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# Understanding Flower Frost Tolerance in Almond (*Prunus dulcis*): The Role of Phenology, Cultivar and Sugars Content

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## ABSTRACT

Freezing temperatures during the flower and fruitlet stages are considered one of the most limiting factors for almond cultivation. Understanding the minimum temperature that reproductive organs can resist without damage is crucial for adapting the crop to different environmental conditions and for breeding cultivars with enhanced tolerance to frost damage. Accordingly, this study examined frost tolerance progression across various phenological stages as well as assessed frost tolerance in 20 almond cultivars during full bloom. Almond cultivars exhibited a noticeable decline in frost tolerance as they advanced through the studied phenological stages, with the highest vulnerability occurring after the fruit set. Phenotyping for frost tolerance at the flowering stage revealed significant differences within cultivars, with most experiencing 50% flower damage at temperatures around  $-4.0^{\circ}\text{C}$ . Among the studied cultivars, 'Vairo', 'Tarraco', 'Lauranne', 'Marinada', 'Tuono' and 'Penta' exhibited the highest tolerance to flower freezing, in contrast to 'Marta', 'Marcona' and 'Francolí' which showed the least. To further explore the relationship between physical and chemical traits and lethal temperatures, ovary and pistil weights were measured, along with the determination of the sucrose, fructose and glucose content in the pistils. Correlation analyses revealed that higher pistil sucrose content was associated with increased flower tolerance to freezing temperatures, suggesting that sucrose content enhances, to a certain extent, tolerance to frost damage at the flowering stage. This study provides valuable insights into assessing freezing tolerance within the almond germplasm, offering growers and breeders crucial information for selecting the most well-adapted cultivars in each environment.

## 1 | Introduction

The low chilling requirements needed for flowering in almond [*Prunus dulcis* (Mill.) D. A. Webb, syn. *P. amygdalus* (L.) Batsch], compared with other temperate fruits like peach, apricot, apple,

pear, or sweet cherry, make almonds one of the most susceptible crops to spring frosts. Among these crops, almonds are also the earliest to bloom (Fadón et al. 2020), typically flowering in January and February in the Mediterranean region hence further increasing the risk of frost damage. Thus, when

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## Summary

- Early flowering of almond significantly increases the risk of spring frost damage at the flower stage.
- This work assesses the levels of susceptibility to spring frost at different phenological stages and cultivars.
- Frost tolerance declines as phenology progresses, with the highest risk occurring after fruit set.
- Differences in tolerance levels to damage caused by simulated frost were observed in the evaluated cultivars.
- Correlation analyses suggest that higher pistil sucrose is associated with greater flower frost tolerance.

one or several frosts occur, the crop is highly susceptible due to its more advanced phenological state compared with other species, resulting in severe economic losses (Felipe et al. 2017). Additionally, the increasing temperatures due to global warming during the winter have produced premature flowering in recent decades, increasing the tree's vulnerability to these spring frosts (Benmoussa et al. 2017; Lorite et al. 2020).

Despite the exposure of almond flowers and fruitlets to lethal freezing temperatures causing significant crop losses worldwide, limited research from a breeding perspective has been conducted to address this problem. For more than 50 years, almond breeding programs have focused on releasing new late-blooming almond cultivars to reduce the risk of overlapping between flowering time and frost events (Batlle et al. 2017). As a result of these efforts, a set of late and extra-late blooming cultivars, with differences of up to one month between the earliest and the latest blooming cultivars, has been released (Martínez-Gómez et al. 2017; Alonso et al. 2017). However, most of the efforts to develop more resilient frost-resistant cultivars have focused on dormancy-related traits, such as increasing the chilling requirements for flowering and/or enhancing late-flowering genes in the almond genome, with just a few investigations focusing on the resistance of flowers and fruitlets to freezing temperatures. As a result, frost protection for flowers and fruitlets in almond orchards has concentrated on planting late-blooming cultivars along with active methods such as ventilators, sprinklers and surface watering (Snyder and Connell 1996; Yu and Lee 2020). These methods complement passive control strategies, such as selecting orchard locations, managing nutrients and irrigation, and utilising cover crops, which are also commonly employed to mitigate or prevent the impact of freezing temperatures.

The minimum temperature at which reproductive organs can survive without damage depends on various factors and determines their adaptability to specific agroecological regions (Ritonga and Chen 2020). This temperature depends primarily on the development stage. During the winter, flower buds are hardy, but as they swell and open into blossoms, they become more vulnerable to freezing damage (Burke et al. 1976; Simons and Doll 1976). Other factors, such as genotype (Kodad et al. 2010; Reig et al. 2013), ice formation (Palta and Weiss 2018), moisture content, amino acids, mineral, sugar and starch levels (Kaya et al. 2021a), and the nutritive status of the pistil (Rodrigo 2000), have been reported to influence the susceptibility of flowers to

frost injury. Moreover, Miranda et al. (2005) concluded that frost tolerance varies not only between orchards, cultivars and species but also within individual trees. For this reason, even flowers that appear similar, are at the same phenological stage, and are in comparable positions on the tree often exhibit differences in cold tolerance.

Numerous studies have investigated the intrinsic tolerance to frost damage of various fruit crops, such as almond, cherry, peach, plum and apricot, during specific phenological stages (Assmann et al. 2008; Kose and Kaya 2022; Miranda et al. 2005; Reig et al. 2013; Rodrigo 2000; Rodrigo et al. 2006; Caglar et al. 2024; Chen et al. 2016; Khorshide et al. 2014; Melgar et al. 2022), with most conducted after natural frosts in field conditions rather than under controlled environments. This variability in phenological stages makes cultivar susceptibility comparison difficult, highlighting the need for controlled studies. While limited research has examined freezing resistance in individual almond cultivars under controlled conditions (Imani et al. 2023; Keleta et al. 2023; Szalay et al. 2022; Moheb et al. 2018; Hadavand et al. 2021), most studies assessed frost injury in field settings (Imani and Mahamadkhani 2011; Imani et al. 2011, 2012; Guillamón et al. 2022; Kodad and Socias i Company 2005). To address these limitations, differential thermal analyses (DTA) have emerged as a reliable method for determining critical temperatures for flower organs and buds (Kose and Kaya 2022; Kaya et al. 2021b), effectively identifying physiological thresholds for frost damage (Kaya and Kose 2019; Kaya et al. 2020). Studies on sweet cherry and apricot demonstrated that DTA-derived characteristics correlate well with field observations (Kaya et al. 2018), though anatomical challenges in some species have been overcome through improved sample preparations (Kaya et al. 2021). When combined with direct viability assessments, these methodologies provide comprehensive insights into frost susceptibility, revealing that almond pistils become increasingly vulnerable from early bud swelling to early fruit development stages (Miranda et al. 2005).

Despite the few existing studies, comprehensive data on the freezing tolerance of flowers and fruitlets in the almond germplasm and its genetic control remain limited. Only a very limited number of studies have investigated the transcriptomic profiles of certain almond cultivars under frost stress, aiming to identify genes that could be targeted for breeding cold-tolerant cultivars during critical phenological stages (Bielsa et al. 2021; Alisoltani et al. 2015; Hosseinpour et al. 2017). Thus, given the lack of detailed information on the freezing tolerance of almond germplasm and the limited available genetic data, it is crucial to evaluate the frost resistance of a wider number of cultivars during key phases such as flowering, fruit set, and early fruit development to further understand the mechanisms behind frost protection. Testing the intrinsic frost tolerance of newly developed almond cultivars is therefore essential for accurately characterising these cultivars. This information enables growers to select cultivars best suited to their environmental conditions, optimising yields and providing breeding programs with critical data for selecting more frost-tolerant parental material for future cultivar development. As a result, this study focused on examining the progression of frost tolerance across different phenological stages and assessing frost tolerance in almond cultivars during full bloom, which is considered a critical phenological

stage. Additionally, we investigated the relationship between some physical and chemical parameters, such as pistil and ovary weight, moisture content and sugar content, with frost susceptibility.

## 2 | Materials and Methods

### 2.1 | Plant Material

Twenty almond cultivars were evaluated for their flower tolerance to freezing temperatures. This material was composed of 17 breeding cultivars from three different almond breeding programs CEBAS-CSIC ('Antoñeta', 'Marta', 'Penta' and 'Tardona'), CITA ('Belona', 'Guara', 'Mardía' and 'Soleta'), IRTA ('Constantí', 'Francolí', 'Glorieta', 'Marinada', 'Masbovera', 'Tarraco' and 'Vairo') and INRAe ('Ferragnès' and 'Lauranne'). Three additional landraces widely planted in the Mediterranean region ('Desmayo Langueta', 'Marcona' and 'Tuono') were also included in the study. Supporting Information S1 provides the pedigree relationships among the cultivars and their ancestors. The study included both self-compatible and self-incompatible cultivars, covering a broad range of flowering times, from very early to extra late (Calle et al. 2025). Detailed characteristics of the selected cultivars are also presented in Supporting Information S2.

The cultivars used in this study were grafted onto 'GF-677' rootstock and planted in 2009 as bare-root trees (~1 m high) at the IRTA Les Borges Blanques research station, NE Spain (41°30'31"N, 0°51'10"E). The experimental plot consisted of 16 trees per cultivar, planted at 4 × 2 m, pruned as a central axis, and randomly distributed. The orchard was drip-irrigated, pruning, soil management and fertilisation were based on the Spanish Integrated Production Management practices (BOE 2002). No fungicide treatments were applied during the experimental period. Climatic data for both years of analysis, including average, maximum and minimum daily temperature, relative humidity, and precipitation are provided in Supporting Information S3.

