

## The relationship between habitat factors and the nutrient contents of wild *Allium victorialis* L. in the Changbai Mountains

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### Abstract

*Allium victorialis* L. (Family: Liliaceae) is an herb with nutritional and medicinal properties. In Jilin Province, China, *A. victorialis* is mainly distributed in the Changbai Mountains and grows in various habitat conditions. However, the relationship between habitat factors and the nutritional quality of *A. victorialis* in the Changbai Mountains has not yet been examined. We assessed the nutritional quality of five *A. victorialis* populations growing in five different habitats in the Changbai Mountains and analyzed the relationship between nutritional quality and habitat factors. *Allium victorialis* populations in this region were primarily found in the undergrowth at elevations above 500 m and within specific ranges of air temperature, air relative humidity, soil temperature, and soil water content. Among the habitat factors investigated, canopy density significantly affected several nutritional components of *A. victorialis*; however, elevation had a significant effect only on the total flavonoid content, and the vitamin C content was not strongly associated with the main habitat factors in this study. During germplasm selection and artificial cultivation, it is important to simulate the growth conditions of the original habitat. Our results provide useful information for site selection and environmental condition optimization for the artificial cultivation of *A. victorialis*.

**Keywords:** *Allium victorialis*; canopy density; elevation; habitat conditions; nutritional components

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### Introduction

*Allium victorialis* L. (Family Liliaceae) is a perennial herb that is widely distributed in the Northern Hemisphere, primarily in Europe, North America and Asia, including China, the Korean Peninsula, and Japan.

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Received: 04 May 2023. Received in revised form: 14 Jul 2023. Accepted: 23 Aug 2023. Published online: 31 Aug 2023.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

In China, this species is mainly found in Heilongjiang, Jilin, Liaoning, Inner Mongolia, Hebei, Shanxi, Shaanxi, and Hubei Provinces. Specifically, in Jilin Province, this species is mainly distributed in the Changbai Mountains. *Allium victorialis* is highly nutritious and has a long history of use as a wild edible herb and functional food (Jeong *et al.*, 2020). Several nutritional components have been identified and extracted from *A. victorialis*, including vitamin C (Golubkina *et al.*, 2010), flavonoids (Woo *et al.*, 2012), sulfur compounds (Nishimura *et al.*, 1988), and anthocyanins (Andersen and Fossen, 1995). These components have various health-related benefits, such as antioxidant (Jeong *et al.*, 2020) and antitumor (Lee *et al.*, 2001) effects. Therefore, research on the active compounds and nutritional components in *A. victorialis* is of considerable interest (Woo *et al.*, 2012; Yang *et al.*, 2014). However, most studies have focused on the extraction and isolation of the active compounds in *A. victorialis*, with limited attention given to the nutritional components.

The nutritional quality and productivity of various crops, such as peppers, strawberries, and figs, are influenced by habitat factors such as elevation, light, and temperature (Lee *et al.*, 2005; Maliníková *et al.*, 2013; Trad *et al.*, 2013). Therefore, it is important to determine the effects of changes in environmental factors on the nutritional quality of plants. *Allium victorialis* is characterized by a wide distribution, considerable habitat variation, and the presence of multiple subspecies or wild types in the same region. Biophysical factors such as latitude, elevation, and forest stand status influence the nutritional quality of this species (Yang *et al.*, 2014).

Although differences in nutritional quality among various *A. victorialis* populations have been extensively studied (Yang *et al.*, 2010, 2014), the relationship between the habitat conditions and nutritional quality of this species remains to be fully elucidated (Jeong *et al.*, 2020). To the best of our knowledge, there have been no studies on this relationship in the *A. victorialis* populations in the Changbai Mountains, although an understanding of this relationship is crucial for the successful cultivation and utilization of this species.

Therefore, the aim of the present study was to determine the relationship between habitat conditions and the nutritional quality of five *A. victorialis* populations from five different habitats in the Changbai Mountains. Additionally, we identified potential habitat factors that influence the nutritional quality of the *A. victorialis* populations in this region. The findings of this study could serve as a reference for the selection of sites and the optimization of environmental conditions for the artificial cultivation of *A. victorialis*.

