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RESEARCH REPORT

Evaluation of freezing tolerance in *Actinidia* germplasm based on relative electrolyte leakage

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Abstract
Cold stress in winter can have a disastrous effect on kiwifruit yield and affect geographical distribution. However, freezing tolerance in *Actinidia* genotypes remains largely unknown. Here, we report changes in metabolite content and enzyme activity in the shoots of *Actinidia* genotypes exposed to low-temperature stress (− 5 °C, − 10 °C, − 15 °C, − 20 °C, − 25 °C and − 30 °C). Moreover, the relative electrolyte leakage method was used to evaluate the freezing tolerance of kiwifruit germplasm; 51 genotypes from 16 species of *Actinidia* were evaluated in total. The data revealed that relative electrolyte leakage, proline (Pro), soluble protein, and catalase (CAT) activity changed with different low temperatures. Results showed that among 16 species, *A. kolomikta*, *A. polygama*, and *A. arguta* had lower LT50 than other species. *A. arguta*, originating from the northeast of China, exhibited stronger freezing resistance than the ones from other places. There was little difference in freezing tolerance between *A. chinensis* and *A. deliciosa*. These findings provide new insights into the freezing tolerance ability and mechanisms of kiwifruit and further contribute to our understanding of the relationship between freezing tolerance and geographic distribution.

Keywords Cold stress · Freezing tolerance · Germplasm resource · Kiwifruit · Relative electrolyte leakage

1 Introduction

Low temperature limits the geographic distribution of plants and reduces agricultural productivity (Su et al. 2015). The injuries caused by low temperature are generally categorized into chilling stress (temperature above 0 °C) and freezing stress (temperature below 0 °C) (Solanke and Sharma 2008). For temperate plants, freezing stress is the main factor threatening in the life of plants during overwintering. Freezing stress results in the formation of ice crystals within the cell, mechanical damage, as well as metabolic dysfunction in plants (Takahashi et al. 2018). However, plants have evolved to employ protective mechanisms to tolerate freezing stresses, such as accumulation of proline and proteins as well as enzyme activities that function to eliminate reactive oxygen species (ROS) production against freezing-induced injury (Zhao et al. 2019; Wang et al. 2006).

Several physiological changes occur under low-temperature stress, including modifications in the cell wall, membrane lipid compositions, increases in proline contents, and protein synthesis (Takahashi et al. 2016). Soluble protein that accumulates in plants serves as a cytoprotective compound that prevents or slow down the formation of ice crystals (Feng et al. 2019). Plants accumulate proline when they are exposed to cold temperatures, which induce osmotic adjustments, maintain turgor in dehydrated cells, and allow plants to tolerate dehydration stress (Ren et al. 2018). In addition to the metabolite content, plants produce excess reactive oxygen species (ROS), such as superoxide radicals, hydrogen peroxide, singlet oxygen, and hydroxyl radicals, that can disrupt the mechanism balance in the cold stress response (Suzuki 2006). Under prolonged oxidative conditions, ROS cause lipid peroxidation, DNA damage, and protein denaturation. The stress response process in plants may be accompanied by increased activity of one or more antioxidative enzyme, such as catalase (CAT), which is

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involved in scavenging ROS. Therefore, the physiological and biochemical changes in plants in response to cold stress can be used as indicators to assess the freezing tolerance in kiwifruit. Variation in freezing tolerance of plants before exposure to subzero temperatures varies within and between species. Furthermore, this variation is obvious along latitudinal and altitudinal gradients. Freezing tolerance is determined fundamentally by inherited genetic traits; therefore, there are significant differences between different species. In *Arabidopsis*, there is significant natural variation in tolerance to subzero temperatures. The origin of the plant contributes to the cold response and survival after exposure to subzero temperatures.

The genus *Actinidia* comprises 54 species that are widely distributed from 0° to 50° north latitude in China (Huang 2009). However, only *A. arguta*, *A. kolomikta*, and *A. polygama* are distributed in the northeast of China, and these species can tolerate lower temperatures. Currently, *A. chinensis* and *A. deliciosa* are the two main species commercially cultivated in the world. These two species are naturally distributed south of the Yellow River, China (103° E–122° E, 23° N–35° N). The weak freezing tolerance of these species (about − 13 °C) in winter could lead to serious damage to shoots (Ding 2018).

The knowledge of the freezing tolerance of other *Actinidia* species is quite limited. Therefore, it is necessary to evaluate the freezing tolerance (FT) of a range of kiwifruit varieties for selection of cultivars with cold tolerance. Our preliminary investigation showed physiological and biochemical changes in different kiwifruit genotypes. The objective of this study was to investigate the response of kiwifruit genotypes against cold stress under controlled environment conditions and assess the ability of freezing tolerance among kiwifruit genotypes.

2 Materials and methods

2.1 Materials

The materials included six genotypes of *Actinidia arguta* ('Changjiang-1', 'Mudanjiang-1', 'RB-4', 'Ruby Star', 'Zhejiang 15–10', and 'Purpurea'), one genotype of *Actinidia valvata* ('Zhongzhen-1'), one genotype of *Actinidia deliciosa* ('Bruno'), and one genotype of *Actinidia chinensis* ('Hort16-A'). The latitude of the original place of these materials is shown in Table 1. One-year-old dormant shoots were collected in early January 2017 in Zhengzhou, China (latitude: 34°71′ N, longitude: 113°71′ E). The weather data of the last 10 years indicated that the lowest temperature in Zhengzhou normally occurred in the end of December to early January during the overwintering period. The shoots were selected on the basis of their uniform appearance, and the detached shoots were packed with polyethylene film for analysis.

Table 1 Distribution of nine *Actinidia* genotypes along with locational lowest temperature (°C)

Species	Genotypes	Location	Locational lowest temperature (°C)
A. arguta	Changjiang-1	126°35′ E 46°40′ N	− 26
	Mudanjiang-1	129°37′ E 44°33′ N	− 24
	RB-4	111°27′ E 33°81′ N	− 5
	Ruby star	111°58′ E 33°31′ N	− 6
	Zhejiang 15–10	120°08′ E 30°16′ N	− 2
	Purpurea	112°58′ E 28° 06′ N	2
A. valvata	Zhongzhen-1	114°20′ E 30° 25′ N	− 1
A. deliciosa	Bruno	113°37′ E 34° 44′ N	− 7
A. chinensis	Hort16A	113°37′ E 34° 44′ N	− 7

Kiwifruit germplasm materials evaluated by verified method included 51 genotypes from 16 species of *Actinidia* genus. All of these materials are maintained in the Zhengzhou kiwifruit germplasm repository; the shoots of these germplasms were collected in early January 2017.