### 2.2 | Freeze Tolerance Phenotyping

The phenotyping of flower freeze tolerance was based on the methodology reported by Miranda et al. (2005), and the frost damage was evaluated as indicated in Torres and Miarnau (2024). For that, one-year-old branches were collected from various parts of the trees when at least 60% of the reproductive organs in the shoot reached the 'F' phenological stage based on Felipe (1977) ('F': full bloom). A total of 150 reproductive organs, distributed over three repetitions of five branches with ten flowers each, were harvested per cultivar and simulated frost temperature (as reported in Miranda et al. 2005). Samples were taken from different trees of each cultivar within the same plot. Sampling was conducted in two different years (2016 and 2019) to confirm the reproducibility of the results. Furthermore, in 2019, 'Desmayo Langueta' and 'Tardona' cultivars were evaluated during the phenological stages 'D' (flower bud open), 'E' (stamens are apparent), 'F' (full bloom), 'G' (petals are falling), 'H' (fruit setting) and 'I' (calyx is falling) (Felipe 1977) to

understand how frost sensitivity evolves throughout these phenological stages.

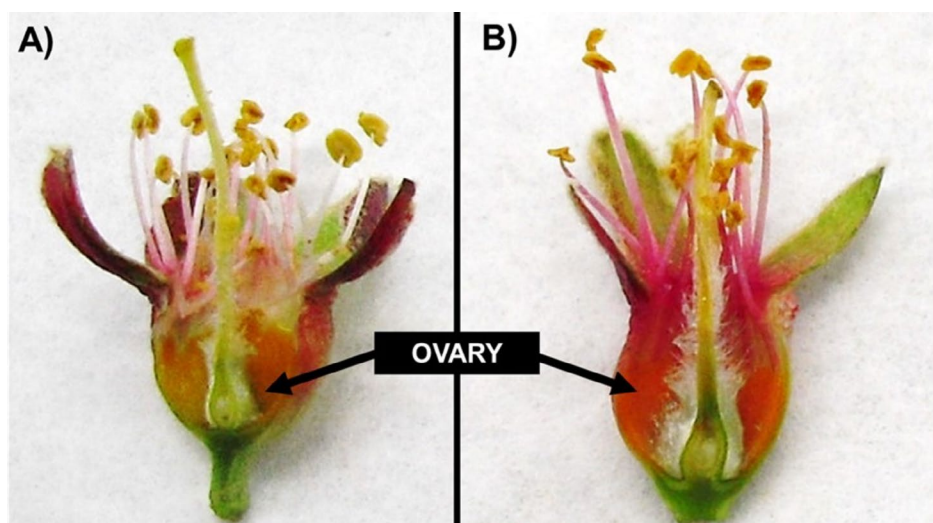
Upon arrival at the laboratory, the branches were arranged to fill three test tube holders, with a total of 50 buds, flowers, or fruits per cultivar in each holder. The test tube holders were selected to support the branches within the chamber (FITOCLIMA 10000HP, Aralab, Portugal), allowing air circulation around the branches. The three holders for each cultivar were positioned in different locations inside the chamber for three repetitions. Holders from the same chamber zones were grouped together, ensuring air could circulate freely between them. Five different frost temperatures (−3.0°C, −4.0°C, −5.0°C, −7.0°C and −9.0°C) were simulated in all evaluated cultivars. Additionally, in some cultivars ('Guara', 'Lauranne', 'Desmayo Langueta', 'Mardía', 'Penta', 'Tuono' and 'Vairo'), a few more temperatures were added for better regression adjustment (−1.0°C, −2.0°C, −2.5°C, −3.0°C, −3.5°C, −4.0°C, −4.5°C, −5.0°C, −7.0°C and −9.0°C). The freezing temperature determination involved a gradual decrease in temperature at a rate of 2°C per hour, starting from 6°C and reaching the desired frost temperature detailed above. After holding at that frost temperature for 30 min, the temperature was then raised back to 6°C at a rate of 3°C per hour. After the frost simulation, the branches were taken out of the climatic chamber and placed in small containers within another chamber maintained at 6°C for 48 h. Following this period, the branches were removed from the chamber, and the corresponding reproductive organs were detached to assess the damage caused by the simulated frost. Then, based on the phenological stage, the respective parts of the organs were examined individually under a magnifier with the help of a scalpel. The frost damage symptoms were identified according to the criteria suggested by Torres and Miarnau (2024). This methodology classified any brownish floral structure (Figure 1B) as indicative of frost damage and considered therefore unviable.

### 2.3 | Physical Characterisation of Pistils

One hundred and sixty flowers at the 'F' stage (Felipe 1977) were collected from each of the 20 cultivars in 2016 and 2019. During transport, the flowers were kept in coolers to maintain a low temperature. In the laboratory, the pistils were separated from the other flower parts using a scalpel, and four groups of forty pistils from each cultivar were placed in separate Petri dishes to ensure sufficient weight for measurement. The ovaries were then separated from the style and stigma, and each part was weighed individually. Once the fresh weights were recorded, the Petri dishes were placed in an oven at 60°C until they reached a constant weight. After this drying period, the samples were removed from the oven to determine the dry matter and humidity percentage of the different parts (pistil and ovary alone).

### 2.4 | Sugar Analysis in Almond Pistils

In 2016, the contents of fructose, glucose and sucrose present in the pistils of 15 cultivars were measured (mg/g of dry weight). To do this, an extraction solution was prepared using 62.5 mL of methanol and 37.5 mL of distilled water as indicated in Giné-Bodonaba and Terry (2010). For each 1 g of pistil sample, 2.5 mL



**FIGURE 1** | Almond flowers unaffected by frost (A) compared to frost-damaged flowers (B).

of the extraction solution was added. The solution containing the pistils was then incubated at 55°C for 15 min in a water bath, with vortex shaking every 5 min. After incubation, the sample was centrifuged at 24,000 g at 20°C for 15 min, and the supernatant was collected into an Eppendorf tube and stored at –80°C until measurement. Analyses were conducted using three replicates. The measurement was made using the commercial kits D-Glucose/D-Fructose (Ref. 12,800; BioSystems; Spain) and Sucrose/D-Glucose/D-Fructose (Ref. 12,819; BioSystems; Spain) following the manufacturer instructions. Determinations were performed using a two-reagent differential determination with a spectrophotometer (Thermo Fisher Scientific, MA, USA) with readings at 340 nm.

## 2.5 | Data Analysis

A sigmoid curve was used to describe the relationship between frost temperature and the percentage of damaged organs for each year (2016 and 2019) and the combined data of both years. A Probit analysis (Finney 1971) was performed using the Specialised Modelling Procedure to generate the relationship curve between frost temperature (dependent variable) and the percentage of damaged organs (independent variable). Subsequently, a regression analysis of flower damage in relation to temperature was fitted for each cultivar. Lethal temperatures at which 10%, 50% and 90% of the organs were damaged ( $LT_{10}$ ,  $LT_{50}$ ,  $LT_{90}$ ) were then estimated. The mean, standard deviation and fiducial limits of each lethal temperature were calculated for each cultivar and year. Additionally, cultivars were grouped into confidence intervals based on their mean lethal temperatures. Confidence intervals were calculated as  $[\bar{x} - E, \bar{x} + E]$ ; where  $\bar{x}$  is the mean value of the lethal temperature for all cultivars, and  $E$  is the margin error at a coefficient level of 95%.

For the physical and chemical traits of the pistils, the mean and standard deviation for each trait were determined. Additionally, mean comparisons (ANOVA and Tukey test;  $p < 0.05$ ) of pistil

