CHILEAN JOURNAL of AGRICULTURAL RESEARCH

CHILEANJAR
CHILEAN JOURNAL of AGRICULTURAL RESEARCH

RESEARCH ARTICLE

Exogenous hormone application regulates dwarf mutant plant height in *Sophora davidii* (Franch.) Skeels by changing endogenous hormone levels

Wen-hui Xie[1], Xiao-Fu Sun[1], Li-Li Zhao[1*], Li-Juan Huang[1], and Pu-Chang Wang[2]

[1]College of Animal Science, Guizhou University, Guiyang 550025, China.
[2]Guizhou Normal University, School of Life Sciences, Guiyang 550001, China.
[*]Corresponding author (zhaolili_0508@163.com).

Received: 9 September 2022; Accepted: 8 November 2022; doi: 10.4067/S0718-58392023000200195

ABSTRACT

Dwarf mutants are valuable materials for investigating plant gene function and developing new crop varieties. In the present study, dwarf mutant (D-CK) and wild-type (W-CK) *Sophora davidii* (Franch.) Skeels plants foliarly sprayed with distilled water were used as controls, and dwarf mutants were treated with different concentrations of gibberellin A3 (GA3; 20, 40 and 60 mg L^{-1}), brassinosteroid (BR; 12.5, 25 and 50 mg L^{-1}); and auxin indole-3-acetic acid (IAA; 12.5, 25 and 50 mg L^{-1}) to study the changes in plant height at 20, 40 and 60 d after exogenous hormone spraying. The changes in endogenous contents of IAA, GA3, BR, abscisic acid (ABA), jasmonic acid (JA), and salicylic acid (SA) and IAA:ABA ratio in the dwarf mutant plants were also measured at 20, 40 and 60 d after exogenous hormone application. The dwarf mutants reverted to the W-CK height phenotype after 60 d of exogenous spraying with different concentrations of GA3 and BR (except 50 mg·L^{-1}), but after exogenous spraying with IAA, dwarf mutants did not revert to the W-CK height phenotype. Compared with D-CK plants, endogenous GA3, BR and IAA contents in dwarf mutant plants increased to some extent with exogenous spraying of GA3, BR and IAA. The results indicated that exogenous spraying of different concentrations of GA3 and BR regulated *S. davidii* plant height by promoting the secretion of endogenous GA3, BR, IAA and SA and inhibiting endogenous ABA secretion in dwarf mutant. The height of mutants was sensitive to exogenous GA3 and BR but not to exogenous IAA.

Key words: Dwarf mutant, hormones, plant height, sensitivity, *Sophora davidii*.

INTRODUCTION

Sophora davidii (Franch.) Skeels, a shrub in the legume family *Sophora*, can reach 2 m in height. It is resistant to drought and barrenness, is highly adaptable, has high nutritional quality and is an important tree species for ecological environment management, forage field establishment and improvement in the karst areas of Guizhou, China (Lin et al., 2019). The taller plants of wild-type *S. davidii* are not conducive to foraging or mechanized harvesting by domestic animals (cattle and sheep, etc.) Previously, we used ^{60}Co-rays for radiation mutagenesis of the dried seeds of *S. davidii* and screened a number of dwarf mutant plants from the progeny population, all of which exhibited slow plant stem growth and shortened internodes (Zhao et al., 2022). These dwarf mutants are important germplasm resources for *S. davidii* dwarf breeding and valuable genetic resources for studying the growth, development and hormonal regulation of the main stem of *S. davidii*. Therefore, studying the genetic basis of the *S. davidii* dwarf traits can help to artificially regulate *S. davidii* strains, which has practical implications for *S. davidii* dwarf breeding and production practices (Tomlinson et al., 2019).

Mutants are consistent with their wild-type genetic background and are important materials for crop genetic improvement and functional gene research (Wang et al., 2018; Wu et al., 2021). Plant height is influenced by internal genetic control as well as various hormones and external environmental factors (Tian et al., 2017). It has been shown that plant height is significantly correlated with gibberellin (GA) and brassinosteroid (BR) compared to other plant hormones with mostly positive regulation (Fernandez et al., 2009). Brassinosteroid is a steroid hormone found throughout the plant kingdom that is similar to animal steroid hormones (Tong and Chu, 2018); it can also promote cell growth by regulating cell division and elongation at lower concentrations (Clouse, 1996). Gibberellin is a cyclic diterpene compound that promotes stem elongation, and biologically active GA can lead to plant dwarfing when either its synthesis or signaling is blocked (Yamaguchi, 2008). Indole-3-acetic acid (IAA) is a naturally occurring form of growth hormone that controls various aspects of plant growth and development, including cell division, elongation and differentiation; leaf differentiation; and morphogenesis (Paciorek et al., 2005). Growth hormone promotes cell expansion, but high concentrations can also inhibit cell elongation (Liu et al., 2016).

To dissect the genetic basis of plant height in relation to hormone regulation, dwarf germplasms (e.g., rice and wheat plants) have been used by many studies for in-depth analyses (Peng et al., 1999; Suzuki et al., 2003). Regarding plant hormone regulation of plant height, dwarf mutants are generally classified into two categories: hormone-sensitive and hormone-insensitive. The hormone synthesis pathway is inhibited or blocked in hormone-sensitive mutants, resulting in a deficiency of endogenous active hormones in the plant body. The external application of the corresponding active hormone will significantly increase plant height to the normal plant level, e.g., under normal conditions and exogenous spraying of 40 mg L^{-1} gibberellin A1 on dwarf mutants of maize (*Zea mays* L.) and rice (*Oryza sativa* L.), the height phenotype of these dwarf mutants could recover to normal levels (Phinney, 1985). In contrast, hormone-insensitive plants are mainly hindered by hormone signaling and do not show significant changes in plant height in response to external hormone application (Best et al., 2016). Under normal conditions, exogenous gibberellin A3 (GA3) was applied during rice seed germination, and the results showed that rice stem elongation and α-amylase yield did not increase significantly, but its endogenous GA level was normal, indicating that they were GA-insensitive mutant rice (Mitsunaga et al., 1994). Harberd and Freeling (1989) also found GA3-insensitive maize plants. Researchers have identified several *Arabidopsis* dwarf mutants. Under normal conditions, the exogenous addition of different concentrations of BR had nonsignificant effect on the plant height of these mutants, but their endogenous BR levels are normal. Therefore, they believe that these *Arabidopsis* mutants are BR-insensitive mutants (Wang et al., 2016).

At present, studies on *S. davidii* in the karst region of Guizhou, China, have focused on the physiological response mechanisms of this plant to adversity stress (Wang et al., 2016; Zhao et al., 2021), and the relevant hormone sensitivity of the *S. davidii* dwarf germplasm has not been reported. Therefore, in this study, we used the wild-type *S. davidii* germplasm and the *S. davidii* dwarf mutant germplasm to determine which exogenous phytohormone the *S. davidii* dwarf mutant was sensitive to. The sensitivities of the *S. davidii* dwarf mutant to three different concentrations of exogenous hormones at 20, 40 and 60 d after foliar spraying were compared, and the effects of the three different concentrations of exogenous hormones on height of the *S. davidii* dwarf mutant were analyzed. These results shed light on the different physiological mechanisms that produce differences between the plant height mutants and the wild type and provide a valid pretheoretical basis for *S. davidii* breeding in the subtropical karst mountains of southwest Guizhou, China.

MATERIALS AND METHODS

Test material

Dwarf mutant plants (hereafter referred to as mutants) were screened from the M3 generation population after radiation mutagenesis of the dried seeds of *Sophora davidii* (Franch.) Skeels were irradiated with ^{60}Co-γ rays, and the wild-type *S. davidii* germplasm was grown for the same number of years under the same growth environment as the mutant plants. The plant hormones auxin (indole-3-acetic acid, IAA), gibberellin A3 (GA3) and brassinosteroid (BR) were purchased from Sigma (China).