## Materials and Methods

### *Plant materials and habitat survey*

Plantlets from the *A. victorialis* populations in the Changbai Mountains were collected from Dunhua city, Antu County, Wangqing County, and Dongfeng County; the populations in these locations are considered local by the local residents. The geographic coordinates, population size, elevation, and forest stand characteristics, including the forest type and canopy density, of the *A. victorialis* populations in each habitat were recorded by Feng *et al.* (2021). Each population was considered a sample. We also recorded the environmental variables at each site, including the air temperature, air relative humidity, soil water content (measured at a depth of 10 cm below the ground surface), and soil temperature, using a portable environmental meter (PC-4; Jinzhou Sunshine Meteorological Technology, Jinzhou, China). All environmental variables were measured three times. The details of the distribution and habitat conditions of each population are presented in Table S1.

### *Sample collection and preparation*

After the habitat survey in early to mid-May 2019, five healthy plantlets were collected from each population during the leaf unfolding stage, which is typically the main harvesting time of this species (*A. victorialis*) in this region. Sampling was conducted in accordance with the relevant laws and regulations in

China and with assistance from the local forestry authorities managing each habitat. All samples were deposited in the herbarium of the Jilin Provincial Academy of Forestry Sciences. The sampled plants were cleaned using distilled water. The five plantlets from each population were separated into three parts: the leaf, the pseudostem, and the bulb with roots. The leaves of these plantlets were stored at  $-80\text{ }^{\circ}\text{C}$  for subsequent analyses.

#### *Determination of nutrient contents*

##### Soluble sugar content

Soluble sugars were analyzed using the anthrone–sulfuric acid colorimetric method (Ebell, 1969; Wang *et al.*, 2011) with slight modifications. A leaf sample (0.2 g) from each population was extracted with distilled water in a boiling water bath for 30 min. The supernatants were filtered and transferred into a 25 ml volumetric flask. Then, a 0.5 ml aliquot was transferred into a graduated test tube and mixed with 0.5 ml of 2% anthrone reagent (prepared by dissolving 2 g of anthrone in 100 ml of ethyl acetate) and 5 ml of concentrated sulfuric acid. The absorbance of the sample was determined at 630 nm using a UV–VIS spectrophotometer (TU-1810, PGENERAL Instrument Co Ltd, Beijing, China).

##### Crude fiber content

Crude fiber was quantified using the anthrone–sulfuric acid colorimetric method (Viles and Silverman, 1949) with slight modifications. The residue of each sample after the extraction of the soluble sugar contents was digested in 60 ml of 60% (v/v) cold sulfuric acid in a cold-water bath for 15 to 30 min. After the extraction, the crude fiber solution was transferred to a 100 ml volumetric flask, and the solution was brought up to a measured volume with 60% cold sulfuric acid. This crude fiber solution was diluted to 50 ml with distilled water in a cool water bath, and the diluent was regarded as the aliquot. Then, a 2 ml aliquot was transferred into a graduated test tube and allowed to cool before adding 0.5 ml of 2% anthrone reagent and 5 ml of 60% (v/v) sulfuric acid. The extraction solution and reagents were intensively mixed and left to stand for 12 min. The crude fiber content was calculated from a standard curve based on the absorbance of the sample, which was determined at 620 nm using the TU-1810 UV–VIS spectrophotometer mentioned above.

##### Soluble protein content

The soluble protein content was measured using a modified Coomassie Brilliant Blue G-250 staining method described by Bradford (1976) and Hussain *et al.* (2017), with a slight modification described by Feng *et al.* (2021). A leaf sample (0.25 g) from each population was extracted in distilled water for 30 min at  $20\text{ }^{\circ}\text{C}$ . The extraction solution was centrifuged at  $5,000 \times g$  for 20 min, and the supernatant was decanted and remixed. The supernatant was used as the sample solution, and a 1 ml solution of each sample and 5 ml of Bradford dye reagent were mixed in a test tube. After 2 min, the absorbance was measured at 595 nm using the TU-1810 UV–VIS spectrophotometer mentioned above.