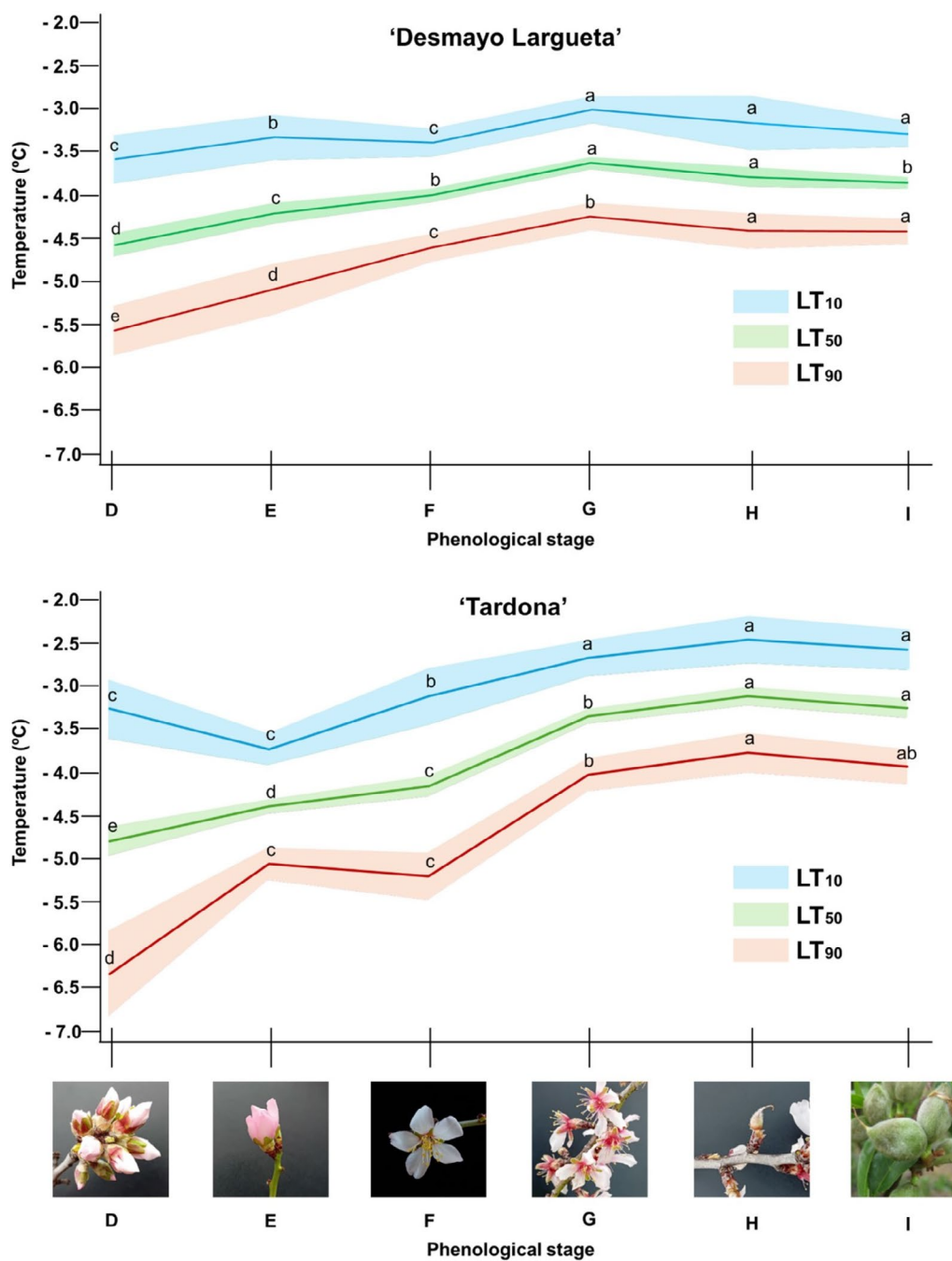
and ovary weight, moisture and sugar content among cultivars were performed to identify differences between genotypes.

Pairwise correlation coefficients were calculated for all physical and chemical parameters, as well as for the lethal temperatures of flowers across all analysed years. Significant correlations were identified using Pearson's correlation coefficient at a 0.01 significance level. All data were analysed using the JMP statistical software package (Version 17; SAS Institute Inc., Cary, North Carolina, USA).

## 3 | Results

### 3.1 | Frost Tolerance Evolution Across Phenological Stages

In frost tolerance evaluation across phenological stages 'D' to 'I', both 'Desmayo Langueta' and 'Tardona' cultivars exhibited a similar pattern, showing a significant decrease in frost tolerance as the organs evolved along the different phenological stages (Figure 2 and Supporting Information S4). In both cultivars, the lethal temperatures at which 10% ( $LT_{10}$ ) of the organs suffered damage at stage 'D' started around –3.50°C (–3.27°C for 'Tardona' and –3.57°C for 'Desmayo Langueta'). For this lethal temperature ( $LT_{10}$ ), the values remained relatively stable throughout the progression of phenological stages ('E' to 'I'), particularly in 'Desmayo Langueta,' where the difference between the lowest and highest lethal temperatures was only 0.58°C (Figure 2). Regarding  $LT_{50}$ , a continuous decrease in frost tolerance was observed as phenology advanced, from –4.81°C and –4.56°C at stage 'D' for 'Tardona' and 'Desmayo Langueta', respectively, to stage 'G', where  $LT_{50}$  values were –3.25°C and –3.61°C (Figure 2 and Supporting Information S4). After the fruit set (stage 'G'),  $LT_{50}$  remained moderately stable in both cultivars, with temperatures slightly above –3.0°C. A similar trend was observed for  $LT_{90}$ , where temperatures required to cause 90% damage started at –6.35°C and –5.56°C at stage 'D' for 'Tardona' and 'Desmayo Langueta', respectively, increased to



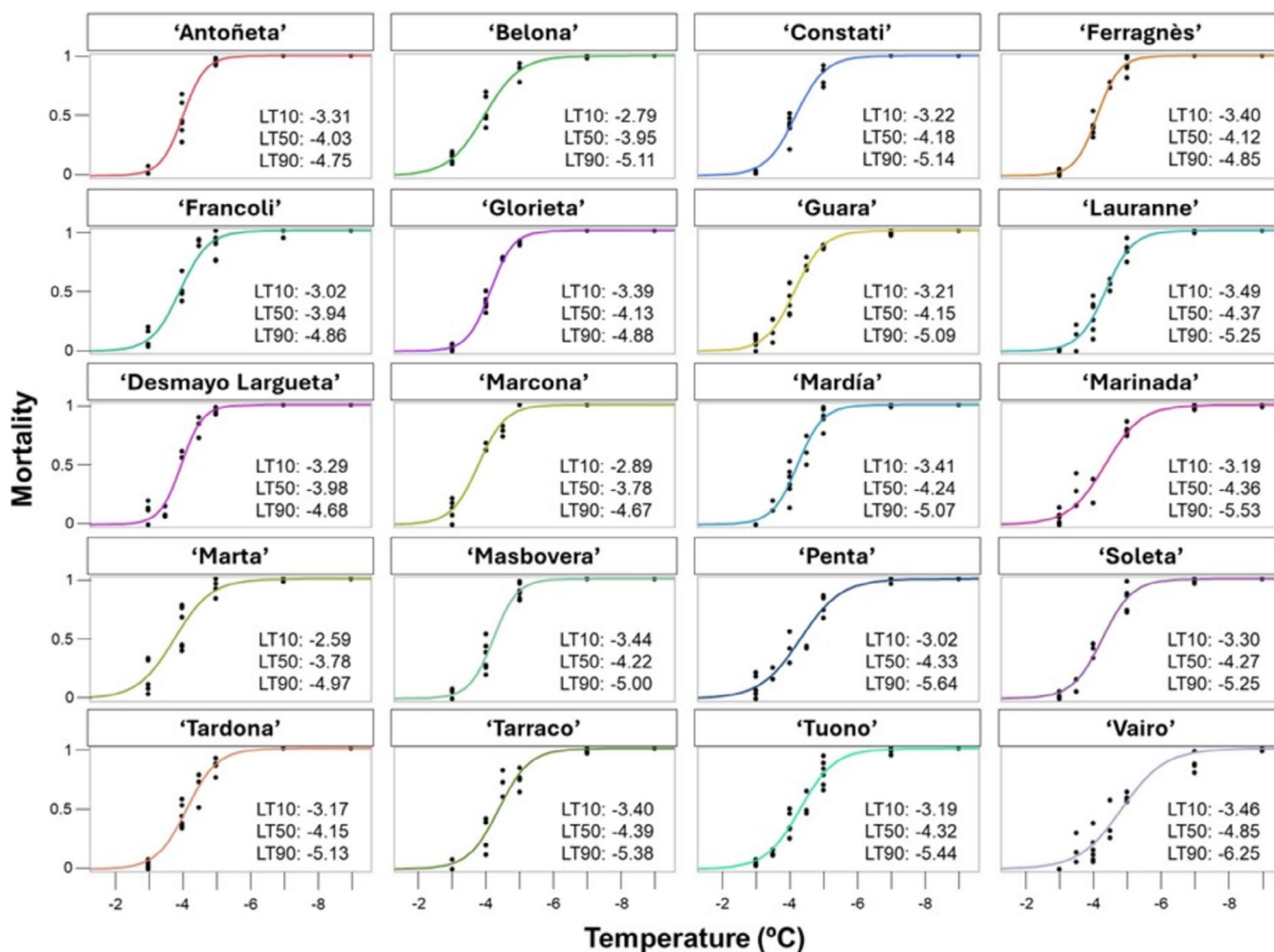
**FIGURE 2** | Frost susceptibility of 'Desmayo Largueta' and 'Tardona' almond cultivars at different phenological stages, ranging from closed buds with visible petals ('D') to small fruit ('I') in 2019. Lethal temperatures causing 10 (LT<sub>10</sub>), 50 (LT<sub>50</sub>) and 90% (LT<sub>90</sub>) of damage to flowers/fruitlets are indicated by solid lines, with the 95% confidence interval shown as shaded areas. Phenological scale based on Felipe (1977). Different letters, in the same lethal temperature, indicate statistically significant differences ( $p < 0.05$ ).

-3.78°C and -4.40°C until stage 'H', and then decreased again by stage 'I'.

Overall, both cultivars showed a clear and significant reduction in frost tolerance as they progressed through development, with the most vulnerable phenological stages occurring after the fruit set ('H' stage). Nonetheless, both cultivars exhibited slightly different LT<sub>10</sub>, LT<sub>50</sub> and LT<sub>90</sub> values, reflecting differing levels of frost tolerance across most stages.