2.2 Low-temperature treatment

The detached shoots of kiwifruit were exposed to low temperatures according to previous methods (Murray et al. 2010). The detached shoots were rinsed with double-distilled water to remove surface contaminants. The middle sections of the shoots (about 10–15 cm long) were wrapped with polyethylene film and placed in a freezing chamber under a controlled environment. The temperature of chamber was gradually decreased to set the temperature with an approximately 10 °C/h fall for 8 h and then it was gradually raised to 25 °C by the 10 °C/h rise. Finally, shoots were taken out and kept at room temperature for 30 min. The materials that originated from the south of the Yellow River (low latitude) were subjected to treatments of − 5 °C, − 10 °C, − 15 °C, − 20 °C, and − 25 °C for 8 h each. Similarly, the materials from the northeast of China (high latitude) were exposed to temperatures of − 5 °C, − 10 °C, − 15 °C, − 20 °C, − 25 °C, and − 30 °C for 8 h each. A part of the sample was used for the measurement of REL, and the others part was used for further analyses of metabolite contents and the antioxidant enzyme activity.

2.3 Budbreak rate

After low-temperature treatment, the morphological lower end of shoots was cut into horseshoe-shaped segments, and the shoots were put into tissue culture bottles. Three repetitions were set for each temperature treatment, and each repetition contained 10 shoots. Bottles were placed in a room at 25 °C with 14 h of fluorescent light, water was changed every 5 d, and budbreak rate was observed after 25 d and was calculated as indicated in Eq. 1 below.

$$Budbreak\ rate\ (\%) = (the\ number\ of\ budbreak)/(the\ total\ of\ bud) * 100\ \% \quad (1)$$

The FT was expressed as LT50 (half lethal temperature at which budbreak rate reaches 50%) by fitting the response curve obtained by the budbreak rate with a logistic sigmoid function (Eq. 2):

$$y = k/\left(1 + ae^{-bx}\right) \quad (2)$$

where x is the treatment temperature, y is the budbreak rate value, k is the extreme value when x approaches infinity, and a and b are the equation parameters.

2.4 Relative electrolyte leakage (REL)

After low-temperature treatment, the shoots without buds were cut into 1- to 2-mm-thick slices. Then, 0.2 g of the slices was incubated in 30 ml of double-distilled water for 2 h, with shaking at 200 rpm at room temperature. The initial electrical conductivity (C_1) was measured using a digital conductivity meter (DDS-307, Rex, China). The samples were heated up to boil for 30 min and then cooled down at room temperature with continuous shaking for 30 min, and the second electrical conductivity (C_2) was taken. The REL was calculated as indicated by Eq. 3:

$$REL(\%) = \left(C_1/C_2\right) * 100\% \quad (3)$$

The LT50 (half lethal temperature at which REL reaches 50%) was determined by fitting the response curve obtained by the REL with a logistic sigmoid function (Eq. 4):

$$y = k/\left(1 + ae^{-bx}\right) \quad (4)$$

where x is the treatment temperature, y is the REL value, k indicates the extreme value when x approaches infinity, and a and b are the equation parameters. If the correlation coefficient r is close to 1, the equation is used to calculate LT50 (He et al. 2015).

2.5 The measurement of antioxidative enzyme activities and metabolite contents

The shoots were ground using an electric grinding miller. We put 0.2 g of the sample into a 2-ml tube, and the frozen powder was immediately used for extraction. The contents of free proline (Pro) were determined using ninhydrin colorimetry (Wang et al. 2013). The soluble protein content was determined using the bicinchoninic acid (BCA) protein concentration test (Hinson and Webber 1988). CAT activity was determined using the ultraviolet absorption method (Bočová et al. 2012). All the measurements were repeated three times.

2.6 Statistical analysis

Proline, soluble protein, and CAT were standardized using the membership function (Yu et al. 2018). The membership degree (U) of proline, soluble protein, and CAT was calculated using formula $U = (X_{ijk} - X_{min})/(X_{max} - X_{min})$, where U represents the membership value for the i_{th} genotype in the j_{th} temperature gradient of the k_{th} index, and $U = [0, 1]$. X_{ijk} represents the k_{th} index value of the i_{th} genotype in the j_{th} temperature gradient, while X_{max} and X_{min} are the maximum and minimum in the k_{th} index among the tested genotypes, respectively.

All data were subjected to two-way ANOVA analysis using SPSS software (v. 22.0 for window; IBM corporation, USA). The treatment means were separated using Duncant's multiple range at the $P < 0.05$ and $P < 0.01$ probability level.

3 Results

3.1 A method for assessing the freezing tolerance in shoots of kiwifruit

3.1.1 Freezing tolerance assessment by budbreak rate

With decreasing temperatures, the budbreak rate of five genotypes decreased (Fig. 1). Under − 5 °C treatment, budbreak rate rank was as follows: 'RB-4' > 'Ruby star' > 'Zhongzhen-1' > 'Zhejiang 15–10' > 'Purpurea'. Under − 15 °C treatment, the rank was 'RB-4' > 'Zhongzhen-1' > 'Ruby star' > 'Purpurea' > 'Zhejiang 15–10'. Under − 25 °C treatment, only 'RB-4' had a lower budbreak rate; the remaining treatments had 0% budbreak rate. Below − 30 °C, all genotypes showed a 0% budbreak rate which indicated