##### Vitamin C content

The vitamin C content was measured using the molybdenum blue colorimetry method described by Isaacs (1942), with some modifications described by Feng *et al.* (2021). A leaf sample (2.5 g) from each population was extracted with oxalic acid–EDTA reagent (prepared by dissolving 6.3 g of oxalic acid and 0.075 g of EDTA in a volume of 1 L with distilled water) before transferring it into a 25 ml volumetric flask. The extraction solution was filtered, and the supernatant was regarded as the aliquot. A 5 ml aliquot, 1 ml of metaphosphoric acid–acetic acid reagent (prepared by dissolving 15 g of metaphosphoric acid into 40 ml of acetic acid and diluting with acetic acid to 500 ml), 2 ml of 5% sulfuric acid, and 4 ml of ammonium molybdate reagent (prepared by dissolving 2 g of ammonium molybdate to 50 ml with distilled water) were mixed. The mixture was then transferred into a 50 ml volumetric flask and brought up to a final volume of 50 ml with

distilled water. Next, this mixture was kept at 30 °C for 20 min, and the absorbance value was measured at 705 nm using the TU-1810 UV–VIS spectrophotometer mentioned above.

#### β-Carotene content

The β-carotene content was measured using the method described by Singh *et al.* (2011) with slight modifications. A leaf sample (0.5 g) from each population was extracted with acetone. The extraction was continued until the residue became colorless. All extracts were pooled and transferred into a separating funnel. Then, 10 ml of petroleum ether (BP 55-60 °C) was added to the extracts, and the mixture was stirred thoroughly. The pigments were transferred into the petroleum ether phase by diluting the acetone with water. Then, the uniform volume was achieved by adding petroleum ether, and the absorbance was measured at 452 nm using the TU-1810 UV–VIS spectrophotometer mentioned above, with 3% acetone in petroleum ether as the blank.

#### Total flavonoid content

Total flavonoids were measured using the method described by Jiang *et al.* (2009), with slight modifications. A leaf sample (1.0 g) from each population was extracted twice with 80% ethanol. For each sample, the extracted solution was filtered, mixed, and evaporated. The supernatant was dissolved in 30% ethanol and diluted to the scale line of a 25 ml volumetric flask. Then, a 5 ml aliquot of this sample solution, 0.5 ml of 5% NaNO<sub>2</sub>, 0.3 ml of 0.1 g/ml Al(NO<sub>3</sub>)<sub>3</sub>, and 3 ml of 0.04 g/ml NaOH were mixed successively and transferred into a 20 ml graduated test tube. Finally, the well-mixed solution was diluted with distilled water to volume and steeped for 12 min. The absorbance was recorded at 510 nm using the TU-1810 UV–VIS spectrophotometer mentioned above.

#### *Statistical analysis*

The quality indicators were analyzed using one-way analysis of variance (ANOVA), and significant differences were assessed using Duncan's multiple-range test. For further statistical analysis, value assignment was performed for certain qualitative indicators (Table 1). After value assignment, Pearson correlation analysis was performed to determine the association between the quality indicators and habitat factors. Stepwise linear regression analysis was also used to assess the relationship between the quality characteristics and habitat factors. Statistical analyses were performed using SPSS v22.0 (SPSS Inc., Chicago, IL, USA), and statistical significance was defined as  $p < 0.05$ .

**Table 1.** Value assignment for the habitat factors and measured nutritional quality characteristics

Characteristic	Code	Representative values
Elevation	x1	1: ≥ 500 m; 2: < 500 m
Forest type	x2	1: Conifer–broadleaf forest; 2: Broadleaf Forest
Canopy density	x3	1: < 60%; 2: ≥ 60%
Air temperature (°C)	x4	Measured values
Air relative humidity (%)	x5	Measured values
Soil temperature (°C)	x6	Measured values
Soil water content (%)	x7	Measured values
Vitamin C content (mg·g <sup>-1</sup> )	y1	Measured values
Total flavonoid content (mg·g <sup>-1</sup> )	y2	Measured values
β-Carotene content (mg·g <sup>-1</sup> )	y3	Measured values
Soluble sugar content (mg·g <sup>-1</sup> )	y4	Measured values
Soluble protein content (mg·g <sup>-1</sup> )	y5	Measured values
Crude fiber content (mg·g <sup>-1</sup> )	y6	Measured values

## Results

### *Distribution and habitat conditions of the A. victorialis populations in the Changbai Mountains*

The *A. victorialis* populations in the Changbai Mountains were distributed across a wide geographical range, with latitude and longitude ranges of 42 to 43°N and 125 to 130°E, respectively (Table 2). This species was mainly found at elevations above 500 m, with most individuals growing at 600 to 700 m above sea level. However, certain populations were found at elevations as low as 400 m in Dongfeng. The *A. victorialis* populations analyzed in this study were mainly distributed in conifer–broadleaf forests in Dunhua and Wangqing and mixed broadleaf forests in Antu and Dongfeng. The canopy density of these forest types ranged from 30% to 70% (Table 2).