### 3.2 | Flower Frost Tolerance Across Cultivars

Almond germplasm displayed varying levels of tolerance to flower freeze damage, as indicated by the percentage of flowers that were injured after exposure to freezing temperatures (Figure 3 and Supporting Information S5). Lethal temperatures (LT<sub>10</sub>; LT<sub>50</sub> and LT<sub>90</sub>) in 2016 and 2019 were calculated for each cultivar (Supporting Information S5). Since there were no statistically significant differences in lethal temperatures between



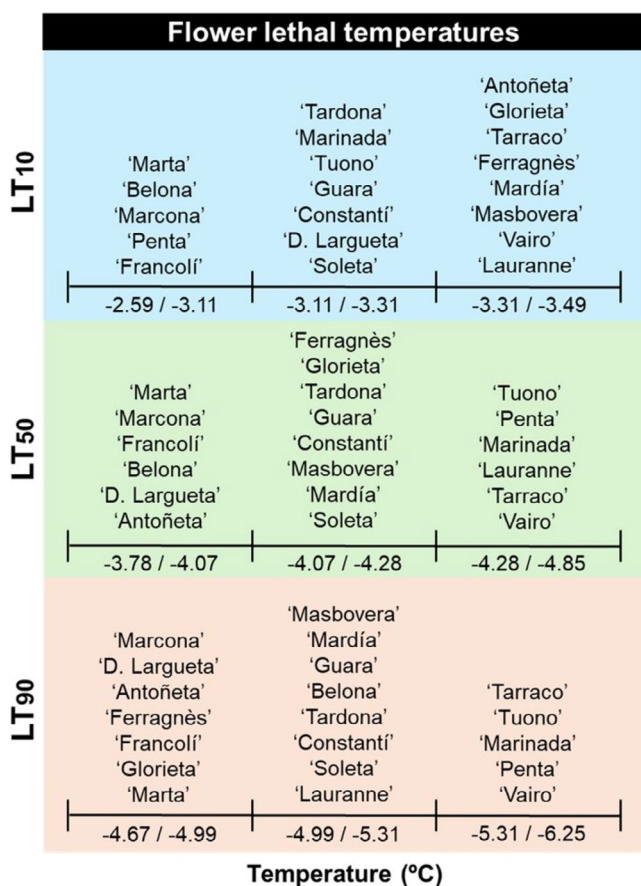
**FIGURE 3** | Susceptibility to frost damage of almond cultivars in phenological stage 'F' (full flowering) (combined data from 2016 and 2019). Lethal temperatures (°C) at which 10 (LT<sub>10</sub>), 50 (LT<sub>50</sub>) and 90% (LT<sub>90</sub>) of the flowers suffered damage (mortality) are indicated for each cultivar. Mortality indicated on a scale from 0 (no flower damage) to 1 (all flowers damage).

the 2 years ( $p < 0.05$ ), all data was pooled into a single regression, and lethal temperatures were calculated for each genotype. Over the combined data of 2 years (2016 and 2019), the temperature at which 10% of the flowers were damaged (LT<sub>10</sub>) ranged from  $-2.59^{\circ}\text{C}$  for 'Marta' to  $-3.49^{\circ}\text{C}$  for 'Lauranne' (Figure 3). Following 'Marta', 'Belona' ( $-2.79^{\circ}\text{C}$ ) and 'Marcona' ( $-2.89^{\circ}\text{C}$ ) were the next most susceptible cultivars. In contrast, 'Vairo' ( $-3.46^{\circ}\text{C}$ ) and 'Masbovera' ( $-3.44^{\circ}\text{C}$ ) showed damage temperatures similar to 'Lauranne'. As the percentage of damaged flowers increases (LT<sub>50</sub> and LT<sub>90</sub>), the temperatures decrease for all cultivars. At LT<sub>50</sub>, 'Vairo' was the most tolerant cultivar, with a temperature of  $-4.85^{\circ}\text{C}$ , followed by 'Tarraco' ( $-4.39^{\circ}\text{C}$ ), 'Lauranne' ( $-4.37^{\circ}\text{C}$ ) and 'Marinada' ( $-4.36^{\circ}\text{C}$ ). In contrast, 'Marta' and 'Marcona' remained the least tolerant cultivars, with LT<sub>50</sub> values of  $-3.78^{\circ}\text{C}$ , followed by 'Francoli' ( $-3.94^{\circ}\text{C}$ ) and 'Belona' ( $-3.95^{\circ}\text{C}$ ). At this temperature, 'Vairo' demonstrated a higher tolerance level than the most susceptible cultivars, 'Marta' and 'Marcona', with a difference of  $1.07^{\circ}\text{C}$ . When considering the temperatures required for 90% damage (LT<sub>90</sub>), 'Vairo' ( $-6.25^{\circ}\text{C}$ ) was once again the most tolerant cultivar, followed by 'Penta' ( $-5.64^{\circ}\text{C}$ ) and 'Marinada' ( $-5.53^{\circ}\text{C}$ ). The least tolerant cultivar at this damage percentage was 'Marcona' ( $-4.67^{\circ}\text{C}$ ), followed closely by 'Desmayo Largueta' ( $-4.68^{\circ}\text{C}$ ) and 'Antoñeta' ( $-4.75^{\circ}\text{C}$ ) (Figure 3).

Considering the distribution of the LT<sub>50</sub> for flowers, it was evident that most cultivars need temperatures between  $-4.0^{\circ}\text{C}$  and  $-4.5^{\circ}\text{C}$  to have 50% of flower damage (Figure 3). However, by comparing the obtained value of LT<sub>50</sub> with the interval estimated based on the distribution of lethal temperatures, the cultivars were clustered into three groups (cultivars with an LT<sub>50</sub> value lower, between, or higher than the interval). Consequently, the first group includes the cultivars 'Marta', 'Marcona', 'Francoli', 'Belona', 'Desmayo Largueta' and 'Antoñeta'. The second group is composed of 'Ferragnès', 'Glorieta', 'Tardona', 'Guara', 'Constanti', 'Masbovera', 'Mardía' and 'Soleta'. And the last group of cultivars with LT<sub>50</sub> higher than the estimated interval is composed of 'Penta', 'Tuono', 'Marinada', 'Lauranne', 'Tarraco' and 'Vairo'. The cultivars were found to cluster into similar groups across the three different lethal temperatures (LT<sub>10</sub>, LT<sub>50</sub> and LT<sub>90</sub>) (Figure 4).

### 3.3 | Physical Characterisation of Pistils

Significant differences ( $p < 0.05$ ) in pistil and ovary weights were observed among the analysed cultivars. The pistil weights ranged from 0.001 mg for 'Tardona' to 0.018 mg for 'Ferragnès', while ovary weights ranged from 0.001 to 0.017 mg (Figure 5).



**FIGURE 4** | Flower lethal temperatures (°C) for evaluated almond cultivars at phenological stage 'F' (combined data from 2016 and 2019). The ranges indicate the lethal temperatures at which 10 (LT<sub>10</sub>), 50 (LT<sub>50</sub>) and 90% (LT<sub>90</sub>) of the flowers suffered damage, with the interval estimated from a means analysis.

'Ferragnès', the cultivar with the statistically heaviest pistil and ovary, stands out from the others followed by 'Francolí' and 'Constantí' which presented pistil weights of 0.008 mg. In contrast, 'Tardona' has the lightest pistils and ovaries, followed by 'Desmayo Largueta' (0.001 mg) and 'Soleta' (0.002 mg). Although some cultivars exhibit nearly double the dry weight of others, very few statistically significant differences among the remaining cultivars were observed. Additionally, very few differences were observed in pistil humidity, which ranged from 68% for 'Francolí' to 82% for 'Desmayo Largueta' (Figure 4). Similarly, ovary humidity values ranged from 63% for 'Guara' to 82% for 'Desmayo Largueta'. No statistically significant differences ( $p < 0.05$ ) between pistil and ovary humidity were observed among the cultivars.

### 3.4 | Sugar Concentration in Almond Pistils

Sugar concentrations in the pistils of 15 almond cultivars were quantified in 2016 (Figure 6). Significant differences ( $p < 0.05$ ) in the content of the three measured sugars were reported between cultivars. Among these sugars, glucose and fructose were the most abundant, with concentrations ranging from 8.9 to 22.1 mg/g and 4.6 to 18.6 mg/g of dry weight, respectively.