Test of mutant strain height sensitivity to exogenous hormones

This experiment was conducted in 2019-2020 in the experimental greenhouse of the Department of Grass Science, College of Animal Science, Guizhou University (26°27′ N, 106°39′ E), where the room temperature was maintained at 23 ± 2 °C (daytime temperature 25 °C/nighttime temperature 21 °C, 16 h light/8 h dark, and 75% relative humidity). The pots used in this study were 30.5 cm in diameter and 25.3 cm high and contained 12 kg culture soil (30% humus+20% vermiculite+50% peat soil) obtained from local loam. Distilled water was used for the test.

The seeds of the *S. davidii* dwarf mutant and wild-type plants were soaked in warm water at 60 °C for 24 h and placed in Petri dishes for germination. After 7 d germination, seedlings of the same size were selected and transplanted to grow in pots. After 60 d growth in pots, exogenous hormone spraying was performed. Four treatments were set for each hormone, i.e., GA3 at 0 (CK), 20, 40, and 60 mg L^{-1}; IAA at 0 (CK), 12.5, 25.0, and 50.0 mg L^{-1}; and BR at 0 (CK), 12.5, 25.0, and 50.0 mg L^{-1}. The mutant (D-CK) and wild-type (W-CK) were sprayed with distilled water and used as controls. Isolation barriers were set up between different exogenous hormone treatments using plastic film to prevent interplot interactions.

Foliar spraying of exogenous hormones was carried out according to the experimental design protocol, and after the spraying experiment started, spraying was carried out once every 2 d at 09:00 h. A total of six sprays were applied within 12 d, and three replicates of each exogenous hormone concentration were set for each spraying with five seedlings in each replicate; i.e., a total of 15 plants were sprayed with each concentration of hormone, applying 150 mL of the corresponding hormone each time. All hormone solutions were first dissolved in a small amount of ethanol and then diluted with distilled water to the appropriate concentration. After the six spraying trials were completed, plant height and changes in the contents of various endogenous hormones, IAA, abscisic acid (ABA), BR, GA3, jasmonic acid (JA), and salicylic acid (SA), were examined and measured at 20 d intervals.

Plant height was determined as the distance from the base of the stem to the growing point of the stem tip of the *S. davidii* plant.

Extraction and analysis of endogenous plant hormones

These procedures were carried out according to Müller and Munné-Bosch (2011) with some modifications. Leaf primordia and growth points were taken from the mutant and wild-type plants, respectively. Five mixed plants were taken from each treatment and ground in liquid nitrogen, and then 1 mL 80% methanol was added. Samples were homogenized by vortexing for 10 s every 15 min for 2 h and then centrifuged at 9600 × g for 15 min. The supernatant was collected.

The standard compounds (purity ≥ 99%) IAA (CAS: 87-51-4), GA3 (CAS: 77-06-5), ABA (CAS: 14375-45-2), BR (CAS: 78821-43-9), SA (CAS: 69-72-7) and JA (CAS: 77026-92-7) were purchased from Sigma (China). These standards were dissolved in 80% methanol, and liquid chromatography-tandem mass spectrometry (LC–MS-MS) (Agilent Technologies 6460 Triple Quad LC/MS, Agilent Technologies, Santa Clara, California, USA) was used for their analysis; each standard was run five times under the same conditions as the extracted samples. An XBridgeTM C18 column was used (2.5 µm, 2.1 mm × 50 mm; Waters, Milford, Massachusetts, USA). Mobile phase A was the aqueous solution, and mobile phase B was methanol. The column temperature was set at 30 °C, and the flow rate was 0.2 mL min^{-1}. The mass spectra were acquired with an electrospray ionization source (ESI source) in negative mode. Indole-3-acetic acid, ABA, GA3, BR, SA and JA were detected in multiple reaction monitoring mode, and the peak areas of each hormone were compared with each standard curve to calculate the concentration of each endogenous hormone. Three replicates were performed for each treatment.

Statistical analysis

One-way ANOVA with the least significant difference (LSD) multiple range test ($P < 0.05$) was performed on the test data using SPSS 20.0 software (IBM, Armonk, New York, USA), and all values are reported as the mean ± standard error.

RESULTS

Effects of exogenous hormones on height of *S. davidii* dwarf mutants

As shown in Figure 1, there were some differences in height of *S. davidii* plants after spraying with different concentrations of GA3, IAA and BR. The effect of IAA on promoting an increase in height of dwarf mutant plants was nonsignificant or significantly different from the height of the D-CK group of dwarf mutants; however, the promotion of height increase caused by IAA was significantly lower than that of the wild-type control. The ability of GA3 to promote an increase in dwarf mutant plant height was significant after treatment with 20, 40 and 60 mg L^{-1} at 40 and 60 d. The promotion in growth of the dwarf mutant plants was also significant after exogenous spraying with 12.5 and 25 mg L^{-1} BR at 40 and 60 d.

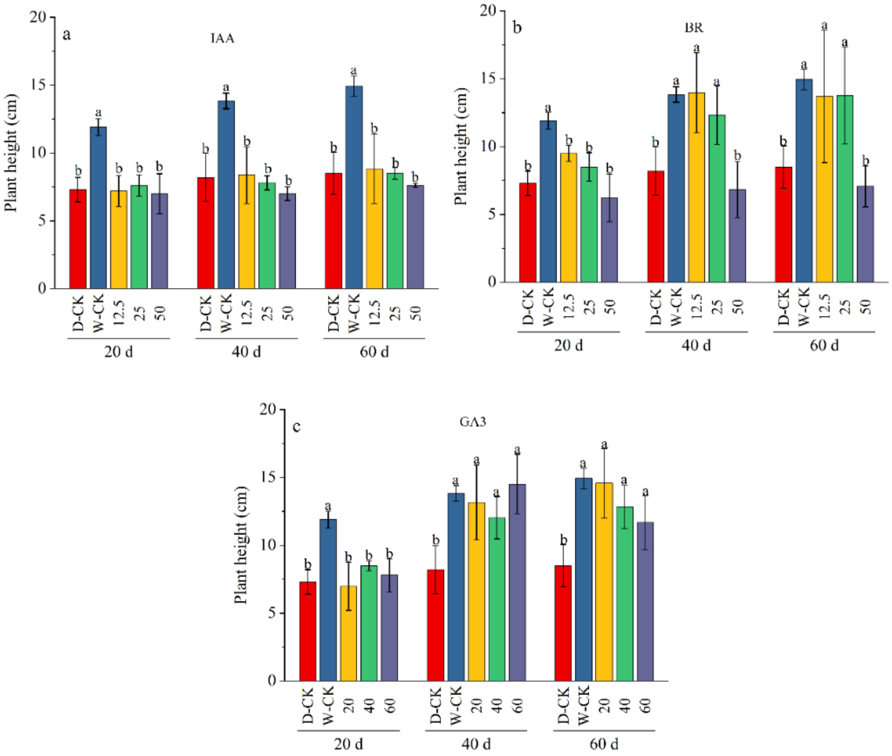

Figure 1. *Sophora davidii* dwarf mutant height (mean ± SD) after the exogenous spraying of different hormones. Change in mutant plant height after the exogenous spraying of auxin (IAA) (a); change in mutant plant height after the exogenous spraying of brassinosteroid (BR) (b); change in mutant plant height after the exogenous spraying of gibberellin A3 (GA3) (c). D-CK: Dwarf mutant control sprayed with distilled water; W-CK: wild-type control sprayed with distilled water; 12.5, 25.0, 50.0 mg L^{-1} corresponding exogenous hormone treatment concentrations; 20, 40, 60 d after treatment. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

Effects of exogenous hormones on endogenous hormones involved in *S. davidii* dwarfing mutations

Endogenous IAA content in mutants. As shown in Figure 2, the IAA content in *S. davidii* dwarf mutant D-CK plants was significantly lower than that in W-CK plants, while IAA content in wild-type plants decreased slightly with growth time. When sprayed with 25 and 50 mg L^{-1} IAA, IAA contents were significantly higher than those in the dwarf mutant D-CK at 20 and 40 d. After spraying with 20, 40 and 60 mg L^{-1} GA3, IAA contents were significantly higher than those in dwarf mutant D-CK at 20, 40 and 60 d. After spraying with 12.5, 25 and 50 mg L^{-1} BR, IAA contents in the plants were significantly higher than those in dwarf mutant D-CK at 20, 40 and 60 d.