**Table 2.** Distribution and habitat conditions of the five *Allium victorialis* populations growing in the Changbai Mountains

Sample	Geographic coordinates <sup>a</sup>	Population size <sup>a</sup>	Elevation (m) <sup>a</sup>	Forest type	Canopy density (%)	Air temperature (°C)	Air relative humidity (%)	Soil temperature (°C)	Soil water content (%)
Dunhua A	43° 10' 27" N, 128° 02' 17" E	> 5,000 plantlets	619.5	Open forest, conifer–broadleaf forest	30	25.2	30.0	15.4	40.6
Dunhua B	43° 10' 27" N, 128° 02' 17" E	> 5,000 plantlets	619.5	Open forest, conifer–broadleaf forest	30	25.2	30.0	15.4	40.6
Wangqing	43° 50' 49" N, 130° 10' 36" E	< 500 plants	632.1	Gentle slopes or lowland, conifer–broadleaf forest	70	23.9	38.2	11.7	33.7
Antu	42° 58' 11" N, 128° 33' 57" E	~1,000 plants	619.7	Gentle slopes on a riverside, sparse mixed broadleaf forest	60	19.1	60.6	11.6	28.5
Dongfeng	43° 04' 24" N, 125° 32' 58" E	~1,000 plants	449.9	Sparse mixed broadleaf forest	30	24.7	45.4	12.3	26.6

<sup>a</sup>Data regarding geographic coordinates, population size, and elevation were based on our previous findings (Feng *et al.*, 2021).

The habitat conditions of the different *A. victorialis* populations in the Changbai Mountains varied. The air temperatures ranged from 19.1 °C (Antu) to 25.2 °C (Dunhua), and the soil temperatures ranged from 11.6 °C (Antu) to 15.4 °C (Dunhua). Furthermore, the air relative humidity levels ranged from 30.0% (Dunhua) to 60.6% (Antu). Additionally, Dongfeng had the lowest soil water content, whereas Dunhua had the highest content (Table 2). Overall, these results indicate that *A. victorialis* can grow under a wide range of environmental conditions in this region.

### *A. victorialis quality characteristics in the Changbai Mountains*

The ANOVA results indicated considerable differences in the nutrients in the plant samples collected from different habitats (Table 3). The Dongfeng population had the highest vitamin C, total flavonoid, and soluble sugar contents (Table 3), whereas the Dunhua B population had the highest  $\beta$ -carotene content ( $p < 0.05$ ). However, there was no significant difference ( $p > 0.05$ ) in the  $\beta$ -carotene content between the Dunhua A and Dongfeng wild populations. In the Dunhua B population, the soluble sugar content was only 85.4% of that in the Dunhua A population. However, the vitamin C, soluble protein, and crude fiber contents were higher in the Dunhua B population than in the Dunhua A population by 117.3%, 105.8%, and 101.7%, respectively. The Wangqing and Antu samples had lower nutrient contents than the Dunhua and Dongfeng samples, whereas the Antu samples had lower vitamin C, total flavonoid, and crude fiber contents than the other samples.

**Table 3.** Nutrient contents in *Allium victorialis* samples

Sample	Vitamin C (mg·g <sup>-1</sup> ) <sup>z</sup>	Total flavonoids (mg·g <sup>-1</sup> ) <sup>z</sup>	β-Carotene (mg·g <sup>-1</sup> ) <sup>z</sup>	Soluble sugars (mg·g <sup>-1</sup> ) <sup>z</sup>	Soluble proteins (mg·g <sup>-1</sup> ) <sup>z</sup>	Crude fiber (mg·g <sup>-1</sup> ) <sup>z</sup>
Dongfeng	3.789±0.064 a <sup>y</sup>	2.453±0.061 a	0.120±0.002 b	37.479±0.254 a	8.064±0.154 a	30.625±0.119 a
Dunhua A	2.350±0.101 c	1.215±0.049 b	0.126±0.003 b	34.924±1.334 a	7.753±0.081 a	26.886±0.098 b
Dunhua B	2.756±0.067 b	1.252±0.018 b	0.149±0.001 a	29.824±1.329 b	8.203±0.245 a	27.331±0.317 b
Antu	1.560±0.162 d	0.820±0.065 c	0.088±0.004 c	16.196±0.900 c	5.033±0.123 b	16.920±0.754 d
Wangqing	2.026±0.027 e	0.915±0.009 c	0.097±0.004 c	16.381±0.621 c	5.181±0.087 b	21.751±0.678 c

<sup>z</sup> Data were obtained from Feng *et al.* (2021).