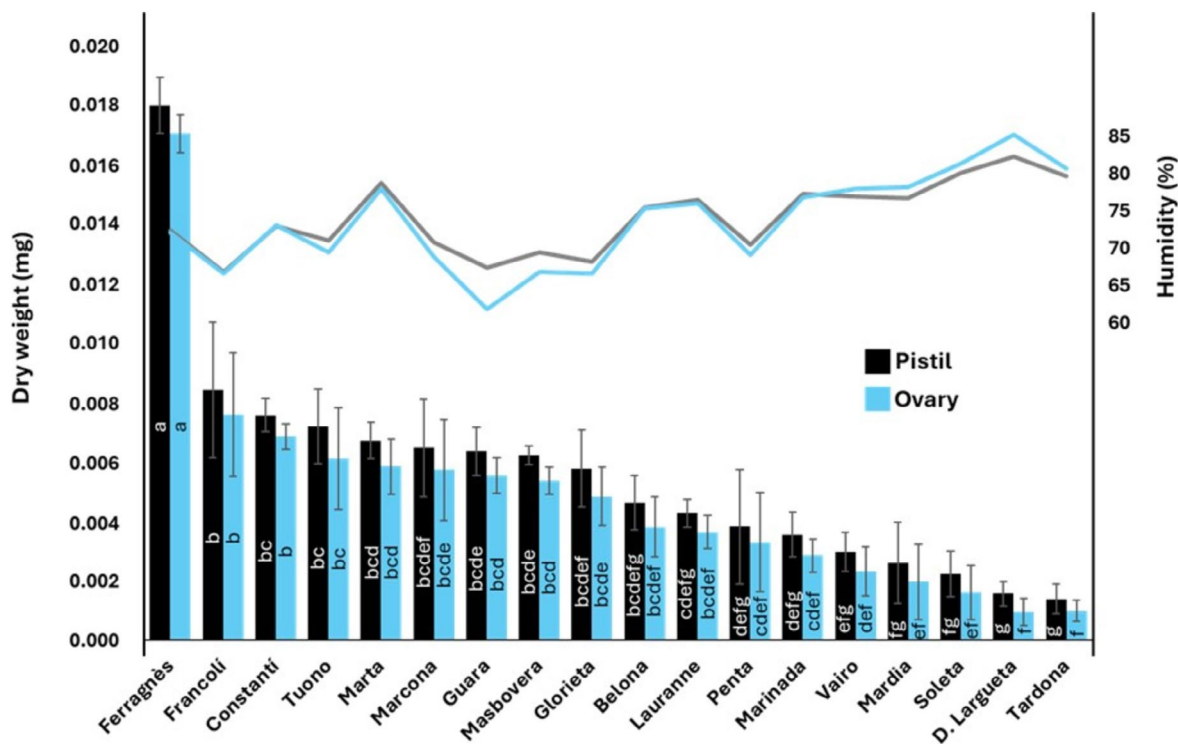
Sucrose was also present in the pistils but at lower concentrations, ranging from 2.5 to 8.7 mg/g (Figure 6). For glucose, 'Mardía' had the lowest concentration at 8.9 mg/g, followed by 'Penta' and 'Ferragnès' at 9.6 and 9.8 mg/g, respectively. In contrast, 'Desmayo Largueta' (22.1 mg/g), 'Vairo' (18.7 mg/g) and 'Lauranne' (18.3 mg/g) exhibited the highest concentrations of glucose. Regarding fructose, the pistils of 'Penta', 'Ferragnès' and 'Mardía' showed the lowest concentrations, ranging from 4.6 to 9.0 mg/g, similar to the glucose pattern. Meanwhile, 'Belona' and 'Soleta' were the only cultivars with fructose concentrations exceeding 17.0 mg/g. For sucrose, 'Penta' (2.5 mg/g), 'Ferragnès' (2.6 mg/g) and 'Desmayo Largueta' (4.0 mg/g) had the lowest concentrations, while 'Soleta' (8.2 mg/g), 'Belona' (8.2 mg/g) and 'Vairo' (8.7 mg/g) recorded the highest concentrations (Figure 6).

### 3.5 | Correlation Between Physical and Chemical Pistil Traits and Lethal Temperatures

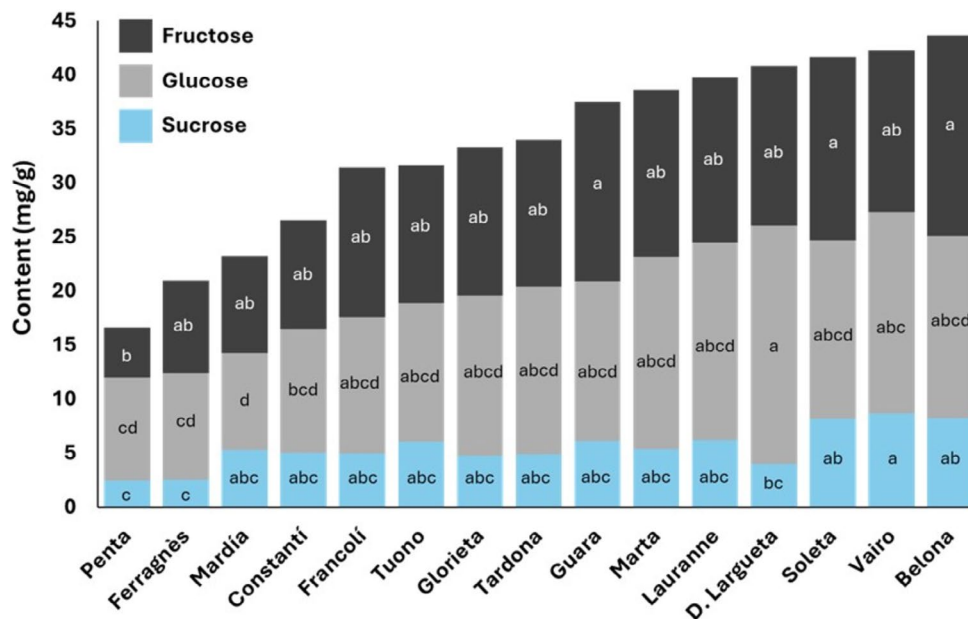
Significant correlations were found between years for most of the evaluated traits (2016 and 2019; Pearson correlation,  $p$ -value  $< 0.01$ ) (Figure 7A and Supporting Information S6). The strongest positive correlations between years were observed for pistil and ovary weights, with values ranging from 0.83 to 0.85. However, no significant correlation was found for pistil humidity between the 2 years. Regarding lethal temperatures, a significant positive correlation was observed at the full bloom stage ('F') in both years, with correlation coefficients ranging from 0.48 to 0.54 (Supporting Information S6).

To further investigate the interaction between physical and chemical traits with the lethal temperatures at the full bloom stage, we analysed trait correlations in 2016, as this was the only year in which all physical and chemical traits were measured together (Figure 7A and Supporting Information S6). A highly significant positive correlation was observed among sugars (glucose, fructose, sucrose and total sugars), ranging from 0.56 to 0.85 (Figure 7A). These sugars showed a negative correlation with pistil and ovary weights; however, only the correlation between sucrose content and ovary weight was significant ( $-0.50$ ). Similarly, pistil and ovary weights were negatively correlated with pistil humidity ( $-0.61$  and  $-0.59$ , respectively), although pistil humidity did not correlate with any other traits. In terms of lethal temperatures for flower damage, significant positive correlations were identified.

An interesting significant negative correlation was observed between pistil sucrose content and lethal temperatures at the full bloom stage (Figure 7A,B). Specifically, pistil sucrose content was significantly negatively correlated with flower LT<sub>50</sub> ( $-0.50$ ) and flower LT<sub>90</sub> ( $-0.65$ ). A negative correlation with flower LT<sub>10</sub> was also noted, although below the significance level. Examining this specific correlation (Figure 7B), there appears to be a tendency for cultivars with higher sucrose levels in the pistil to exhibit lower lethal temperatures. Specifically, cultivars with pistil sucrose content exceeding 6 mg/g were the only ones that exhibited a flower LT<sub>90</sub> below  $-5.0^{\circ}\text{C}$ , while those with sucrose levels below 6 mg/g did not reach this temperature (Figure 7B).



**FIGURE 5** | Mean and standard deviation values of pistil and ovary dry weights (bars) and humidity (lines) (combined data from 2016 and 2019). Different letters in the bars, for each organ (pistil and ovary), indicate statistically significant differences ( $p < 0.05$ ).

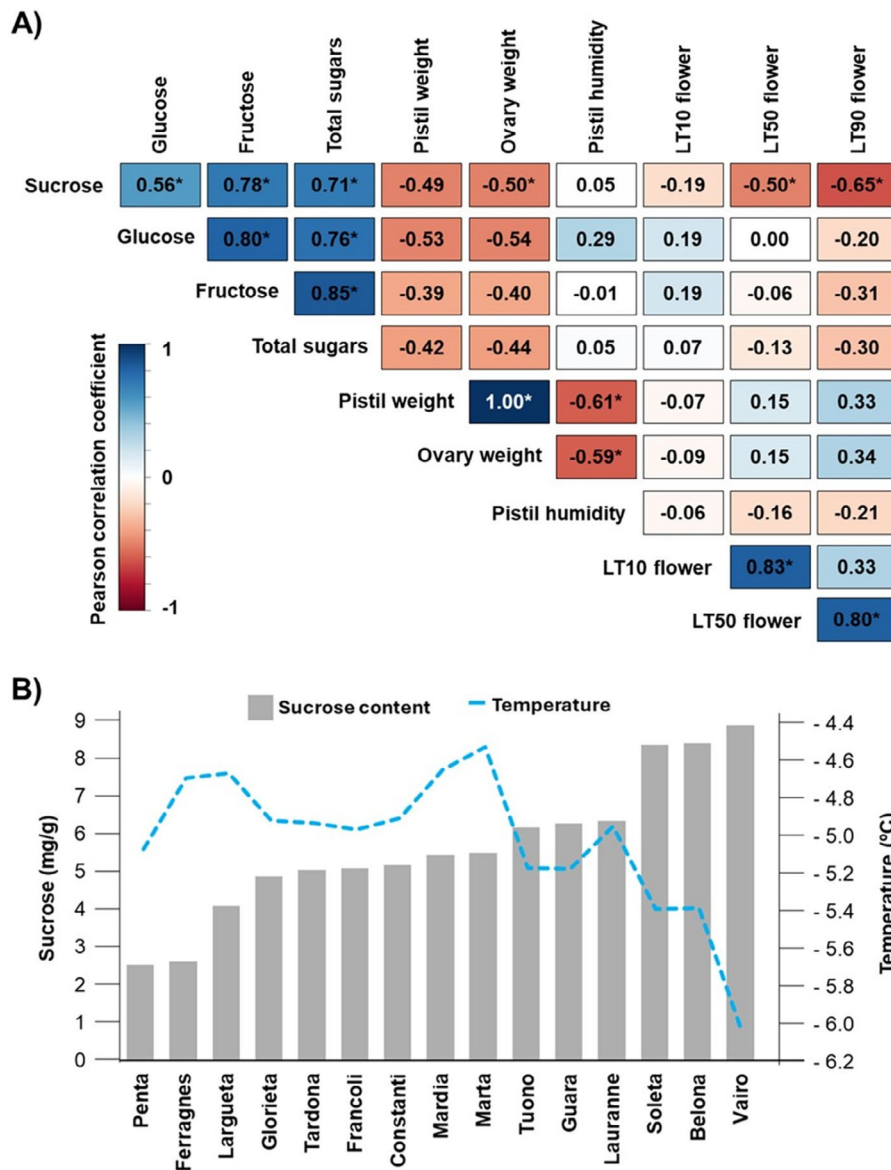


**FIGURE 6** | Content of sucrose, glucose and fructose (mg/g of dry weight) in the pistils of almond cultivars analysed in 2016. Different letters indicate statistically significant differences ( $p < 0.05$ ).