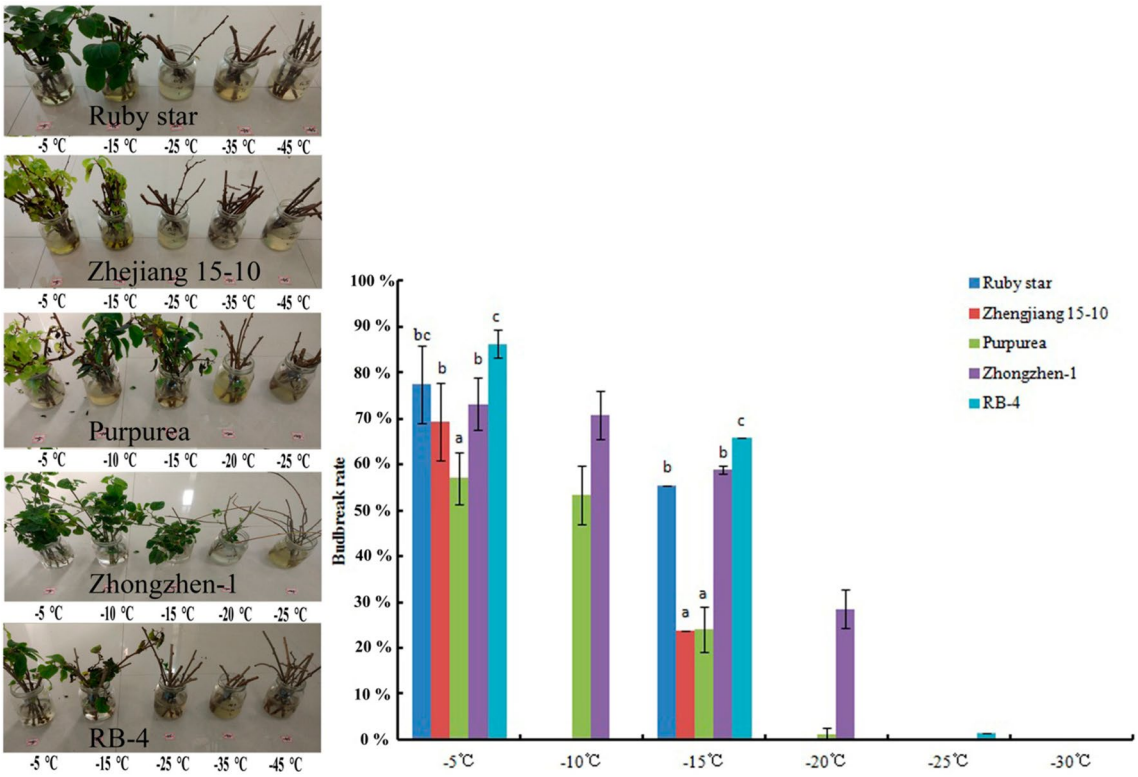

Fig. 1 The influence of low temperature on budbreak rate in the shoots of kiwifruit. Bars represent standard errors (n = 3). Different letters indicate significant differences according to Duncant's test ($p < 0.05$)

shoot damage and mortality. The trend of the budbreak rate presented as an inverted S shape within the gradient low low-temperature treatment, which fit the logistic function analysis. Using a logistic function, we calculated FT of five genotypes and got the following FT ranking: 'RB-4' > 'Ruby star' > 'Zhongzhen-1' > 'Zhejiang 15–10' > 'Purpurea' (Table 2).

3.1.2 Freezing tolerance assessment by relative electrolyte leakage method

Within the range of the low-temperature treatment, the REL of all the genotypes increased with the decrease of temperature (Fig. 2) and change of REL showed a flat-steep-flat trend under gradient low temperature. In short, the increase of REL was not obvious when the temperature was above − 15 °C. Moreover, different genotypes showed the different increase trends for REL under decreasing temperature, i.e. 'Hort 16A' and 'Zhongzhen-1' showed a sharp increase in REL between − 15 and − 20 °C, 'Zhejiang 15–10' and 'Purpurea' had a quick increase from − 15 °C to − 30 °C, and 'Bruno' had a quick increase at − 20 °C. The four *A. arguta* genotypes 'Changjiang-1', 'Mudanjiang-1', 'RB-4', and 'Ruby Star' had a sharp increase in REL below − 25 °C. The two *A. arguta* genotypes that originated in northeastern China, 'Changjiang-1' and 'Mudajiang-1', had the lowest LT50, which was lower than − 30 °C, while 'Hort16A' and 'Bruno' had the highest LT50 (above − 20 °C, Table 3).

Table 2 LT50 of five *Actinidia* genotypes calculated by the budbreak rate method

Species	Genotypes	Logistic equation	LT50/°C	Correlation coefficient
A. arguta	RB-4	$y = 1/(1 + 0.00076e^{-0.5493x})$	− 13.08	0.8580*
	Ruby star	$y = 1/(1 + 0.00111e^{-0.7615x})$	− 8.93	0.8910*
	Zhejiang 15–10	$y = 1/(1 + 0.00261e^{-0.7341x})$	− 8.10	0.9030*
	Purpurea	$y = 1/(1 + 0.00225e^{-0.9693x})$	− 6.29	0.9240**
A. valvata	Zhongzhen-1	$y = 1/(1 + 0.00052e^{-0.8668x})$	− 8.73	0.8700*