Endogenous ABA content in mutants. As shown in Figure 3, the ABA content in wild-type *S. davidii* plants showed an overall decreasing trend with plant growth. However, there was a different trend in the ABA contents in *S. davidii* dwarf mutant plants exogenously sprayed with different concentrations of GA3, IAA and BR. Compared with that in the dwarf mutant D-CK plants, spraying different concentrations of IAA, GA3 and BR reduced the ABA contents in the plants to different degrees (except at 60 d when 50 mg·L^{-1} BR was applied), and significant differences were reached at 20, 40 and 60 d (except at 40 d when 40 mg L^{-1} GA3 was used and at 60 d when 25 mg L^{-1} BR was employed). The ABA contents in dwarf mutants were significantly lower than those in the W-CK plants at 20 d following treatment with different concentrations of GA3, IAA and BR, at 40 d with different concentrations of BR, and at 60 d after the application of different concentrations of GA3 and IAA.

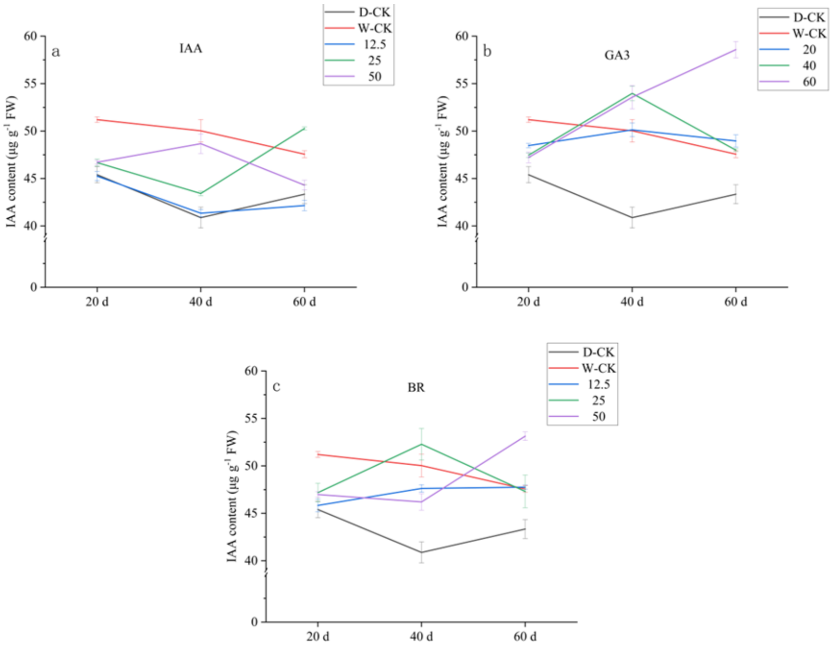

Figure 2. Effects of the exogenous spraying of different hormones on the endogenous auxin (IAA) contents in *Sophora davidii* dwarf mutants (mean ± SD). Change in the endogenous IAA contents in the mutants after exogenous spraying of IAA (a); change in the endogenous IAA contents in the mutants after exogenous spraying of gibberellin A3 (GA3) (b); change in endogenous IAA contents in the mutants after exogenous spraying of brassinosteroid (BR) (c).

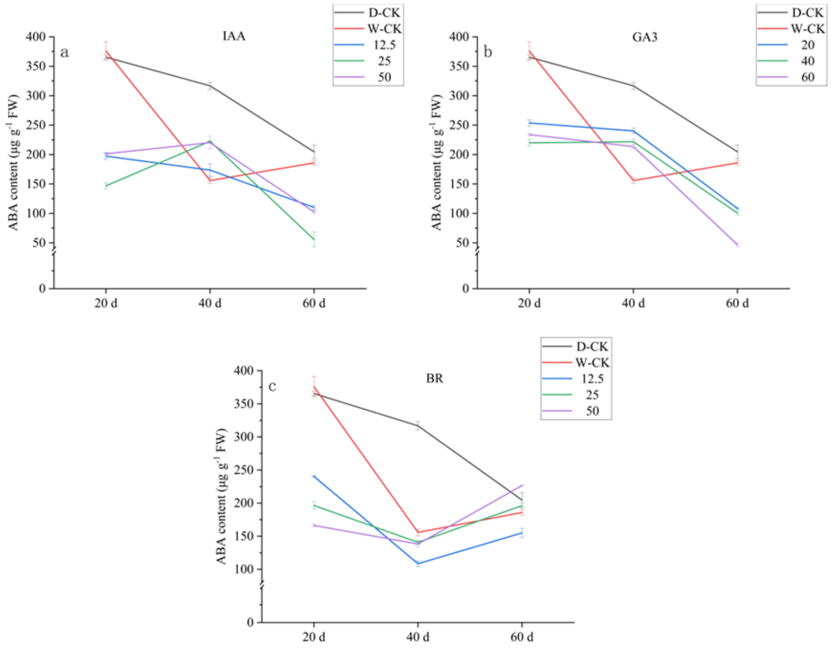

Figure 3. Effects of the exogenous spraying of different hormones on the endogenous abscisic acid (ABA) contents in *Sophora davidii* dwarf mutants (mean ± SD). Change in the endogenous ABA contents after exogenous spraying of auxin (IAA) (a); change in the endogenous ABA contents after exogenous spraying of gibberellin A3 (GA3) (b); change in the endogenous ABA contents after exogenous spraying of brassinosteroid (BR) (c).

Endogenous GA3 content in mutants. As shown in Figure 4, after the exogenous spraying of different concentrations of IAA, the GA3 contents in the *S. davidii* dwarf mutant plants were significantly higher than those in the D-CK dwarf mutants at 20, 40 and 60 d after spraying (except at 40 d when 12.5 and 25 mg L^{-1} IAA were applied). At 20 and 60 d, each group sprayed with IAA had significantly lower GA3 contents than the W-CK plants. The GA3 contents in the dwarf mutants were significantly higher than those in the D-CK mutants throughout the growth period when different concentrations of GA3 were sprayed, while the GA3 content in the dwarf mutant was significantly lower than that in the W-CK group throughout the growth period (except at 40 d when 40 mg L^{-1} GA3 was sprayed). After the exogenous spraying of 25 and 50 mg L^{-1} BR at 20 and 60 d, GA3 contents in the dwarf mutants were significantly higher than those in dwarf mutant D-CK and significantly lower than those in W-CK.

Endogenous BR content in mutants. As shown in Figure 5, after the exogenous spraying of different concentrations of IAA and GA3, BR contents in the *S. davidii* dwarf mutant plants were significantly higher than those in the D-CK plants at 20, 40 and 60 d after spraying (except at 20 d after 20 mg L^{-1} GA3 application). Additionally, BR contents in all of the treated mutants were significantly lower than those in the W-CK plants. After spraying different concentrations of BR, BR contents in the *S. davidii* dwarf mutant plants were significantly higher than those in the D-CK mutants at 20, 40 and 60 d after spraying (except for 25 mg L^{-1} BR treatment at 20 d after application). The endogenous BR contents in the D-CK mutant showed a decreasing and then increasing trend throughout the growth period, whereas the endogenous BR contents in the W-CK plants showed a continuous increasing trend.