#### 4 | Discussion

Spring frosts during flowering can cause significant crop losses by damaging sensitive plant tissues and organs (Felipe 1977). One approach to minimising these losses is the use of late-flowering cultivars that avoid frost exposure, combined with active protection methods (Snyder and Connell 1996; Battle

et al. 2017; Yu and Lee 2020). Additionally, the use of chemical products for crop protection against freezing stress has also been proposed as an approach to reduce the impact of frost damage with different results (Román-Figueroa et al. 2021; Torres and Miarnau 2024). However, these methods alone are insufficient, and the use of certain plant growth regulators, nutrients, or anti-transpirant products for frost protection has shown inconsistent



**FIGURE 7** | (A) Pearson correlation coefficient among traits in 2016. Asterisks indicate correlation significance ( $*p < 0.01$ ). Positive and negative correlations are highlighted in blue and orange, respectively. (B) Relationship between the sucrose content and lethal temperature for frost damage of 90% of the flowers ( $LT_{90}$ ) at phenological stage 'F' for fifteen almond cultivars in 2016.

results (Perru 1998; Smith 2019). Moreover, several disadvantages have been reported, including high energy costs for some strategies and environmental impacts associated with these products. Similarly, even late-flowering almond cultivars have been affected by frost, despite their more delayed phenological development compared to earlier-blooming varieties (Guillamón et al. 2022). Thus, breeding for cultivars with enhanced frost tolerance during critical phenological stages, such as flowering and early fruit development, is still essential.

Nevertheless, despite the importance of frost tolerance in flowers and fruitlets, there has been limited research to support breeding for more resilient cultivars to this abiotic stress within the almond germplasm. Traditionally, the frost resistance of almond trees was assessed after natural frost events in field conditions (Kodad and Socias i Company 2005; Guillamón et al. 2022). This study evaluated flowers from each cultivar at the same phenological stage under controlled chamber conditions, enabling

easy comparisons between cultivars, as previously demonstrated in almond (Miranda et al. 2005; Imani et al. 2011), peach (Reig et al. 2013; Caglar et al. 2024), apricot (Viti et al. 2010; Kaya et al. 2018), plum (Szalay et al. 2017), apple (Salazar-Gutiérrez et al. 2016; Szalay et al. 2019) or sweet cherry (Kose and Kaya 2022; Kaya et al. 2021b). Additionally, the correlation between the lethal temperatures at the flowering stage observed across different years for the same cultivar remained consistently stable, highlighting the accuracy of this method for assessing frost tolerance phenotyping as earlier reported (Miranda et al. 2005). These approaches, developed under controlled conditions to understand plant responses to freezing temperatures, have demonstrated their effectiveness (Salazar-Gutiérrez et al. 2016; Kaya et al. 2018; Kaya and Kose 2019). In this context, the methodology proposed in this study, which simulates frost events using different thermal regimes under controlled conditions, has previously been shown to effectively replicate the effects of natural frosts. Models employing differential thermal

analysis have proven successful in simulating freezing conditions and predicting cell death in various floral organs, closely mirroring field conditions (Kodad et al. 2010; Reig et al. 2013; Caglar et al. 2024).

The relationship between frost damage to flowers and fruitlets and freezing temperatures has been previously studied across different phenological stages under controlled conditions in the almond cultivars 'Marcona' and 'Ferragnès' (Miranda et al. 2005). In this study, the frost tolerance patterns in 'Tardona' and 'Desmayo Langueta' from stages 'D' to 'I' were similar to those observed in almonds and other *Rosaceae* species during the same stages (Szalay et al. 2019). The tolerance level decreases from the budbreak stage (phenological stage D) to the fruitlet stage (H) with the most critical stage found after flowering, particularly during the fruit set stage. Throughout the winter, temperate fruit trees protect their reproductive organs within buds. These buds are hardy during winter, but once they approach dormancy release, they begin to swell and open into blossoms, exposing the reproductive organs and increasing the impact of low temperatures on them (Rodrigo 2000). The temperatures causing damage varied between cultivars and phenological stages. In Miranda et al. (2005), temperatures required to cause 10% damage in the productive organs (flower buds, flowers and fruitlets) ranged from  $-6.6^{\circ}\text{C}$  to  $-2.4^{\circ}\text{C}$  with the less and most susceptible stages being 'B' and 'I', respectively. These temperatures were similar to that observed for 'Tardona' and 'Desmayo Langueta' in the current study. However, more severe damage (90%) requires lower temperatures. For example, in the assessment of 'Marcona' and 'Ferragnès' (Miranda et al. 2005), the lethal temperatures causing 90% damage at stages 'D', 'F' and 'I' were  $-10.3^{\circ}\text{C}$ ,  $-4.8^{\circ}\text{C}$  and  $-3.6^{\circ}\text{C}$ , respectively, closely matching the results from our study for 'Tardona' ('D':  $-6.3^{\circ}\text{C}$ ; 'F':  $-5.2^{\circ}\text{C}$ ; 'I':  $-3.9^{\circ}\text{C}$ ) and 'Desmayo Langueta' ('D':  $-5.5^{\circ}\text{C}$ ; 'F':  $-4.6^{\circ}\text{C}$ ; 'I':  $-4.4^{\circ}\text{C}$ ). Based on these temperatures, we may conclude that almond tolerance to freezing temperatures exhibits a common pattern within the species. Initially, dormant buds show high frost tolerance, followed by a transition period where the buds begin to swell and gradually lose their freezing tolerance and a final stage in which flowers, and especially fruitlets, become highly susceptible to frost damage. The critical vulnerability of fruitlets is connected to the fact that frost damage is primarily caused by ice formation rather than the low temperatures themselves (Pramsohler and Neuer 2013). As fruitlets contain a higher water content than other organs, such as flowers, they are more susceptible to ice crystal formation, finally causing cellular injuries that result in frost damage.

While a common pattern of freezing tolerance has been observed in almonds, susceptibility levels vary among cultivars, as demonstrated in this study and previous research (Imani et al. 2023; Keleta et al. 2023; Szalay et al. 2022; Moheb et al. 2018; Hadavand et al. 2021). The critical temperature at which a reproductive organ becomes susceptible to frost damage influences its adaptability, helping to identify the cultivars best suited for specific conditions. In this study, the lethal temperatures causing flower damage showed a consistent pattern across cultivars in both years, suggesting potential genetic control of this trait, as already indicated for peach (Caglar et al. 2024). In peach, analysis of a dataset comprising 51 cultivars indicated that 52% to 85% of the phenotypic variability in fruitless freeze tolerance could be

attributed to genetic factors (Caglar et al. 2024). Unfortunately, due to the limited number of cultivars evaluated and the restricted years of phenotypic data collection in the present study, it was not possible to quantify the proportion of variability attributable to genetic effects. Among the cultivars studied, 'Vairo' emerged as the most tolerant, followed by other cultivars such as 'Tarraco', 'Lauranne', 'Marinada', 'Tuono' and 'Penta'. In contrast, cultivars like 'Marta', 'Marcona' and 'Desmayo Langueta' exhibited higher susceptibility to freezing temperatures. These results align with previous findings for some Spanish almond cultivars (Kodad et al. 2010). In both studies, traditional cultivars like 'Marcona' and 'Desmayo Langueta' showed a high level of susceptibility to freezing, whereas 'Masbovera', one of the most tolerant cultivars in Kodad et al. (2010), also proved to be highly tolerant in our study. This pattern was further confirmed by field data observed after natural frost events (Kodad and Socias i Company 2005). It is noteworthy that no correlation was observed between flowering dates and tolerance to freezing temperatures (data not shown), suggesting that separate mechanisms control these two important traits.