* and ** indicate 0.05 and 0.01 significance level, respectively

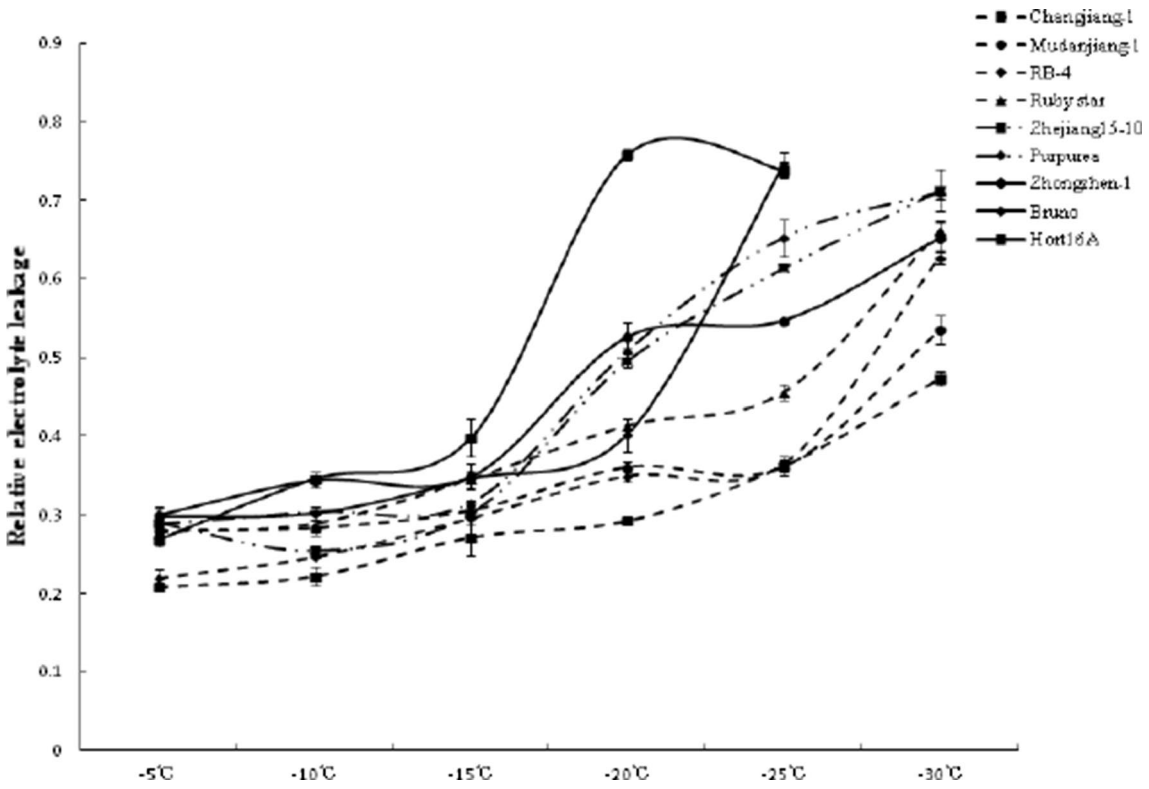

Fig. 2 The influence of low temperature on relative electrolyte leakage in the shoots of kiwifruit. Bars represent standard errors (n = 3). Different letters indicate significant differences according to Duncant's test ($p < 0.05$)

Table 3 LT50 of nine *Actinidia* genotypes calculated by REL in shoots of kiwifruit genotypes

Species	Genotypes	Logistic equation	LT50/°C	Correlation coefficient
A. arguta	Changjiang-1	$y = 1/(1 + 5.3951e^{-0.0474x})$	− 35.53	0.9680**
	Mudanjiang-1	$y = 1/(1 + 3.6283e^{-0.0385x})$	− 33.50	0.8880*
	RB-4	$y = 1/(1 + 5.6996e^{-0.0622x})$	− 27.98	0.9130**
	Ruby star	$y = 1/(1 + 4.2880e^{-0.0607x})$	− 23.98	0.9400**
	Zhejiang 15–10	$y = 1/(1 + 5.4228e^{-0.0827x})$	− 20.44	0.9440**
	Purpurea	$y = 1/(1 + 4.8383e^{-0.0810x})$	− 19.46	0.9540**
A. valvata	Zhongzhen-1	$y = 1/(1 + 3.5272e^{-0.0606x})$	− 20.80	0.9650**
A. deliciosa	Bruno	$y = 1/(1 + 5.0903e^{-0.0862x})$	− 18.88	0.8440*
A. chinensis	Hort16A	$y = 1/(1 + 5.6602e^{-001173x})$	− 14.78	0.9310*

* and ** indicate 0.05 and 0.01 significance level, respectively

The LT50 of the other genotypes was between − 19.46 and − 27.98 °C.

3.1.3 Freezing tolerance assessment by the Proline (Pro) method

Shoot proline content generally increased with the reduction of temperature in *A. arguta*. The proline content of five *A. arguta* genotypes and *A. valvata* 'Zhongzhen-1' showed a rising trend (Fig. 3), except 'Mudanjiang-1', which showed a significant decrease at − 30 °C. Moreover, the Pro contents of 'Changjiang-1' and 'Mudanjiang-1' were significantly higher than the other genotypes. 'Bruno' and 'Hort16A' presented a decreasing trend. Using membership function standardized Pro data, the ranking of freezing tolerance between genotypes was as follows: 'Changjinag-1' > 'Mudanjiang-1' > 'Zhejiang 15–10' > 'Hort 16A' > 'Zhongzhen-1' > 'Purpurea' > 'Bruno' > 'Ruby star' > 'RB-4' (Table 4). For 'RB-4', this was inconsistent with the field observation, which

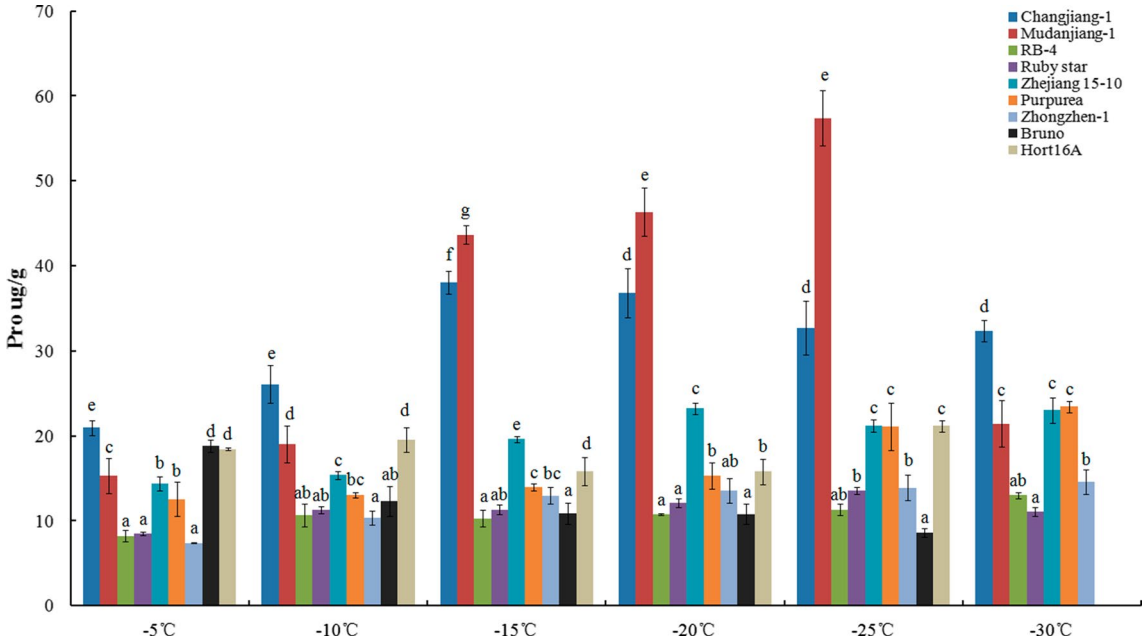

Fig. 3 Effects of low temperature on Pro contents of kiwifruit shoots. Bars represent standard errors (n = 3). Different letters indicate significant differences according to Duncant's test ($p < 0.05$)

Table 4 U value of proline in nine *Actinidia* genotypes

Species	Genotypes	U value	Ranking
A. arguta	Changjiang-1	0.5247	1
	Mudanjiang-1	0.4713	2
	RB-4	0.0581	9
	Ruby star	0.0705	8
	Zhejiang 15–10	0.2291	3
	Purpurea	0.0907	6
A. valvata	Zhongzhen-1	0.1689	5
A. deliciosa	Bruno	0.0883	7
A. chinensis	Hort16A	0.1908	4

showed a stronger freezing tolerance than *A. chinensis* and *A. deliciosa*.