Endogenous SA content in mutants. As shown in Figure 6, after exogenous spraying with different concentrations of IAA, there were nonsignificant differences between endogenous SA contents in *S. davidii* dwarf mutant plants and D-CK plants at 20 d after spraying. At 40 d after spraying, 25 mg L^{-1} IAA significantly increased endogenous SA content in the plants, whereas at 60 d, 25 and 50 mg L^{-1} IAA significantly decreased endogenous SA contents in the plants. After exogenous spraying of different concentrations of GA3, at 20 d, the endogenous SA contents in *S. davidii* dwarf mutant plants significantly increased, and at 40 d, spraying 20 and 40 mg L^{-1} GA3 significantly increased endogenous SA contents, but spraying 60 mg·L^{-1} GA3 significantly decreased the endogenous SA content. The SA contents in the plants sprayed with 20 and 40 mg L^{-1} GA3 were not significantly different from those in the D-CK plants. When different concentrations of BR were exogenously sprayed, the endogenous SA content in the dwarf mutant plants was significantly higher than that in the D-CK plants at 20 d. Moreover, at 40 d, 25 and 50 mg L^{-1} BR significantly increased endogenous SA contents in the dwarf plants, while the difference in SA content between the 12.5 mg·L^{-1} BR-treated group and D-CK group was nonsignificant. At 60 d, spraying 50 mg L^{-1} BR significantly increased the endogenous SA content in the dwarf plants, while differences in SA contents between 12.5 and 25 mg L^{-1} BR spraying and D-CK treatment were nonsignificant. The SA contents in the dwarf mutants were significantly lower than those in the W-CK plants at both 20 and 60 d.

Endogenous JA content in mutants. As shown in Figure 7, after exogenous spraying of 12.5 mg L^{-1} IAA, the endogenous JA content in the *S. davidii* dwarf mutant plants was significantly higher than that in the D-CK plants 20 d after spraying, and at 40 d, JA contents in dwarf mutant plants were significantly higher than those in D-CK plants after treatment with 12.5 and 50 mg L^{-1} IAA. At 60 d, 25 and 50 mg L^{-1} IAA significantly reduced the JA contents in the plants. On days 20 and 40 after spraying 20 and 40 mg L^{-1} GA3, JA contents in the plants significantly increased, while at 60 d, spraying different concentrations of GA3 significantly reduced the endogenous JA contents in the dwarf plants. On day 20 after spraying with 50 mg·L^{-1} BR, the JA content in the plants significantly increased; however, at 40 and 60 d, spraying with different concentrations of BR significantly decreased the JA contents in the dwarf plants. The endogenous JA content was significantly lower in the D-CK group than in the W-CK group after 20 d of growth, but the difference in JA content between these groups was nonsignificant after 40 d of growth.

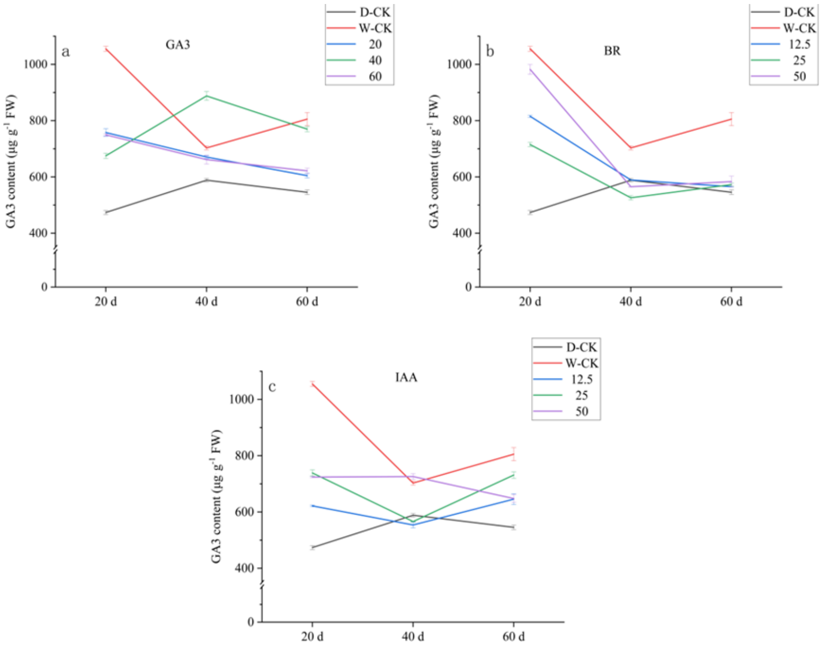

Figure 4. Effects of the exogenous spraying of different hormones on the endogenous gibberellin A3 (GA3) contents in *Sophora davidii* dwarf mutants (mean ± SD). Change in the endogenous GA3 contents in the mutants after exogenous spraying of auxin (IAA) (a); change in the endogenous GA3 contents in the mutants after exogenous spraying of GA3 (b); change in the endogenous GA3 contents in the mutants after exogenous spraying of brassinosteroid (BR) (c).

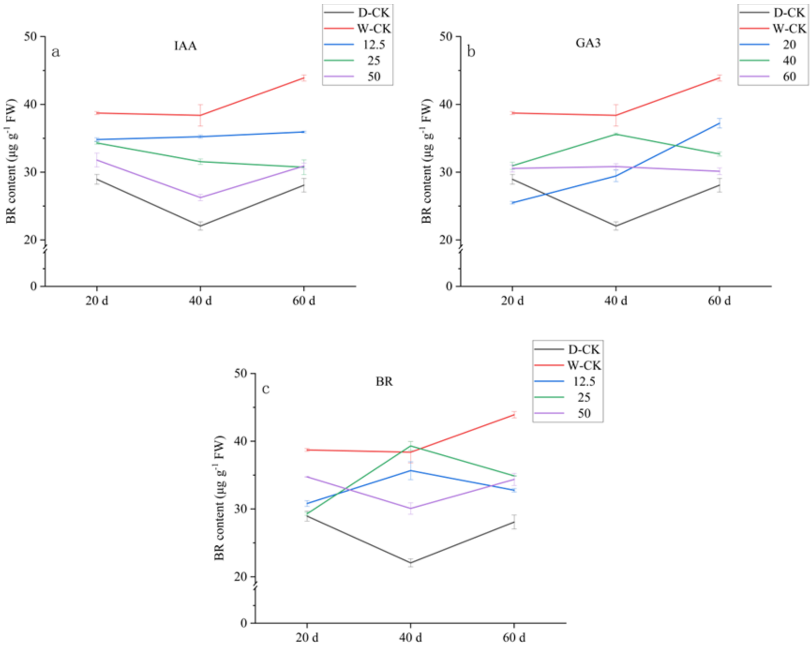

Figure 5. Effects of the exogenous spraying of different hormones on the endogenous brassinosteroid (BR) contents in *Sophora davidii* dwarf mutants (mean ± SD). Change in the endogenous BR contents in the mutants after exogenous spraying of auxin (IAA) (a); change in the endogenous BR contents in the mutants after exogenous spraying of gibberellin A3 (GA3) (b); change in the endogenous BR contents in the mutants after exogenous spraying of BR (c).