<sup>y</sup> Nutrient content values that are expressed as means ± SE within the same column followed by different lowercase letters are significantly different ( $p < 0.05$ ; assessed using Duncan's multiple range test).

#### *Correlation between nutrient contents and habitat factors*

Pearson correlation analysis was performed to determine the association between the nutrient contents and habitat factors of *A. victorialis* growing in the Changbai Mountains. There was a significant positive correlation between elevation and the total flavonoid content in the populations ( $r = 0.959$ ,  $p < 0.05$ ). Additionally, the soluble sugar ( $r = -0.962$ ) and protein ( $r = -0.994$ ) contents in the *A. victorialis* populations in the Changbai Mountains were significantly negatively correlated ( $p < 0.01$ ) with the canopy density. Moreover, the β-carotene ( $r = -0.885$ ) and crude fiber ( $r = -0.910$ ) contents were significantly negatively correlated ( $p < 0.05$ ) with the canopy density. However, neither the β-carotene content nor the soluble sugar content was significantly affected by the habitat factors (Table 4).

**Table 4.** Correlation between nutrient contents and habitat factors

Habitat factor	Vitamin C	Total flavonoids	β-Carotene	Soluble sugars	Soluble proteins	Crude fiber
Elevation	0.855	0.959*	0.092	0.581	0.426	0.615
Forest type	0.193	0.426	-0.452	-0.011	-0.170	-0.158
Canopy density	-0.759	-0.647	-0.885*	-0.962**	-0.994**	-0.910*
Air temperature	0.650	0.458	0.773	0.709	0.765	0.869
Air humidity	-0.291	-0.053	-0.768	-0.491	-0.617	-0.622
Soil temperature	0.195	0.001	0.867	0.600	0.744	0.526
Soil water content	-0.159	-0.387	0.651	0.211	0.391	0.224

\* Significant at  $p < 0.05$ .

\*\* Significant at  $p < 0.01$ .

#### *Stepwise linear regression analysis of nutrient contents and habitat factors*

The relationship between the nutrient contents and main habitat factors was analyzed using linear regression models (Table 5). The total flavonoid content increased significantly with an increase in elevation ( $R^2 = 0.997$ ; Table 5), whereas there was no significant effect of elevation on the vitamin C content. The β-carotene, soluble sugar, soluble protein, and crude fiber contents were substantially affected by canopy density. Additionally, the *A. victorialis* populations growing in sparse forest stands with low canopy densities had high β-carotene, soluble sugar, soluble protein, and crude fiber contents, with  $R^2$  values of 0.783, 0.926, 0.989, and 0.828, respectively (Table 5).

**Table 5.** Regression analysis between nutrient contents and main habitat factors

Nutrient content	R2	Regression equation	F	p
Total flavonoids	0.997	$y_2 = -1.796 + 1.522 \times x_1 + 0.098 \times x_7$	395.827	0.003
$\beta$ -Carotene	0.783	$y_3 = 0.171 - 0.039 \times x_3$	10.846	0.046
Soluble sugars	0.926	$y_4 = 51.803 - 17.787 \times x_3$	37.471	0.009
Soluble proteins	0.989	$y_5 = 10.906 - 2.900 \times x_3$	258.417	0.001
Crude fiber	0.828	$y_6 = 23.886 - 8.945 \times x_3$	14.394	0.032

## Discussion

The nutritional quality of several crops, including kidney beans (Kigel, 1999; Murube *et al.*, 2021), globe artichokes (Cappelletti *et al.*, 2016), and apples (Jing *et al.*, 2020), is influenced by environmental factors. The results of the present study showed that canopy density was significantly negatively correlated with the  $\beta$ -carotene, soluble sugar, soluble protein, and crude fiber contents, indicating that canopy density considerably affected the nutritional quality of *A. victorialis* growing in the Changbai Mountains. This finding was consistent with the regression analysis results. Therefore, a decrease in canopy density can increase the soluble sugar and soluble protein contents in *A. victorialis* tissues.