3.1.4 Freezing tolerance assessment by the soluble protein method

A. arguta and *A. valvata* had less variation in soluble proteins than 'Bruno' and 'Hort 16A', in which the soluble protein contents were higher and began to decrease significantly at − 10 °C (Fig. 4). Among the *A. arguta* genotypes, the soluble protein contents of 'Changjiang-1' and 'Mudanjiang-1' were higher than the genotypes originating from the south and middle regions of China. Moreover, the soluble protein content of 'Zhongzhen-1' was lower and did not present a remarkable change. Both 'Bruno' and 'Hort 16A' had higher soluble protein; however, both showed immense fluctuation in results. So, we can speculate that the rate of soluble protein change was an important index for FT and a greater change represented weak FT. Using membership function standardized soluble protein data, the ranking of freezing tolerance of these genotypes was as follows: 'Bruno' > 'Hort 16A' > 'Mudanjiang-1' > 'Changjinag-1' > 'Ruby star' > 'RB-4' > 'Zhongzhen-1' > 'Purpurea' > 'Zhejiang 15–10' (Table 5). The results were inconclusive for evaluating the freezing tolerance of *A. chinensis*, *A. deliciosa*, and *A. arguta* by the soluble protein method.

3.1.5 Freezing tolerance assessment by the CAT method

The CAT activity initially represented a declining trend but finally it increased in *A. arguta* genotypes ('Changjiang-1', 'RB-4', 'Purpurea') and *A. valvata* ('Zhongzhen-1') (Fig. 5). Among *A. arguta* genotypes, the ones with low LT50 showed greater CAT activity. No significant change was observed between *A. deliciosa* and *A. chinensis*. 'Hort 16A' produced the lowest CAT activity than all other genotypes. Moreover, 'Bruno' resulted in relatively stable and higher CAT activity. We obtained the following ranking of freezing tolerance for kiwifruit genotypes using membership function standardized CAT data: 'Mudanjiang-1' > 'RB-4' > 'Bruno' > 'Changjiang-1' > 'Purpurea' > 'Zhejiang 15–10'. > 'Ruby star' > 'Zhongzhen-1' > 'Hort 16A' (Table 6). Although the freezing tolerance of *A. arguta* cultivars could be determined by the CAT method, it was

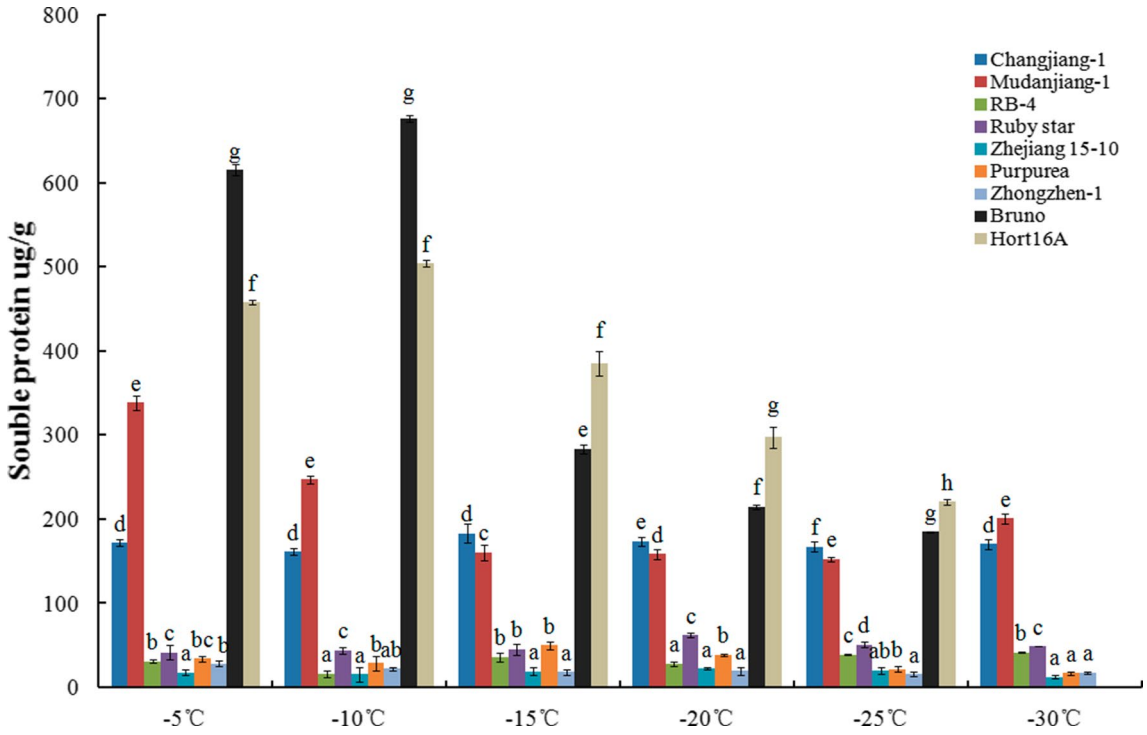

Fig. 4 Effects of low temperatures on soluble protein contents of kiwifruit shoots. Bars represent standard errors (n = 3). Different letters indicate significant differences according to Duncant's test ($p < 0.05$)

Table 5 U value of soluble protein in nine *Actinidia* genotypes

Species	Genotypes	U value	Ranking
A. arguta	Changjiang-1	0.2391	4
	Mudanjiang-1	0.2969	3
	RB-4	0.0292	6
	Ruby star	0.0547	5
	Zhejiang 15–10	0.0080	9
	Purpurea	0.0116	8
A. valvata	Zhongzhen-1	0.0288	7
A. deliciosa	Bruno	0.5767	1
A. chinensis	Hort16A	0.5432	2

inconsistent with field observations as 'Bruno' showed less freezing tolerance compared to *A. arguta*.