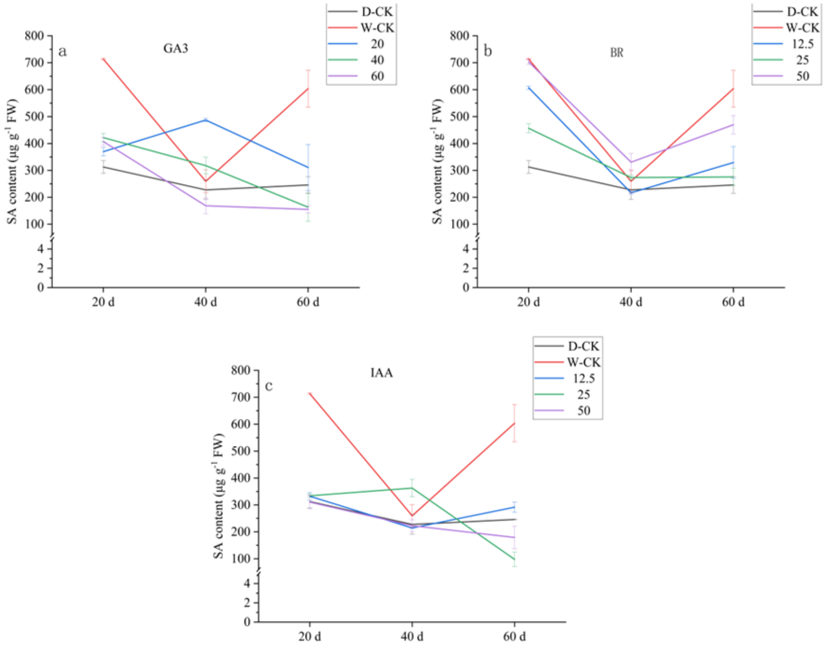

Figure 6. Effects of the exogenous spraying of different hormones on the endogenous salicylic acid (SA) contents in *Sophora davidii* dwarf mutants (mean ± SD). Change in the endogenous SA contents in the mutants after exogenous spraying of auxin (IAA) (a); change in the endogenous SA contents in the mutants after exogenous spraying of gibberellin A3 (GA3) (b); Change in the endogenous SA contents in the mutants after exogenous spraying of brassinosteroid (BR) (c).

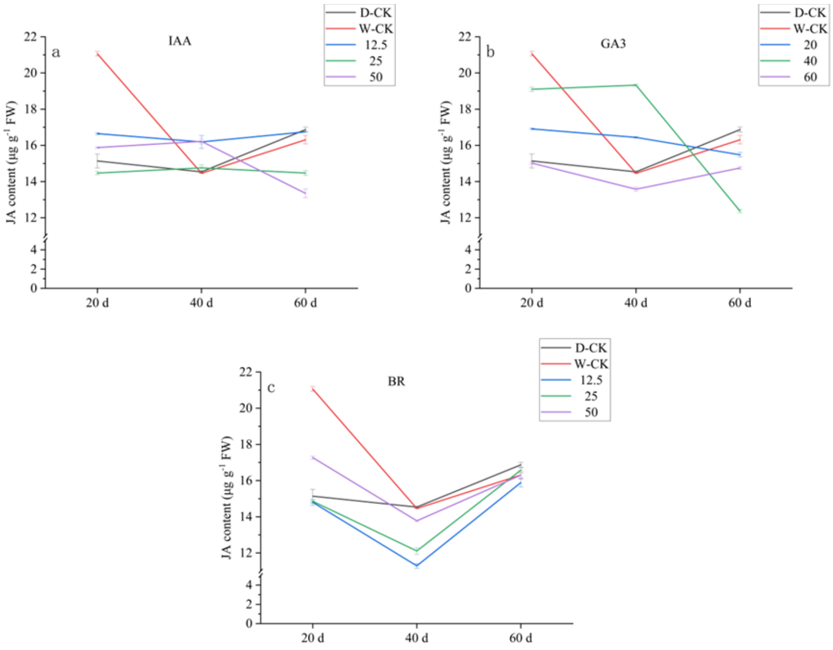

Figure 7. Effects of the exogenous spraying of different hormones on the endogenous jasmonic acid (JA) contents in *Sophora davidii* dwarf mutants (mean ± SD). Change in the endogenous JA contents in the mutants after exogenous spraying of auxin (IAA) (a); change in the endogenous JA contents in the mutants after exogenous spraying of gibberellin A3 (GA3) (b); change in the endogenous JA contents in the mutants after exogenous spraying of brassinosteroid (BR) (c).

Changes in the relative content of endogenous hormones in the mutant plants

As shown in Figure 1, different concentrations of GA3, IAA and BR were applied to *S. davidii* plants. At 20 d, the IAA:ABA ratios in the treated plants were significantly higher than those in D-CK plants for all treatments except GA-20 and BR-50, but this increase was not observed in W-CK plants. At 40 d, the highest IAA:ABA ratio among all treatments was found in the BR-12.5 group, and with the exceptions of IAA-25, GA-20 and GA-40 groups, the IAA:ABA ratios were significantly higher than that of D-CK for all treatments. At 60 d, exogenous spraying of different concentrations of GA3 and IAA significantly increased the IAA:ABA ratio, while spraying different concentrations of BR decreased the IAA:ABA ratio, although the difference was nonsignificant.

Correlation analysis of mutant plant height and endogenous hormone content

Correlation analysis showed that at 20 d, heights of the *S. davidii* dwarf mutants were highly significantly correlated with the endogenous ABA and IAA contents ($p < 0.01$), significantly correlated with the endogenous JA and SA contents ($p < 0.05$), and significantly negatively correlated with the IAA:ABA ratio. At 40 d, mutant plant height was highly significantly correlated with endogenous BR and IAA contents ($p < 0.01$).

DISCUSSION

Sophora davidii dwarf mutant plant height sensitivity to different exogenous hormones

The effects of exogenous hormones on plant height are mostly measured as the difference between the treated plant height and that of the control. Brassinosteroid, GA3 and IAA are closely related to plant dwarfism (Shahnejat-Bushehri et al., 2016; Xing et al., 2020). In this study, the promotion of plant height increase in mutant plants sprayed with different concentrations of IAA throughout the growth period was nonsignificant, nor was the plant height significantly different from the plants in the mutant D-CK group; however, heights of the IAA-treated plants were significantly lower than those of the W-CK plants (Table 1). This result indicates that spraying of the IAA solution did not restore plant height to normal in this dwarf germplasm plant and that the mutant plant height was not sensitive to exogenous IAA (Zanewich et al., 1991). Spraying GA3 and low and medium concentrations of BR (12.5 and 25 mg L^{-1}) promoted an increase in heights of dwarf mutant plants but not in the early stages of plant growth. This is probably because the internal physiological and biochemical plant pathways only affected the middle and late stages of plant growth, suggesting that dwarf plants also differ in their response to hormones at different reproductive stages (Can et al., 2006). The continuous external application of GA3 and low to medium concentrations of BR restored plant height to normal in this dwarf germplasm, and the mutant plant height was sensitive to exogenous GA3 and BR treatment.