These findings suggest that sufficient light conditions may be necessary for the optimal growth of *A. victorialis* in the Changbai Mountains, whereas a closed canopy may be unsuitable. The zonal plant communities in the Changbai Mountain region mainly comprise broadleaf Korean pine forests; however, *A. victorialis* mainly grows in the understory of conifer–broadleaf or deciduous broadleaf forests at elevations above 500 m in this area. Korean pine and other tall tree species within these communities are predominantly distributed at elevations below 1,100 m (Hao *et al.*, 2002; Zhang *et al.*, 2009). These trees provide a shaded environment for the growth of *A. victorialis*, which can be regarded as a part of the understory plant community. Canopy density is closely correlated with light intensity in forest environments (Tyagi *et al.*, 2011). An environment with low canopy density receives a relatively large amount of light, which causes increases in the air and soil temperatures to some extent and is also beneficial for photosynthesis, as well as the accumulation of photosynthetic products such as soluble sugars (Abeysinghe *et al.*, 2019; Singh *et al.*, 2019). This explains the relatively high nutrient contents in the Dongfeng samples, although the *A. victorialis* habitat in Dongfeng is at a lower elevation (< 500 m). The relatively high nutrient contents in the Dongfeng and Dunhua samples could be attributed to the low canopy density, which in turn may be associated with the high soil temperature and water content at these sites. This observation further corroborates the correlation between plant nutritional quality and habitat factors.

Furthermore, the results of the present study showed that elevation was positively correlated with the total flavonoid content in *A. victorialis* tissues, which is inconsistent with the results of Mphahlele *et al.* (2014), who found that fruits harvested at relatively low elevations, characterized by a Mediterranean climate, had significantly higher levels of total flavonoids than fruits harvested at higher elevations. However, there was no significant correlation between the elevation and the nutritional components studied. Although some correlations were observed between the air temperature, air relative humidity, or soil temperature and the nutrient contents of *A. victorialis* in the Changbai Mountains, the correlations were nonsignificant, suggesting a need for further studies.

In the *A. victorialis* samples from the Changbai Mountains, the nutrient contents were associated with habitat conditions; however, the vitamin C content was not strongly associated with the main habitat factors. A major limitation of the present study was the small sample size, which may have influenced the results. Additionally, there was no significant relationship between the air and soil indicators (air temperature, air

relative humidity, and soil temperature) and nutrient contents. The exact reason for this result remains unknown and requires further studies with larger sample sizes.

### **Conclusions**

In conclusion, the findings of this study show that the *A. victorialis* populations in the Changbai Mountains mostly grow as understory vegetation at elevations above 500 m in areas that have the optimal ranges for key environmental factors, such as air temperature, air relative humidity, soil temperature, and soil water content. Additionally, the nutritional quality of *A. victorialis* was influenced by habitat conditions, among which canopy density exerted a significant effect, whereas elevation significantly affected only the total flavonoid content. Overall, these findings can be helpful for the selection of sites and the optimization of environmental conditions for cultivating *A. victorialis* in the Changbai Mountains. However, further research is needed to elucidate the relationship between habitat factors and the levels of active compounds in *A. victorialis* in this region, such as sulfur compounds and anthocyanins. Jeong *et al.* (2020) found that the contents of sulfur compounds in *A. victorialis* populations from the Korean Peninsula were significantly influenced by forest stand characteristics and soil physicochemical properties. However, similar research on *A. victorialis* in the Changbai Mountains has not yet been performed, indicating that further research is needed.

### **Authors' Contributions**

Conceptualization (BF and SS); Formal analysis (BF, GX, XW, XS and JL); Funding acquisition (BF, JR, SS); Investigation: (BF, XW, CL, JZ, KZ); Methodology (BF); Writing – original draft preparation (BF); Writing – review & editing (SS); Resources (SS); Supervision (SS). All authors have read and approved the final manuscript.

### **Ethical approval** (for researches involving animals or humans)

Not applicable.

### **Acknowledgements**

This work was supported by the Department of Agriculture and Rural Affairs of Jilin Province under the project “Construction and Demonstration of Production Technology for Quality Improvement and Efficiency Improvement of Specialty Vegetables in Jilin Province”, as well as by the Jilin Province Department of Finance under grant agreement No. GY-2020-09.

## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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