3.2 Freezing tolerance evaluation of kiwifruit germplasm

Budbreak rate is the most important freezing tolerance index and has been extensively used to evaluate freezing tolerance in previous studies. We assessed freezing tolerance of different genotypes by determining budbreak rate, REL, Pro, soluble protein, and CAT. Correlation analysis between budbreak rate and other indices showed that relative electrolyte leakage measurement in kiwifruit shoots was an appropriate method to assess freezing tolerance (Table 7). The LT50 of *Actinidia* species was calculated by applying a logistic function to relative electrolyte leakage, and it varied in kiwifruit genotypes when we decreased temperature from − 10.05 to − 37.61 °C (Table 8). The cold treatment temperature had significant correlation with relative electrolyte leakage of 51 *Actinidia* genotypes, suggesting that the calculated LT50 of these genotypes by the logistic function was reliable. The LT50 of *A. kolomikta*, *A. polygama*, and *A. arguta* from the northeast of China was lower than − 27.00 °C, while the LT50 of *A. chinensis* and *A. deliciosa* was above − 20.79 °C. The LT50 of the other 11 *Actinidia* species was between − 11.18 and − 22.66 °C. Within species, variation of LT50 represents an enormous change. Among the *A. chinensis* genotypes, 'Hort 16A' had the weakest hardiness, while 'Hongyang' had the strongest hardiness at LT50 of − 20.79 °C with a variation of 6.01 °C between both species. Among the *A. deliciosa* genotypes, 'Jinkui' had the weakest hardiness, while 'Bruno' had the greatest hardiness with a variation of 8.75 °C between both species. The species (11–17) from *A. arguta* had the weakest hardiness, while 'LD133' had the greatest hardiness with a variation of 17.05 °C. *A. latifolia*, *A. eriantha*, *A. longicarpa*, *A. rufa*, *A. tetramera*, *A.macrosperma*, *A. callosa*, *A. chrysantha*, *A. hemsleyana*, and *A. valvata* had a similar LT50 to *A. chinensis* and *A. deliciosa*.

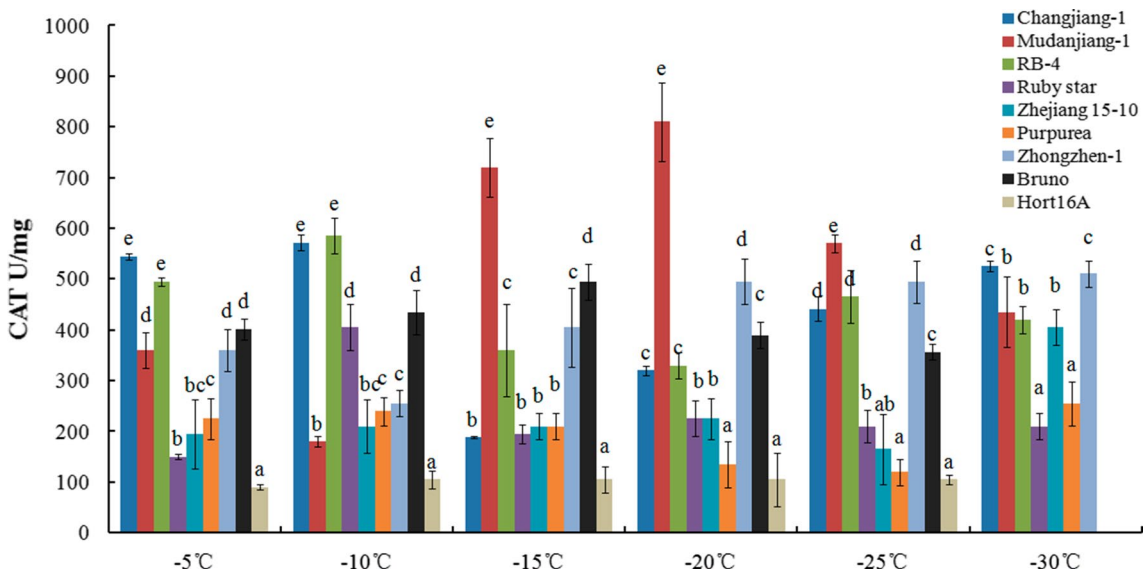

Fig. 5 Effects of low temperatures on CAT activity of kiwifruit shoots. Bars represent standard errors (n = 3). Different letters indicate significant differences according to Duncant's test ($p < 0.05$)

Table 6 U value of CAT in nine *Actinidia* genotypes

Species	Genotypes	U value	Ranking
A. arguta	Changjiang-1	0.4618	4
	Mudanjiang-1	0.5868	1
	RB-4	0.4896	2
	Ruby star	0.1979	7
	Zhejiang 15–10	0.2014	6
	Purpurea	0.4583	5
A. valvata	Zhongzhen-1	0.1493	8
A. deliciosa	Bruno	0.4625	3
A. chinensis	Hort16A	0.0167	9

Table 7 Correlation analysis between budbreak rate and other indices

Indexes	REL	Pro	Soluble Protein	CAT
Budbreak rate	0.944*	−0.709*	0.179	−0.660

* and ** indicate 0.05 and 0.01 significance level, respectively

4 Discussion

Temperature is a main environmental factor determining the natural latitudinal and altitudinal distribution of plants. The native distribution of kiwifruit in China extends to diverse latitudes from 0° N to 50° N, which shows kiwifruit has greater freezing tolerance germplasm. On the basis of our preliminary investigation, we chose nine representative genotypes from different geographical distributions. In this study, we further confirmed that genotypes that are native of higher latitudes showed higher freezing tolerance, as indicated by LT50.