Table 1. Effects of spraying different exogenous hormones, auxin (IAA), gibberellin A3 (GA3), and brassinosteroid (BR), on the relative contents of endogenous hormones in *Sophora davidii* dwarf mutants (mean ± SD). D-CK: Dwarf mutant control sprayed with distilled water; W-CK: wild-type control sprayed with distilled water; 12.5, 25, 50 mg L^{-1} corresponding exogenous hormone treatment concentrations; 20, 40, 60 d after treatment. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

	IAA:ABA ratio		
Treatments	20 DAT	40 DAT	60 DAT
D-CK	2.85 ± 0.01fg	2.65 ± 0.05gh	3.77 ± 0.15cd
W-CK	2.81 ± 0.08fg	4.52 ± 0.06b	4.34 ± 0.06bcd
IAA-12.5	3.15 ± 0.05de	3.20 ± 0.09ef	5.87 ± 0.20b
IAA-25	5.04 ± 0.06a	2.54 ± 0.01h	13.70 ± 2.18a
IAA-50	3.60 ± 0.01b	3.30 ± 0.08e	6.34 ± 0.03b
GA-20	2.99 ± 0.01ef	2.79 ± 0.02g	5.58 ± 0.07bc
GA-40	3.07 ± 0.06de	2.81 ± 0.0067g	5.43 ± 0.01bc
GA-60	3.20 ± 0.03d	3.10 ± 0.07f	12.27 ± 0.44a
BR-12.5	3.39 ± 0.01c	5.44 ± 0.13a	3.65 ± 0.10cd
BR-25	3.64 ± 0.04b	3.73 ± 0.02d	3.17 ± 0.01d
BR-50	2.69 ± 0.01g	4.10 ± 0.08c	2.58 ± 0.04d

In conclusion, this mutant may be a GA3- or BR-sensitive dwarf mutant germplasm or an IAA-insensitive dwarf mutant germplasm. Hormone-sensitive plants lack endogenous hormones due to the loss of plant hormone synthesis function, but spraying dwarf plants with the corresponding exogenous hormone can restore their height to a normal level (Feng et al., 2015). Hormone-insensitive plants mainly suffer from losses in hormone sensing or signaling functions, resulting in nonsignificant changes in plant height in response to external hormone application (Clouse et al., 1996; Davière and Achard, 2016). No similar studies related to hormone synthesis and signal transduction pathways in *S. davidii* have been reported, and further genetic localization and gene function cloning studies are needed to confirm the mutant type.

Relationship between endogenous hormones and plant height in *S. davidii* dwarf mutants

Exogenous hormones often trigger physiological effects in plants by altering endogenous hormone contents and thus affecting changes in plant morphology (Michael and Beringer, 1980). In this study, we found that after exogenous spraying of different concentrations of GA3, BR and IAA solutions, the endogenous GA3, BR and IAA contents in the plants increased to some extent throughout the experimental period compared to those in the D-CK group (except for 12.5 mg L^{-1} IAA treatment). This result is probably because plants are stimulated by hormonal signals after being subjected to exogenous hormone spraying, and the plants absorb the sprayed exogenous hormones to promote their growth and development (Shao et al., 2020). In addition, exogenous spraying of different concentrations of GA3 and BR up to 60 d showed an overall increasing trend in the endogenous IAA content in the plants throughout the growth period, and related studies have suggested that exogenous spraying of GA3 could effectively inhibit IAA oxidase (IAAO) activity to reduce the decomposition of IAA, resulting in a significant increase in the endogenous IAA content (Bagatharia and Chanda, 1998). Numerous studies have shown that taller plants have higher endogenous IAA levels than dwarf plants (Weijers et al., 2018), which is consistent with the results of the present study. However, the results of this study on the heights of *S. davidii* plants showed that directly spraying different concentrations of IAA did not significantly promote an increase in the height of mutant plants, and there were nonsignificant differences between any of the treatment groups compared with the plants in the mutant D-CK group. This finding indicates that although the active endogenous IAA content in hormone-insensitive dwarf plants increased after the external application of IAA and was higher than that of the wild-type at some time points, the IAA hormone signaling function was lost, and endogenous IAA could still not directly regulate the heights of the mutant plants to return to the wild-type phenotype (Liu et al., 2010).

The exogenous spraying of different concentrations of IAA, GA3 and BR solutions resulted in higher endogenous BR contents in the mutants than in D-CK plants, which, together with plant height growth performance, indicated that exogenous spraying of GA3 and BR indirectly regulated plant height by affecting the endogenous BR content. Brassinosteroid regulates the expression of genes related to GA metabolism and promotes its accumulation, which in turn stimulates the elongation of plant cells (Rademacher, 2000). Throughout the growth period, spraying different concentrations of exogenous IAA (with the exceptions of spraying medium and low concentrations of IAA at 20 d) and BR significantly increased the endogenous GA3 contents in plants compared to D-CK plants. Exogenously applied IAA inhibits GA3 inactivation and catalyzes the synthesis of GA1, encoded by the *Le* gene, while IAA promotes the production of LE mRNA and thus GA3 synthesis; therefore, exogenous spraying of IAA can significantly increase the endogenous GA3 content in plants (Petzold et al., 1992). However, after the exogenous spraying of IAA, the mutant endogenous GA3 content was lower than that in the W-CK plants, which indicated that the mutant endogenous hormone content reached a certain concentration to stimulate plant height recovery. Correlation analysis (Table 2) showed that the endogenous GA3, BR and IAA contents in *S. davidii* were positively correlated with plant height throughout the growth period, indicating that these three endogenous hormones promoted and increased the plant height of the *S. davidii* dwarf mutant.

In addition, the endogenous ABA content in D-CK was significantly higher than that in W-CK at both 40 and 60 d in this study. Abscisic acid is a hormone that performs a variety of functions during plant growth and development, often regulating water exchange by affecting stomatal conductance and inhibiting seed germination (Chen et al., 2022). It has been shown that dwarfism in apples (*Malus ×domestica* Borkh.) is due to an elevated endogenous ABA content (Lordan et al., 2017). The endogenous ABA contents in *S. davidii* plants after spraying different concentrations of IAA, GA3 and BR were lower than those in D-CK plants (with the exception of 60 d after exogenous application of 50 mg L^{-1} BR), which was due to the antagonistic effects of ABA with GA3, IAA and BR on plant growth and development. The elongation of internodes was found to be inhibited by ABA treatment in rice supplemented with different ABA concentrations during water immersion treatment (Hoffmann-Benning and Kende, 1992). The present study showed that exogenously spraying different concentrations of IAA, GA3 and BR inhibited the synthesis of endogenous ABA in the mutant and reduced the inhibitory effect of endogenous ABA on plant height.

Table 2. Correlation between endogenous hormone content and plant height in the dwarf mutant of *Sophora davidii*. *, **Significant correlations at the 0.05 and 0.01 levels ($P \leq 0.05$, $P \leq 0.01$), respectively. ABA: Abscisic acid; BR: brassinosteroid; GA3: gibberellin A3; IAA: indole-3-acetic acid auxin; JA: jasmonic acid; SA: salicylic acid.

	Time		
Hormone	20 d	40 d	60 d
ABA	-0.461**	-0.231	0.031
BR	0.340	0.484**	0.102
GA3	0.340	0.223	-0.099
IAA	0.467**	0.633**	0.098
JA	0.410*	-0.205	0.176
SA	0.399*	-0.022	0.265
IAA/ABA	-0.372*	-0.071	0.331

Jasmonic acid is a class of cyclopentane compounds present in plant tissues that have physiological effects similar to those of ABA during plant growth and development and show antagonistic effects with GA3, BR and IAA. In this experiment, there was no consistent change in the endogenous JA content in the *S. davidii* dwarf mutant after the plants were exogenously sprayed with different concentrations of IAA. However, the endogenous JA contents showed an overall decreasing trend when different concentrations of GA3 were applied, and the endogenous JA contents showed an overall decreasing trend followed by an increasing trend after treatment with different concentrations of BR. In this study, exogenous spraying of the plants with different concentrations of GA3 and BR inhibited the synthesis of endogenous JA, which decreased the inhibitory effect of JA on the plants and finally promoted plant growth. It has been suggested that JA can increase plant height and root growth by promoting related enzymatic activities in plants when they are subjected to adversity stress (Xue and Zhang, 2007). Correlation analysis (Table 2) showed that *S. davidii* plant height was significantly and positively correlated with endogenous JA content only at 20 d. The reason for this may be because plant growth and development are the result of interactions between multiple hormones, and further and deeper studies are needed. It has been suggested that in rice, jasmonic acid can conjugate with isoleucine (jasmonoyl-L-isoleucine, JA-Ile), and overexpression of certain enzymes accelerates the inactivation of JA-Ile, leading to a decrease in the effectiveness of JA signaling and ultimately to an increase in rice plant height. A previous study indicated that this is related to possible crosstalk between the plant endogenous GA signaling pathway and JA-Ile activity at certain times to control rice internode growth (Kurotani et al., 2015).