In this study, physiological and chemical parameters were measured to assess their potential influence on frost tolerance. Despite variations in pistil and ovary weights among almond cultivars, no correlations were found between these differences and increased resistance to freezing temperatures at the flower stages. Similarly, previous research investigated the effect of pistil moisture content on susceptibility to freezing temperatures (Rodrigo 2000; Reig et al. 2013). While some early studies noted that higher water content negatively impacted ovary cold hardiness, the differences were insufficient to explain the variation in frost tolerance between cultivars, as reported in this work, but also in peach (Reig et al. 2013). This suggests that although almond pistil humidity and weight vary within the germplasm, they do not significantly affect susceptibility to freezing temperatures. On the other side, differences in sugar content were observed in the pistils of the evaluated almond cultivars, consistent with previous studies that showed variation in sugar levels across species, genotypes and flower organs (Shin et al. 2015; Chai et al. 2019; Kaya, et al. 2021a). These sugars play a crucial role in protecting flower organs against frost by lowering osmotic potential, which helps plants to respond to low temperatures. Among these sugars, fructose and glucose levels were higher than sucrose, as also reported in apricot and pear cultivars (Pedryc et al. 2006; Kaya et al. 2021a; Sekozawa et al. 2003). However, despite being the least abundant sugar in the pistils, sucrose showed the strongest correlation with frost tolerance at the flowering stage. Higher sucrose content in the pistil was associated with greater protection against freezing temperatures. Sucrose acts as a cryoprotectant for proteins and membranes during freezing stress, which is considered a key factor in enhancing frost tolerance, and it has been reported to function as a signalling molecule that modulates the expression of genes involved in stress responses, which could explain the high potential of this sugar on low temperature protection (Renaut et al. 2004; Pagter et al. 2015). This relationship was previously noted in apricot cultivars and other plant species, where increases in sugar content were inversely related to the temperatures that cause cell death (Wang et al. 2020). Among sugars, sucrose has been identified as the most responsive to freezing temperatures in the buds of many deciduous trees

(Guy et al. 1992; Palonen et al. 2000; Pedryc et al. 2006; Pagter et al. 2015; Lee et al. 2012). Moreover, sucrose has been observed to be one of the disaccharides offering the largest protection against solute leakage in the cell from membrane fusion (van den Gogaart et al. 2007). Thus, sucrose appears to be a key factor in enhancing cultivar tolerance to freezing temperatures by regulating osmotic pressure within cells and likely inhibiting the formation of ice crystals, which are the main cause of frost damage. However, since sugar content was measured in only one of the evaluated years in this work, further studies are needed to confirm this finding, despite the strong correlation observed and its support in the existing literature.

## 5 | Conclusions

This study highlights the susceptibility variation to freezing temperatures in the flowers of almond cultivars, where both physiological and chemical traits seem to play crucial roles in determining their resilience to this abiotic stress. While a general pattern of freezing susceptibility was observed across cultivars, starting with high tolerance in dormant buds and declining through the flowering and fruitlet stages, significant variation was noted across cultivars. Additionally, the findings from this study confirm that traits like pistil weight and moisture content are not reliable indicators of frost resistance in almond, whereas sucrose emerges as a key factor likely conferring tolerance to freezing temperatures at the flowering stage. This sugar may enhance frost tolerance by regulating osmotic pressure and preventing ice crystal formation, offering valuable insight into breeding programmes aimed at improving almond tolerance to frost. The varying sucrose levels in the pistils of the evaluated cultivars, combined with differences in lethal temperatures and the correlation between these traits, also highlight the genetic basis of frost tolerance and the potential for breeding more resilient cultivars.

### Author Contributions

Conceptualization: X.M.; methodology: A.C., P.G.B., L.T., J.G.-B., G.R., X.M.; data curation: A.C., P.G.B., L.T., J.G.-B., G.R., X.M.; investigation: A.C., P.G.B., L.T., J.G.-B., G.R., X.M.; validation: A.C., P.G.B.; formal analysis: A.C., P.G.B., L.T., J.G.-B., G.R., X.M.; funding acquisition: X.M.

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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### References

Alisoltani, A., B. Shiran, H. Fallahi, and E. Ebrahimie. 2015. "Gene Regulatory Network in Almond (*Prunus dulcis* Mill.) in Response to Frost Stress." *Tree Genetics & Genomes* 11: 100.

Alonso, J. M., R. Socias i Company, and O. Kodad. 2017. "Late-Blooming in Almond: A Controversial Objective." *Scientia Horticulturae* 224: 61–67.

Assmann, A. P., I. Citadin, M. C. Locatelli, S. Scariot, M. A. Danner, and M. Raseira. 2008. "Fruit Frost Tolerance in Peach." *Revista Brasileira de Fruticultura* 30: 1030–1035.

Batlle, I., F. Dicenta, R. Socias i Company, et al. 2017. "Classical Genetics and Breeding." In *Almonds: Botany, Production and Uses*, edited by R. Socias i Company, and T. M. Gradziel. CABI.

Benmoussa, H., M. Ghrab, M. Ben, and E. Luedeling. 2017. "Chilling and Heat Requirements for Local and Foreign Almond (*Prunus dulcis* Mill.) Cultivars in a Warm Mediterranean Location Based on 30 Years of Phenology Records." *Agricultural and Forest Meteorology* 239: 34–46.

Bielsa, B., J. I. Ávila-Alonso, Á. Fernández i Martí, J. Grimplet, and M. J. Rubio-Cabetas. 2021. "Gene Expression Analysis in Cold Stress Conditions Reveals *BBX20* and *CLO* as Potential Biomarkers for Cold Tolerance in Almond." *Horticulturae* 7, no. 12: 527.

BOE. 2002. *Real Decreto 1201/2002, de 20 de Noviembre, Por el Que se Regula la Producción Integrada de Productos Agrícolas*. Boletín Oficial del Estado.

Burke, M. J., L. V. Gusta, H. A. Quamme, C. J. Weiser, and P. H. Li. 1976. "Freezing and Injury in Plants." *Annual Review of Plant Physiology* 27: 507–528.

Caglar, U., J. M. Lawton, J. C. Melgar, and K. Gasic. 2024. "Fruitlet Freeze Tolerance in Peach Germplasm." *Agronomy* 14: 302.

Calle, A., L. Aparicio-Durán, I. Batlle, I. Eduardo, and X. Miarnau. 2025. "Review of Agronomic and Kernel Quality Traits of 273 Almond Cultivars." *Genetic Resources and Crop Evolution* 72: 3783–3828. <https://doi.org/10.1007/s10722-024-02183-0>.

Chai, F., W. Liu, Y. Xiang, et al. 2019. "Comparative Metabolic Profiling of *Vitis vinifera* During Cold Acclimation." *Horticulture Research* 6, no. 1: 1–12.

Chen, C., W. R. Okie, and T. G. Beckman. 2016. "Peach Fruit Set and Buttoning After Spring Frost." *HortScience* 51, no. 7: 816–821.

Fadón, E., S. Herrera, B. I. Guerrero, M. E. Guerra, and J. Rodrigo. 2020. "Chilling and Heat Requirements of Temperate Stone Fruit Trees (*Prunus* sp.)." *Agronomy* 10, no. 3: 409.

Felipe, A. J. 1977. "Almendro: Estados Fenológicos." *ITEA—Información Técnica de Economía Agraria* 27: 8–9.

Felipe, A. J., X. Rius, and M. J. Rubio-Cabetas. 2017. "El Cultivo del Almendro. El Almendro II." 978-0-646-85851-7."

Finney, D. J. 1971. *Probit Analysis*, 333. Cambridge University Press.

Giné-Bodonaba, J., and L. A. Terry. 2010. "Manipulating the Taste-Related Composition of Strawberry Fruits (*Fragaria × ananassa*) From Different Cultivars Using Deficit Irrigation." *Food Chemistry* 122: 1020–1026.

Guillamón, J. G., J. Egea, F. Mañas, J. A. Egea, and F. Dicenta. 2022. "Risk of Extreme Early Frosts in Almond." *Horticulturae* 8, no. 8: 687.

Guy, C. L., J. L. Huber, and S. C. Huber. 1992. "Sucrose Phosphate Synthase and Sucrose Accumulation at Low Temperature." *Plant Physiology* 100, no. 1: 502–508.

Hadavand, N., A. Imani, M. Esnaashari, A. Ershadi, and R. Haghjooyan. 2021. "Evaluation of Cold Tolerance of Almond Cultivars and Genotypes in Open Flower Stage." *Acta Horticulturae* 1315: 319–322.

Hosseinpour, B., S. Sepahvand, K. Kamali Aliabad, et al. 2017. "Transcriptome Profiling of Fully Open Flowers in a Frost-Tolerant Almond Genotype in Response to Freezing Stress." *Molecular Genetics and Genomics* 293: 151–163.

Imani, A., K. Barzegar, and S. Piripireivatlou. 2011. "Relationship Between Frost Injury and Ion Leakage as an Indicator of Cold Hardiness