Cold stress causes an increase of electrolyte leakage. Hence, the cellular membrane, which has higher selective permeability, is the first damaged part of a plant under cold stress (Miki et al. 2018). Under cooling stress, a dynamic balance is maintained by the intracellular and extracellular ion levels. Damage in the membrane results in increased extracellular ion concentration (Su et al. 2015), which ultimately increases electrolyte leakage. Our results were consistent with other studies where the electrolyte leakage level of plants was positively correlated with low-temperature stress (An et al. 2018). In our evaluation of freezing tolerance among nine genotypes, *A. arguta* originating from different geographical distributions exhibited a wide range of LT50, which became lower with an increase in latitude. Some researches evaluated kiwifruit species freezing tolerance through tissue activity and bud sprouting of dormant shoots and got the following order: *A. arguta* > *A. deliciosa* > *A. chinensis* (Lawes et al. 1995), while other researchers used anatomical structure of shoots method and got the following freezing tolerance order: *A. arguta* > *A. chinensis* > *A. deliciosa* (Qi et al. 2011). These studies showed consistent results with our field observations and predictions because the *A. arguta* genotypes that came from north of China were highly tolerant to freezing.

Proline and soluble protein are important osmotic regulation substances that can enhance the osmotic potential of cells under cold stress and thus improve the hardiness of the plant. These two substances have some contradictions with

Table 8 LT50 of different kiwifruit genotypes based on REL in shoots of kiwifruit genotypes

Species	Genotype	Logistic equation	LT50/ °C	Correlation coefficient
A. chinensis	Hort16A	$y = 1/(1 + 5.6630e^{-0.1173x})$	− 14.78	0.9310*
	Taishan-1	$y = 1/(1 + 4.9999e^{-0.1066x})$	− 15.10	0.8900*
	Chuhong	$y = 1/(1 + 6.7230e^{-0.1160x})$	− 16.43	0.8900*
	Taishanhuang	$y = 1/(1 + 5.8259e^{-0.1034x})$	− 17.05	0.9170*
	Longzanghong	$y = 1/(1 + 6.2148e^{-0.1015x})$	− 17.40	0.9420*
	Xinmei	$y = 1/(1 + 7.0535e^{-0.1114x})$	− 17.54	0.9070*
	Jintao	$y = 1/(1 + 6.6286e^{-0.1055x})$	− 17.93	0.9710**
	Beiyuan04207	$y = 1/(1 + 9.1830e^{-0.1200x})$	− 18.48	0.9190*
	Hongmeiren	$y = 1/(1 + 7.3144e^{-0.1075x})$	− 18.51	0.9230*
	Wanhong	$y = 1/(1 + 6.8831e^{-0.1001x})$	− 19.27	0.9170*
	Hongyang	$y = 1/(1 + 6.9699e^{-0.0934x})$	− 20.79	0.9820**
A. deliciosa	Jinkui	$y = 1/(1 + 3.6467e^{-0.1288x})$	− 10.05	0.9000*
	Hayward	$y = 1/(1 + 9.5656e^{-0.1533x})$	− 14.73	0.9310*
	Miliang-1	$y = 1/(1 + 7.6010e^{-0.1327x})$	− 15.28	0.9360*
	Xuxiang	$y = 1/(1 + 6.7421e^{-0.1154x})$	− 16.54	0.9130*
	Hayward♂	$y = 1/(1 + 5.0476e^{-0.0884x})$	− 18.30	0.8840*
	Bruno	$y = 1/(1 + 5.0903e^{-0.0862x})$	− 18.80	0.8440*
A. arguta	11–17	$y = 1/(1 + 2.8951e^{-0.0677x})$	− 18.2	0.9480*
	11–19	$y = 1/(1 + 3.6789e^{-0.0677x})$	− 19.23	0.9150*
	Zhejiang 15–10	$y = 1/(1 + 5.4228e^{-0.0827x})$	− 20.44	0.9440**
	Zhejiang 15–7	$y = 1/(1 + 3.5825e^{-0.0577x})$	− 22.11	0.9220*
	E-1	$y = 1/(1 + 3.0700e^{-0.0555x})$	− 23.59	0.9930**
	Ruby star	$y = 1/(1 + 4.1706e^{-0.0540x})$	− 23.98	0.9400**
	E-2	$y = 1/(1 + 2.9942e^{-0.0450x})$	− 24.34	0.9390*
	E-3	$y = 1/(1 + 3.2690e^{-0.0473x})$	− 25.04	0.9370*
	Kuilv	$y = 1/(1 + 6.6626e^{-0.0690x})$	− 27.47	0.9460**
	RB-4	$y = 1/(1 + 5.6996e^{-0.0622x})$	− 27.98	0.9130**
	RB-3	$y = 1/(1 + 3.4692e^{-0.0441x})$	− 28.23	0.9410*
	Kuilv♂	$y = 1/(1 + 3.1400e^{-0.0395x})$	− 28.99	0.9390*
	Yongfeng-4	$y = 1/(1 + 5.3045e^{-0.0561x})$	− 29.72	0.8080*
	KJBR-22	$y = 1/(1 + 4.6899e^{-0.0512x})$	− 30.17	0.9550*
	E-4	$y = 1/(1 + 4.0323e^{-0.0456x})$	− 30.56	0.9240*
	Yongfeng-1	$y = 1/(1 + 3.7072e^{-0.0421x})$	− 31.13	0.9570**
	Mudanjiangruan	$y = 1/(1 + 4.4716e^{-0.0464x})$	− 32.24	0.9780*
	Mudanjiang-1	$y = 1/(1 + 3.6283e^{-0.0385x})$	− 33.5	0.8880*
	You-4	$y = 1/(1 + 4.1097e^{-0.0421x})$	− 33.54	0.974*
	LD133	$y = 1/(1 + 3.6430e^{-0.0367x})$	− 35.25	0.9540*
A. latifolia	S-1	$y = 1/(1 + 17.7859e^{-0.2574x})$	− 11.18	0.9020*
A. eriantha	S-2	$y = 1/(1 + 7.075e^{-0.1503x})$	− 12.97	0.9220*
	Zaoxu	$y = 1/(1 + 4.7464e^{-0.0899x})$	− 17.33	0.9060*
A. longicarpa	S-3	$y = 1/(1 + 4.7594e^{-0.1200x})$	− 13.00	0.9410*
A. rufa	S-4	$y = 1/(1 + 7.1224e^{-0.1503x})$	− 13.06	0.9150*
A. tetramera	S-5	$y = 1/(1 + 2.8532e^{-0.0745x})$	− 14.07	0.9830**
A.macrosperma	S-6	$y = 1/(1 + 3.3404e^{-0.0441x})$	− 14.50	0.9440*
A. callosa	S-7	$y = 1/(1 + 12.3300e^{-0.1660x})$	− 15.14	0.9010*
A. chrysantha	S-8	$y = 1/(1 + 4.4500e^{-0.0905x})$	− 16.50	0.8810*
A. hemsleyana	S-9	$y = 1/(1 + 5.4413e^{-0.0962x})$	− 17.62	0.9530*
A. valvata	S-10	$y = 1/(1 + 3.5272e^{-0.0606x})$	− 20.80	0.9650**
A. melanandra	S-11	$y = 1/(1 + 2.3448e^{-0.0299x})$	− 22.66	0.9600**
A. polygama	S-12	$y = 1/(1 + 2.8516e^{-0.0388x})$	− 27.00	0.8870*