Salicylic acid is widely found in plants, directly or indirectly regulates physiological and biochemical activities and is an important signaling molecule for physiological processes such as growth and development, maturation and aging, and stress resistance (Raskin et al., 1990). In this study, in the early stage of plant growth (20 d), exogenous application of IAA, GA3 and BR caused an increase in the mutant plant endogenous SA content, but the height of the mutant did not differ significantly from the D-CK plant height. At 40 and 60 d after the exogenous spraying of BR solution, the dwarf mutants showed overall higher endogenous SA contents than the D-CK plants, which promoted an increase in mutant plant height, which is consistent with the findings of Kovácik et al. (2009). This result showed that only the external application of appropriate concentrations of exogenous hormones was more beneficial for increasing plant height. In the late growth period, the endogenous SA content in the plants was significantly higher than that in the D-CK plants after spraying only high concentrations of BR, indicating that the physiological response of the plants to low and medium concentrations of exogenous hormones was significantly weaker as the growth period of the plants was extended. Some studies (Kunkel and Brooks, 2002; Bari and Jones, 2009) have suggested that SA may also act synergistically with other phytohormones, such as ABA, JA, and GA3, to promote plant growth during certain periods and antagonistically to protect plants in some environments, although this needs to be investigated more in depth.

Relationship between relative content of endogenous hormones and mutant plant height

The interactions between and cross-effects of plant hormones can significantly affect plant growth processes (Zhang et al., 2015), and it has been suggested that the ratio of the endogenous content of IAA to ABA in plants is closely related to plant dwarfing (Tworkoski and Fazio, 2016). Previous investigators found that crabapple dwarfing was due to a decrease in the endogenous IAA content and changes in the IAA:ABA ratio under both growth conditions (Rudikovskii et al., 2019). Our experimental results showed that after external application of IAA, GA3 and BR, at 20 d, the IAA:ABA ratio in the treated plants were significantly higher than those in D-CK plants, and the IAA:ABA ratio was significantly and positively correlated with mutant plant height, which further confirmed that exogenous hormones affected endogenous hormones in dwarfing mutants and regulated plant height. It has been shown that ABA can increase the activity of IAA oxidase and thus reduce the high IAA activity in the dwarf mutant. Changes in endogenous hormone levels in plants can lead to disturbances in endogenous hormone metabolism and involve the collaborative expression of some key genes, which act together at the molecular and physiological metabolic levels to cause the appearance of the dwarf phenotype. In the present study, we only discussed and investigated the endogenous hormone differences in dwarf mutants, but the molecular mechanism of dwarfing and the related physiological and biochemical metabolic pathways of dwarfing need to be further investigated.

CONCLUSIONS

In this study, for the first time, by exogenously spraying different concentrations of auxin (indole-3-acetic acid, IAA), gibberellin A3 (GA3) and brassinosteroid (BR) on dwarf mutants of the high-quality forage shrub *Sophora davidii*, we found significant differences in the endogenous hormone content and plant height changes in response to exogenous IAA, GA3 and BR in dwarf mutant plants. The dwarf mutant plant height could not be restored to the normal wild-type phenotype after exogenous application of different concentrations of IAA, and the mutant endogenous IAA, GA3 and BR contents were lower than those of the wild-type throughout the experimental period. After exogenous application of different concentrations of GA3 and BR, the mutants showed increased plant growth and reverted to the wild-type phenotype by increasing the endogenous IAA, GA3, BR and salicylic acid (SA) contents and decreasing the endogenous abscisic acid (ABA) content. In addition, this study also showed that high concentrations of BR inhibited normal plant growth. The dwarf mutant in this study, which was a typical dwarf mutant of *S. davidii*, was significantly lower in height than the wild type, and the mutant was insensitive to exogenous IAA but sensitive to exogenous GA3 and BR. These results may provide preliminary information for the analysis of the genetic basis of *S. davidii* plant height in relation to hormonal regulation.

Author contributions

Methodology: W.X., L.Z. Software: X.S. Validation: L.H., P.W. Formal analysis: W.X. Investigation: X.S. Resources: L.Z. Data curation: W.X. Writing-original draft: W.X. Writing-review & editing: L.Z. Funding acquisition: L.Z. All co-authors reviewed the final version and approved the manuscript before submission.

Acknowledgments

This work was funded through projects of Science and Technology Project of Guizhou Province (QKHJC[2020]1Z026, QKHZC[2021]YB155, QKHPTRC[2021]5636), the National Natural Science Foundation of China (32260340, 32060391), and the Guizhou Province Graduate Research Fund (YJSKYJJ[2021]012).

References

Bagatharia, S.B., Chanda, S.V. 1998. Changes in peroxidase and IAA oxidase activities during cell elongation in *Phaseolus* hypocotyls. Acta Physiologiae Plantarum 20:9-13.

Bari, R., Jones, J.D. 2009. Role of plant hormones in plant defence responses. Plant Molecular Biology 69(4):473-488.

Best, N.B., Hartwig, T., Budka, J., Fujioka, S., Johal, G., Schulz, B., et al. 2016. *nana plant2* encodes a maize ortholog of the Arabidopsis brassinosteroid biosynthesis gene *DWARF1*, identifying developmental interactions between brassinosteroids and gibberellins. Plant Physiology 171(4):2633-2647.

Can, C., Wu, Y., Liu, B., Tong, J., Wu, J., Zhang, Y., et al. 2006. Expression of plant height and sensitivity to GA_3 of dominant semi-dwarfing gene in rice. Chinese Journal of Rice Science 20(1):25-30.

Chen, G., Zheng, D., Feng, N., Zhou, H., Mu, D., Liu, L., et al. 2022. Effects of exogenous salicylic acid and abscisic acid on growth, photosynthesis and antioxidant system of rice. Chilean Journal of Agricultural Research 82:21-32.

Clouse, S.D. 1996. Molecular genetic studies confirm the role of brassinosteroids in plant growth and development. The Plant Journal 10(1):1-8.

Clouse, S.D., Langford, M., McMorris, T.C. 1996. A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. Plant Physiology 111(3):671-678.

Davière, J.M., Achard, P. 2016. A pivotal role of DELLAs in regulating multiple hormone signals. Molecular Plant 9(1):10-20.

Feng, Y., Yin, Y., Fei, S. 2015. Down-regulation of *BdBRI1*, a putative brassinosteroid receptor gene produces a dwarf phenotype with enhanced drought tolerance in *Brachypodium distachyon*. Plant Science 234:163-173.

Fernandez, M.G.S., Becraft, P.W., Yin, Y., Lübberstedt, T. 2009. From dwarves to giants? Plant height manipulation for biomass yield. Trends in Plant Science 14(8):454-461.

Harberd, N.P., Freeling, M. 1989. Genetics of dominant gibberellin-insensitive dwarfism in maize. Genetics 121(4):827-838.

Hoffmann-Benning, S., Kende, H. 1992. On the role of abscisic acid and gibberellin in the regulation of growth in rice. Plant Physiology 99(3):1156-1161.

Kovácik, J., Grúz, J., Bačkor, M., Strnad, M., Repčák, M. 2009. Salicylic acid-induced changes to growth and phenolic metabolism in *Matricaria chamomilla* plants. Plant Cell Reports 28(1):135-143.