- in 60 Almond Selections." *International Journal of Nuts and Related Sciences* 2, no. 1: 22–26.
- Imani, A., M. Ezaddost, F. Asgari, S. H. Masoumi, and I. Raeisi. 2012. "Evaluation the Resistance of Almond to Frost in Controlled and Field Conditions." *International Journal of Nuts and Related Sciences* 3, no. 1: 29–36.
- Imani, A., and Y. Mahamadkhani. 2011. "Characteristics of Almond Selections in Relation to Late Frost Spring." *International Journal of Nuts and Related Sciences* 2, no. 2: 31–34.
- Imani, A., H. Torkaman, V. Rasoli, and K. Zandifar. 2023. "Screening of Almond Cultivars and Genotypes in Relation to Frost Stress Tolerance." *Iranian Journal of Plant Physiology* 3, no. 13: 4599–4608.
- Kaya, O., and C. Kose. 2019. "Cell Death Point in Flower Organs of Some Apricot (*Prunus armeniaca* L.) Cultivars at Subzero Temperatures." *Scientia Horticulturae* 249: 299–305.
- Kaya, O., C. Kose, V. Donderalp, T. Gecim, and S. Taskın. 2020. "Last Updates on Cell Death Point, Bud Death Time and Exothermic Characteristics of Flower Buds for Deciduous Fruit Species by Using Differential Thermal Analysis." *Scientia Horticulturae* 270: 109403.
- Kaya, O., C. Kose, and T. Gecim. 2018. "An Exothermic Process Involved in the Late Spring Frost Injury to Flower Buds of Some Apricot Cultivars (*Prunus Armeniaca* L.)." *Scientia Horticulturae* 241: 322–328.
- Kaya, O., C. Kose, A. Esitken, M. Turan, and O. Utku. 2021a. "Can Organic Acid and Sugar Compositions Be Used to Predict Cell Death Point Limits? Receptacle and Pistil Organs of Apricot (*Prunus armeniaca* L.)." *Rendiconti Lincei. Scienze Fisiche e Naturali* 32: 493–509.
- Kaya, O., C. Kose, and M. Sahin. 2021b. "The Use of Differential Thermal Analysis in Determining the Critical Temperatures of Sweet Cherry (*Prunus avium* L.) Flower Buds at Different Stages of Bud Burst." *International Journal of Biometeorology* 65: 1125–1135.
- Keleta, B. T., Z. Békefi, J. L. Bakos, D. Örsi, and L. Szalay. 2023. "Frost Hardiness of Almond Flower Buds During Dormancy." *Acta Biologica Szegediensis* 66, no. 2: 170–179.
- Khorshide, S., G. Davarynejad, F. Azmoode, and M. Kamlei. 2014. "Evaluation of Susceptibility of Pear and Plum Cultivars to Winter Frost." *Folia Horticulturae* 26, no. 2: 103–108.
- Kodad, O., F. Morales, and R. Socias i Company. 2010. "Evaluación de la Tolerancia de las Flores de Almendro a las Heladas por la Fluorescencia de la Clorofila." *ITEA - Información Técnica de Economía Agraria* 106, no. 2: 142–150.
- Kodad, O., and R. Socias i Company. 2005. "Daños Diferenciales por Heladas en Flores y Frutos y Criterios de Selección Para la Tolerancia a Heladas en el Almendro." *ITEA - Información Técnica de Economía Agraria* 101, no. 4: 349–363.
- Kose, C., and O. Kaya. 2022. "Differential Thermal Analysis Reveals the Sensitivity of Sweet Cherry Flower Organs to Low Temperatures." *International Journal of Biometeorology* 66: 987–994.
- Lee, J. H., D. J. Yu, S. J. Kim, D. Choi, and H. J. Lee. 2012. "Intraspecies Differences in Cold Hardiness, Carbohydrate Content and  $\beta$ -Amylase Gene Expression of *Vaccinium corymbosum* During Cold Acclimation and Deacclimation." *Tree Physiology* 32, no. 12: 1533–1540.
- Lorite, I. J., J. M. Cabezas-Luque, O. Arquero, et al. 2020. "The Role of Phenology in the Climate Change Impacts and Adaptation Strategies for Tree Crops: A Case Study on Almond Orchards in Southern Europe." *Agricultural and Forest Meteorology* 295: 108142.
- Martínez-Gómez, P., A. S. Prudencio, T. M. Gradziel, and F. Dicenta. 2017. "The Delay of Flowering Time in Almond: A Review of the Combined Effect of Adaptation, Mutation and Breeding." *Euphytica* 213: 197.
- Melgar, J. C., Q. Zhou, B. T. Lawrence, K. Gasic, J. E. Preece, and C. DeBuse. 2022. "Tolerance of Peach Fruitlets to Late Spring Freezes." *Acta Horticulturae* 1346: 191–194.
- Miranda, C., L. G. Santesteban, and J. B. Royo. 2005. "Variability in the Relationship Between Frost Temperature and Injury Level for Some Cultivated *Prunus* Species." *HortScience* 40, no. 2: 357–361.
- Moheb, M. B., A. Imani, and M. Shamili. 2018. "The Evaluation of Almond Progenies of Cold-Susceptible and Cold-Tolerant Parents (Filippo Ceo x Shahrood 12)." *Scientia Horticulturae* 234: 176–186.
- Pagter, M., U. B. Andersen, and L. Andersen. 2015. "Winter Warming Delays Dormancy Release, Advances Budburst, Alters Carbohydrate Metabolism and Reduces Yield in a Temperate Shrub." *AoB Plants* 7: plv024.
- Palonen, P., D. Buszard, and D. Donnelly. 2000. "Changes in Carbohydrates and Freezing Tolerance During Cold Acclimation of Red Raspberry Cultivars Grown *In Vitro* and *In Vivo*." *Physiologia Plantarum* 110, no. 3: 393–401.
- Palta, J. P., and L. S. Weiss. 2018. "Ice Formation and Freezing Injury: An Overview on the Survival Mechanisms and Molecular Aspects of Injury and Cold Acclimation in Herbaceous Plants." In *Advances in Plant Cold Hardiness*, ISBN 9781351069526. CRC Press.
- Pedryc, A., J. Korbuly, and E. Sárdi. 2006. "Relationship Between Sugar Composition of the Buds and Frost Tolerance in Apricot Cultivars." *Acta Horticulturae* 701: 57–62.
- Perru, K. B. 1998. "Basic of Frost and Freeze Protection for Horticultural Crops." *Hort Technology* 8: 10–15.
- Pramsohler, M., and G. Neuer. 2013. "Dehydration and Osmotic Adjustment in Apple Stem Tissue During Winter as It Relates to the Frost Resistance of Buds." *Tree Physiology* 33, no. 8: 807–816.
- Reig, G., I. Iglesias, C. Miranda, F. Gatiús, and S. Alegre. 2013. "How Does Simulated Frost Treatment Affect Peach [*Prunus persica* (L.)] flowers of Different Cultivars From Worldwide Breeding Programmes?" *Scientia Horticulturae* 160: 70–77.
- Renaut, J., S. Lutts, L. Hoffmann, and J. F. Hausman. 2004. "Responses of Poplar to Chilling Temperatures: Proteomic and Physiological Aspects." *Plant Biology* 6: 81–90.
- Ritonga, F. N., and S. Chen. 2020. "Physiological and Molecular Mechanism Involved in Cold Stress Tolerance in Plants." *Plants* 9, no. 5: 560.
- Rodrigo, J. 2000. "Spring Frost in Deciduous Fruit Trees. Morphological Damage and Flower Hardiness." *Scientia Horticulturae* 92: 155–173.
- Rodrigo, J., C. Julián, and M. Herrero. 2006. "Spring Frost Damage in Buds, Flowers and Developing Fruits in Apricot." *Acta Horticulturae* 717: 87–88.
- Román-Figueroa, C., L. Bravo, M. Paneque, R. Navia, and M. Cea. 2021. "Chemical Products for Crop Protection Against Freezing Stress: A Review." *Journal of Agronomy and Crop Science* 207: 391–403.
- Salazar-Gutiérrez, M. R., B. Chaves, and G. Hoogenboom. 2016. "Freezing Tolerance of Apple Flower Buds." *Scientia Horticulturae* 198: 344–351.
- Sekozawa, Y., S. Sugaya, H. Gemma, and S. Iwahori. 2003. "Cold Tolerance in 'Kousui' Japanese Pear and Possibility for Avoiding Frost Injury by Treatment With n-Propyl Dihydrojasmonate." *HortScience* 38, no. 2: 288–292.
- Shin, H., Y. Oh, and D. Kim. 2015. "Differences in Cold Hardiness, Carbohydrates, Dehydrins and Related Gene Expressions Under an Experimental Deacclimation and Reacclimation in *Prunus persica*." *Physiologia Plantarum* 154, no. 4: 485–499.
- Simons, R. K., and C. C. Doll. 1976. "Morphological and Anatomical Response of Apples to a Late Spring Frost in Relation to Stage of Fruit Development." *Journal of the American Society for Horticultural Science* 101: 315–320.
- Smith, E. D. 2019. "Cold Hardiness and Options for the Freeze Protection of Southern Highbush Blueberry." *Agriculture* 9: 9.

Snyder, R. L., and J. H. Connell. 1996. "Frost Protection." In *Almond Production Manual*, edited by W. C. Micke, 155–166. University of California.

Szalay, L., Z. György, and M. Tóth. 2019. "Frost Hardiness of Apple (*Malus × domestica*) Flowers in Different Phenological Phases." *Scientia Horticulturae* 253: 309–315.

Szalay, L., B. T. Keleta, J. L. Bakos, and Z. Békefi. 2022. "Frost Hardiness of Flower Buds of Three Hungarian Almond Cultivars During Dormancy." *Acta Agriculturae Slovenica* 118, no. 1: 1–9.

Szalay, L., A. Molnár, and S. Kovács. 2017. "Frost Hardiness of Flower Buds of Three Plum (*Prunus domestica* L.) Cultivars." *Scientia Horticulturae* 214: 228–232.

Torres, E., and X. Miarnau. 2024. "Frost Damage Mitigation in Flowers and Fruitlets of Peach and Almond From the Application of a Multi-Attribute Approach Biostimulant." *Plants* 13, no. 12: 1603.

van den Gogaart, G., N. Hermans, V. Krasnikov, A. H. de Vries, and B. Poolman. 2007. "On the Decrease in Lateral Mobility of Phospholipids by Sugars." *Biophysical Journal* 92: 1598–1605.

Viti, R., S. Bartolini, and L. Andreini. 2010. "Flower Bud Frost Tolerance of Several Italian Apricot Genotypes." *European Journal of Horticultural Science* 75, no. 5: 185–192.

Wang, J., M. Lv, H. He, Y. Jiang, J. Yang, and S. Ji. 2020. "Glycine Betaine Alleviated Peel Browning in Cold-Stored 'Nanguo' Pears During Shelf Life by Regulating Phenylpropanoid and Soluble Sugar Metabolisms." *Scientia Horticulturae* 262: 109100.

Yu, D. J., and H. J. Lee. 2020. "Evaluation of Freezing Injury in Temperate Fruit Trees." *Horticulture, Environment, and Biotechnology* 61: 787–794.

### Supporting Information

Additional supporting information can be found online in the Supporting Information section.