Table 8 (continued)

Species	Genotype	Logistic equation	LT50/ °C	Correlation coefficient
A. kolomikta	S-13	$y = 1/(1 + 2.7306e^{-0.0197x})$	− 37.61	0.9700**

* and ** indicate 0.05 and 0.01 significance level, respectively

freezing tolerance. An increase in proline content indicates that the Pro metabolism pathway is activated in response to low temperature, in addition to up-regulation of the related gene. Hence, the Pro metabolism pathway is also important for freezing tolerance investigations. An increased activity of antioxidant enzymes can mitigate the damaging effect of ROS in plants under cold stress (Cho and Park 2000). SOD can catalyze $:O^{2-}$ to generate H_2O_2, which can be decomposed by CAT or POD. All the antioxident enzymes play an important role in plant cold hardiness. Our analysis showed that CAT was a vital enzyme to protect the cell against cold stress. CAT activity indicated that an initial low temperature could cause damage in kiwifruit, but subsequent low temperature enabled kiwifruit to have improved freezing tolerance.

The results of budbreak rate showed that the freezing tolerance of 'RB-4' and 'Ruby star' was stronger than that of 'Zhejiang 15–10' and 'Purpurea'. These results were consistent with the results of the LT50 method based on REL, which indicated that the evaluation method of LT50 based on REL was accurate enough. However, the experiment assessing budbreak rate was not feasible for large-scale evaluation of kiwifruit germplasm because it was time-consuming and laborious. Pro, soluble protein, and CAT had minor contradictions in their evaluation ranking and can be correlated with the freezing tolerance of plants in general. The LT50 method based on REL was better than Pro, soluble protein, and CAT in evaluating freezing tolerance. Therefore, LT50 based on REL was suitable for evaluating kiwifruit germplasm resources.

This study showed that there was significant intraspecific variation in freezing tolerance. Moreover, there were significant differences in freezing tolerance of plants from different geographic origins. The similar LT50 of *A. eriantha*, *A. longicarpa*, *A. rufa*, *A. tetramera*, *A.macrosperma*, *A. callosa*, *A. chrysantha*, *A. hemsleyana*, *A. valvata*, *A. chinensis*, and *A. deliciosa* suggested that these species may have the same genetic background in terms of freezing tolerance. The genetic makeup contributed to freezing tolerance of *Actinidia* species, in which *A. kolomikta*, *A. polygama*, and *A. arguta* had much lower LT50 than the other species (Table 3). From an evolution standpoint, *A. kolomikta* and *A. polygama* had similar hardiness with *A. arguta* because they had ancestral association with it. Apart from these three species, LT50 of *A. melanandra* was lower than that of other species, and *A. melanandra* was considered to have the same evolutionary level with *A. arguta*. The correlation between evolution and hardiness has been discussed previously (Liu et al. 2017). A previous study suggested that the anatomical structure of shoots may explain the difference in cold hardiness (Matisons et al. 2020). The xylem thickness of the four species evaluated in the present study was thinner than that of other *Actinidia* species.

However, within the species, the geographic origin of kiwifruit genotypes played an important role in cold hardiness. *A. arguta* had the most extensive geographic distribution in the genus *Actinidia*. In this study, 20 genotypes from A. *arguta* were evaluated, from which 8 originated from the northeast China, 6 from the middle region of China, 2 from southern China, and 4 from Europe (Table 9). The range of LT50 was − 27.47 to − 35.25 °C, − 18.20 to − 30.17 °C, − 20.44 to − 22.11 °C, and − 23.59 to − 30.56 °C, respectively. The relationship between hardiness and geographic distribution should be investigated further, and research on the molecular markers or genes related to the hardiness would benefit the future breeding work.

Table 9 LT50 of *A.arguta* from different origins based on REL of different areas

Origin	Average temperature in Dec. and Jan.	Genotypes	LT50
North	− 30.9 to − 14.7 °C	Kuilv	− 27.47
		Kuilv♂	− 28.99
		Yongfeng-4	− 29.72
		Yongfeng-1	− 31.13
		Mudanjiangruan	− 32.24
		Mudanjiang-1	− 33.5
		You-4	− 33.54
		LD133	− 35.25
Middle	− 12 to − 5 °C	11–17	− 18.2
		11–19	− 19.23
		Ruby star	− 23.98
		RB-4	− 27.98
		RB-3	− 28.23
		KJBR-22	− 30.17
South	2 to − 7 °C	Zhejiang 15–10	− 20.44
		Zhejiang 15–7	− 22.11
Europe		E-1	− 23.59
		E-2	− 24.34
		E-3	− 25.04
		E-4	− 30.56

5 Conclusions

In the present study, we assessed FT in 51 genotypes from 16 species of *Actinidia*. The calculation of LT50 based on the REL method was consistent with budbreak rate. Additionally, the REL method was appropriate for assessing FT of kiwifruit germplasm. The proline, soluble protein, and CAT methods were not applicable to assessing FT in kiwifruit genotypes. According to assessment results of kiwifruit germplasm, genotypes that were native to northeast China showed a stronger freezing tolerance, whereas the genotypes distributed from the north to south of China showed varied freezing tolerance. Based on these results, our data strongly indicate differences in freezing tolerance among genotypes. In addition, the data indicate that freezing tolerance is strongly correlated with geographic distribution.

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Author contributions FANG Jinbao and LIN Miaomiao designed experiments; SUN Shihang carried out experiments; WANG Ran and QI Xiujuan gave instruction in language grammar. SUN Shihang analyzed experimental results and wrote the manuscript.

Data availability All data generated or analyzed during this study are included in this article.

Compliance with ethical standards

Conflict of interests The authors declare no competing financial interests.

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