cultivars of 'Riesling' and 'Velteliner Grun', as well as some rootstock varieties (Williams and Ayars, 2005). Thus, GA₃ could be applicable to rabbiteye blueberries for this type of postponed culture strategy. The impact of GA₃ treatment on the cluster depends frequently on both the concentration and development stage when used. Later GA₃ application inhibits the formation of flowering and inflorescence primordial development, though GA₃ is required for the formation and induction of the inflorescence axis. For example, using 20 mg/L of GA₃ in bloom results in a significant decrease in fruit yield of Table grape because the subsequently increased flower abscission has a negative effect on successful fertilization (Baydar and Harmankaya, 2005). But in the case of wine grape, GA₃ treatment led to higher fruit quality with higher Brix and increased rate of berry skin/berry flesh (Teszlák et al., 2013).

To date the effect of GA₃ on phenological pattern, return bloom, fruit quality of rabbiteye blueberry cultivars remains unknown. Therefore, we evaluated the effect of GA₃ on phenological pattern of three rabbiteye blueberry cultivars widely grown in Zhejiang province of China. The present study had three objectives: implementing GA₃ application as a postponed culture strategy in existing rabbiteye blueberry orchards, with reference to defining an orchard's optimal harvest date; monitoring plant response to ensure that supplied GA₃ does not result in irreversible stress that leads to loss of production or fruit quality in the long term; determining whether fruit quality characteristics is influenced to better understand the physiological response of rabbiteye blueberry plants to GA₃ application regimes.

2. Materials and methods

2.1. Field condition, plant material and GA3 treatment

This study was carried out in 2013 using five-year-old rabbiteye blueberry plants (cv. Powder blue, Garden blue, and Climax) cultivated in Zhejiang province of China. All the plants were positioned at a distance of 2.0 m between rows and 1.5 m within the rows. Plants of 1.2 m height and 1.2 m width with similar growth vigor were selected as test materials. Soil properties were tested as pH 5.52, 22.3% organic matter, 1.45 g/kg available phosphorus, 1.86 g/kg available nitrogen, 4.08 g/kg available potassium. The plants were placed at yearly average temperature 15.8 °C, sunshine hours 1498.4 h, average humidity 76%, precipitation 1613.9 mm. All the plants were grown under the same environmental conditions with the same doses of irrigation, fertilization and phytosanitary treatments. A standard randomized block design with six replications was used. The plants to be tested were isolated with at least one untreated guard plant. Foliar GA₃ applications, along with the water treatment served as control, were performed by triple spray treatments with 500 mg/L GA₃ at 15 L/six plants in the morning,

starting from 30 DAH (days after harvest of fruit) at 5-day intervals between the sprays.

2.2. Measurements

Return bloom (indicated by number of inflorescence per plant and flower number per inflorescence) in GA₃-treated and untreated control plants was evaluated on February 25 and March 15, 2014, respectively. Leaf unfolding, full bloom stage, beginning of ripe fruit, and 50% fruit ripening were observed from February to August in 2014. Vegetative growth parameters (leaf fresh weight, leaf dry weight, leaf area, total chlorophyll, chlorophyll a, chlorophyll b) were determined in late June 2014. Fully ripening fruits were randomly selected from the plants on July 22, August 1, and August 7 in 2014, and they were then transferred to laboratory for quality determination of individual fruit weight, fertile seed count, length, diameter, L/D ratio, soluble solid concentration (SSC), titratable acidity (TA), superoxide dismutase (SOD) activity in peel and pulp on 50 berries per treatment.

2.3. Determination of leaf area, leaf fresh weight, dry weight, and chlorophyll content

One hundred mature healthy leaves of rabbiteye blueberry plants per treatment were selected in July and were divided into two groups. One was used for determination of leaf area, leaf fresh weight, and leaf dry weight. Another was used for measuring total chlorophyll, chlorophyll a and b content. Leaf area was measured using a LI-COR Li-3100 leaf area meter (LI-COR, Lincoln, NE, USA). Leaf dry weight was determined after drying for 24 h at 80 °C after leaf fresh weight was measured. Total chlorophyll, chlorophyll a and b were extracted with 80% acetone from the samples of fresh leaves and their contents were determined at 645 and 665 nm by the spectrophotometer as mg/100 g FW (Lichtenthaler, 1987).

2.4. Determination of SSC and TA in rabbiteye blueberry

SSC readings were collected on a sample of fresh fruit (100 berries per treatment) using a digital refractometer (PR-101, Cat. No. 3412, ATAGO, Japan). Measurement of TA was carried out using a digital fruit acidity analyzer (Model: GMK-708, GVK, South Korea).

2.5. Determination of SOD activity in rabbiteye blueberry

SOD activity was measured according to the method of Prochazkova et al. (2001). Ten milliliter extraction buffer (0.1 mol/L phosphate buffer, pH7.5, containing 0.5 mmol/L EDTA and 1 mmol/L ascorbic acid) was added to 0.5 g sample that was grinded under frozen condition. The homogenate was centrifuged at $15000 \times g$ for 15 min at 4 °C, and the supernatants were used for enzyme assays.

SOD activity was estimated by recording the decrease in optical density of nitro-blue tetrazolium (NBT) dye using a spectrophotometer (VIS7200A, China). Reaction mixture contained 13 mmol/L methionine, 25 mmol/L nitroblue tetrazolium chloride, 0.1 mmol/L EDTA, 50 mmol/L phosphate buffer (pH 7.8), 50 mmol/L sodium carbonate, and 0.1 mL enzyme extracts. Reaction was started by adding 0.2 mL of 2 μ mol/L riboflavine to a 3-mL reaction mixture and placing the tubes under 15 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme, which gave the maximal color, served as control. Reaction was stopped by switching off the light and putting the tubes into dark. A non-irradiated complete reaction mixture served as a blank. The absorbance was recorded at 560 nm, and one unit of enzyme activity was taken as the amount of enzyme,

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