Kunkel, B.N., Brooks, D.M. 2002. Cross talk between signaling pathways in pathogen defense. Current Opinion in Plant Biology 5(4) 325-331.

Kurotani, K.I., Hattori, T., Takeda, S. 2015. Overexpression of a CYP94 family gene *CYP94C2b* increases internode length and plant height in rice. Plant Signaling & Behavior 10(7):e1046667.

Lin, B.B., Liu, X., Wu, S.Q., Zheng, H.X., Huo, K.K., Qi, S.S., et al. 2019. Phytochemicals content, antioxidant and antibacterial activities of *Sophora viciifolia*. Chemistry & Biodiversity 16(7):e1900080.

Liu, A., Chen, J., Li, R., Yi, J., Feng, Z. 2010. Response of plant height to exogenous hormones of dwarf cotton (*Gossypium hirsutum*) cv. Lu-ai No. 1. Cotton Science 22(3):248-253.

Liu, X., Wei, X., Sheng, Z., Jiao, G., Tang, S., Luo, J., et al. 2016. Polycomb protein OsFIE2 affects plant height and grain yield in rice. PLOS ONE 11(10):e0164748.

Lordan, J., Fazio, G., Francescatto, P., Robinson, T. 2017. Effects of apple (*Malus ×domestica*) rootstocks on scion performance and hormone concentration. Scientia Horticulturae 225:96-105.

Michael, G., Beringer, H. 1980. The role of hormones in yield formation. p. 85-116. In Physiological aspects of crop productivity. Proceedings of the 15th Colloquium of the International Potash Institute, Wageningen, The Netherlands. International Potash Institute, Zug, Switzerland.

Mitsunaga, S., Tashiro, T., Yamaguchi, J. 1994. Identification and characterization of gibberellin-insensitive mutants selected from among dwarf mutants of rice. Theoretical and Applied Genetics 87(6):705-712.

Müller, M., Munné-Bosch, S. 2011. Rapid and sensitive hormonal profiling of complex plant samples by liquid chromatography coupled to electrospray ionization tandem mass spectrometry. Plant Methods 7(1):1-11.

Paciorek, T., Zažímalová, E., Ruthardt, N., Petrášek, J., Stierhof, Y.D., Kleine-Vehn, J., et al. 2005. Auxin inhibits endocytosis and promotes its own efflux from cells. Nature 435(7046):1251-1256.

Peng, J., Richards, D.E., Hartley, N.M., Murphy, G.P., Devos, K.M., Flintham, J.E., et al. 1999. 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400(6741):256-261.

Petzold, U., Peschel, S., Dahse, I., Adam, G. 1992. Stimulation of source-applied ^{14}C-sucrose export in *Vicia faba* plants by brassinosteroids, GA_3 and IAA. Acta Botanica Neerlandica 41(4):469-479.

Phinney, B.O. 1985. Gibberellin A_1 dwarfism and shoot elongation in higher plants. Biologia Plantarum 27(2):172-179.

Rademacher, W. 2000. Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. Annual Review of Plant Biology 51(1):501-531.

Raskin, I., Skubatz, H., Tang, W., Meeuse, B.J. 1990. Salicylic acid levels in thermogenic and non-thermogenic plants. Annals of Botany 66(4):369-373.

Rudikovskii, A.V., Stolbicova, A.V., Rudikovskaya, E.G., Dudareva, L.V. 2019. Role of phytohormones in the formation of dwarf and tall Siberian crabapple (*Malus baccata* L. Borkh.) Zemdirbyste-Agriculture 106(2):167-172.

Shahnejat-Bushehri, S., Tarkowska, D., Sakuraba, Y., Balazadeh, S. 2016. *Arabidopsis* NAC transcription factor JUB1 regulates GA/BR metabolism and signalling. Nature Plants 2:16013.

Shao, X., Wu, S., Dou, T., Zhu, H., Hu, C., Huo, H., et al. 2020. Using CRISPR/Cas9 genome editing system to create *MaGA20ox2* gene-modified semi-dwarf banana. Plant Biotechnology Journal 18(1):17-19.

Suzuki, Y., Saso, K., Fujioka, S., Yoshida, S., Nitasaka, E., Nagata, S., et al. 2003. A dwarf mutant strain of *Pharbitis nil*, Uzukobito (*kobito*), has defective brassinosteroid biosynthesis. The Plant Journal 36(3):401-410.

Tian, X., Wen, W., Xie, L., Fu, L., Xu, D., Fu, C., et al. 2017. Molecular mapping of reduced plant height gene *Rht24* in bread wheat. Frontiers in Plant Science 8:1379.

Tomlinson, L., Yang, Y., Emenecker, R., Smoker, M., Taylor, J., Perkins, S., et al. 2019. Using CRISPR/Cas9 genome editing in tomato to create a gibberellin-responsive dominant dwarf DELLA allele. Plant Biotechnology Journal 17(1):132-140.

Tong, H., Chu, C. 2018. Functional specificities of brassinosteroid and potential utilization for crop improvement. Trends in Plant Science 23(11):1016-1028.

Tworkoski, T., Fazio, G. 2016. Hormone and growth interactions of scions and size-controlling rootstocks of young apple trees. Plant Growth Regulation 78(1):105-119.

Wang, B., Smith, S.M., Li, J. 2018. Genetic regulation of shoot architecture. Annual Review of Plant Biology 69:437-468.

Wang, H.H., Wang, P.C., Zhao, G., Sun, Q., Long, Z., Zhang, Y. 2016. Seed size and germination strategy of *Sophora davidii* under drought stress. Acta Ecologica Sinica 36(2):335-341.

Weijers, D., Nemhauser, J., Yang, Z. 2018. Auxin: small molecule, big impact. Journal of Experimental Botany 69(2):133-136.

Wu, S., Kong, L., Li, B., Tu, X., Guo, Z., Ye, J. 2021. Identification and characterization of CD152 in Nile tilapia (*Oreochromis niloticus*) playing roles in host immune response against bacterial pathogens. Aquaculture 545:737160.

Xing, M., Su, H., Liu, X., Yang, L., Zhang, Y., Wang, Y., et al. 2020. Morphological, transcriptomics and phytohormone analysis shed light on the development of a novel dwarf mutant of cabbage (*Brassica oleracea*). Plant Science 290:110283.

Xue, R., Zhang, B. 2007. Increased endogenous methyl jasmonate altered leaf and root development in transgenic soybean plants. Journal of Genetics and Genomics 34(4):339-346.

Yamaguchi, S. 2008. Gibberellin metabolism and its regulation. Annual Review of Plant Biology 59:225-251.

Zanewich, K.P., Rood, S.B., Southworth, C.E., Williams, P.H. 1991. Dwarf mutants of *Brassica*: Responses to applied gibberellins and gibberellin content. Journal of Plant Growth Regulation 10(1):121-127.

Zhang, H., An, H.S., Wang, Y., Zhang, X.Z., Han, Z.H. 2015. Low expression of *PIN* gene family members is involved in triggering the dwarfing effect in M9 interstem but not in M9 rootstock apple trees. Acta Physiologiae Plantarum 37(5):1-18.

Zhao, X., Sun, X.F., Zhao, L.L., Huang, L.J., Wang, P.C. 2022. Morphological, transcriptomic and metabolomic analyses of *Sophora davidii* mutants for plant height. BMC Plant Biology 22:144.

Zhao, X., Zhao, L.L., Huang, L.J., Sun, X.F., Wang, P.C. 2021. Response of growth characteristics and endogenous hormones of *Sophora davidii* to low-phosphorus stress. Acta Physiologiae Plantarum 43:118.