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Fruitlet Freeze Tolerance in Peach Germplasm

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Abstract: Climate change is affecting the production of temperate fruit crops. Freeze damage, particularly in spring, has resulted in significant economic losses in peach production in the southeastern United States. Research efforts in peach and other *Prunus* species have primarily focused on dormancy-related traits associated with bloom time, such as chill and heat requirement, with fruitlet freeze tolerance not equally represented. This study reports fruitlet freeze tolerance in 75 peach and nectarine accessions at six freezing temperatures (0 to −10 °C) using electrolyte leakage method over two seasons (2022–2023). Fruitlet freeze tolerance ranged from −3.9 to −10.2 °C with an estimated freeze damage ranging from 16–48% of fruitlet tissue with the majority of the accessions showing tolerance to cold temperatures in the −4 to −6 °C and 25–35% range. Variability in tolerance was noted across years, including some inconsistencies between tolerance group assignments. Grouping based on the estimated damage showed better stability and some accessions changed their grouping from the extremes to an intermediate tolerance group. Interestingly, nectarine accessions were among the most tolerant in both seasons. Broad-sense heritability of 0.52 and 0.85, estimated for freeze tolerance and % tissue damage, respectively, suggested genetic control of this trait with a potential for improvement via breeding.

Keywords: broad-sense heritability; climate change; cold stress; electrolyte leakage; late spring frosts; *Prunus persica*

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1. Introduction

Climate change, manifested as an increase in temperature and or episodes of temperature spikes during winter, and insufficient cold exposure during dormancy, increases tree vulnerability to spring frosts, and threatens fruit production worldwide [1,2]. Freeze damage poses a significant ecological risk to stone fruit crops, and destructive spring frosts are projected to intensify due to global climate change [3]. Peach (*Prunus persica* [L.] Batsch) is a highly economically valuable temperate fruit tree that is widely cultivated in moderate climatic zones throughout the world and, therefore, affected by climate change. Peach trees exhibit a remarkable adaptation to seasonal climatic variations by experiencing a period of dormancy during the winter months [4]. This evolutionary process involves shedding their leaves and entering a stage of reduced metabolic activity (endo-dormancy). Then, once trees have experienced adequate chilling temperatures, they are released from dormancy (eco-dormancy) and start developing and blooming in response to warm temperatures. Increasingly warmer winters, as well as weather patterns with more severe winter and spring temperature fluctuations, disrupt this normal pattern. Warmer winters can cause trees to bloom prematurely and expose their flowers or fruitlets to lethal freezing temperatures. This scenario has caused disastrous losses of the peach crop in the southeastern U.S. in recent years. Numerous research has focused on assessing the susceptibility of trees, buds, and flowers to freezing events [5–7], but the fruitlet freeze tolerance is relatively understudied [8–10].

Various methods are available for assessing freeze damage, such as visual evaluation of tissue discoloration, thermal analysis, measure of electrolyte leakage, and triphenyl tetrazolium chloride reduction analysis, the selection and implementation of which mostly depend on the organ or tissue that is being assessed [7]. Dexter et al. [11] first noted that freezing temperatures led to the destabilization of cellular membranes and an accelerated release of symplastic solutes from cells, e.g., electrolyte leakage. The electrolyte leakage has evolved into a standard method for assessing the relative quantity of cell damage in many species in reaction to biotic and abiotic stresses, including cold stress [12–16]. The electrolyte leakage, and temperature at which 50% of tissue experiences damage (LT50), used to evaluate fruitlet freeze tolerance in selected peach germplasm from the National Clonal Germplasm Repository (NCGR) in Davis, CA, and Clemson University [10] revealed freeze tolerance to temperatures as low as −10 °C, with the majority of accessions exhibiting tolerance within −5 and −6 °C. The highest tolerance, <−8 °C, was observed in several cultivars from various breeding programs released during last five decades ('White Lady', 'Scarletpearl', 'Raritan Rose', 'Manon', 'MA Blake', 'Canadian Harmony', 'Harrow Diamond', and 'Sugar Giant') suggesting that diversity for this trait in peach germplasm could be explored in breeding. However, the graphical presentation of the LT50 results revealed that the two accessions with similar LT50 values have distinctively different asymmetric sigmoid curve patterns. The authors suggested that further investigation is needed to determine how best to describe the level of freeze damage in the fruitlets. The research highlighted the possibility of harnessing the genetic potential for freeze tolerance in peach breeding to address the effects of changing climate, and predicted more frequent late spring frosts on stone fruit production [17–20]. However, they did not investigate the heritability of this trait to support the suggestion that breeding for improved fruitlet freeze tolerance in peaches might be possible.

The breeding goals in peach breeding programs have evolved significantly over the decades [21,22]. Initially, the focus was primarily on improving traits related to yield, disease resistance, and adaptability to specific growing regions. However, as consumer preferences and environmental concerns evolved, breeding goals shifted towards quality attributes, such as fruit flavor, appearance, and nutritional content [23,24]. Importance of breeding for climate resilience or plasticity became more emphasized with production disruptions caused by climate change. However, focus was on the dormancy related traits, such as lowering chilling requirement and delaying bloom time [25,26], and not fruitlet freeze tolerance. Genetic studies of the traits associated with climate resilience, such as chilling and heat requirement, and bloom time, have shown that these traits are controlled by several genes on different chromosomes and that some regions of the genome contribute more to the phenotypic variance than others [26–29]. Despite extensive genetic research in *Prunus* and peach [30] there is no information on genetic control of fruitlet freeze tolerance. In addition, due to a narrow genetic base of peach [31], breeders could lack adaptation genes in breeding germplasm [32]. Therefore, we expanded the Melgar et al. [10] study by evaluating fruitlet freeze tolerance and heritability in modern peach breeding germplasm with the specific goal to determine if fruitlet freeze tolerance is genetically controlled, and identify potential donors of this trait. Our hypothesis was that observed diversity in peach fruitlets' ability to tolerate low temperatures is genetically controlled and can be used in breeding to incorporate fruitlet freeze tolerance in newly developed cultivars. As an added benefit, fruitlet freeze tolerance of the currently grown cultivars when provided to growers could aid in orchard management. This is the first comprehensive study of the fruitlet freeze tolerance in peach that provides the foundation for further understanding the genetics behind this trait and breeding of new peach cultivars with enhanced freeze tolerance.

2. Materials and Methods

2.1. Peach and Nectarine Germplasm

Fifty-one peach and nine nectarine cultivars from the Clemson University *Prunus* germplasm collection and 15 advanced selections from Clemson University peach breeding program (CUPBP) were used for evaluation of their fruitlet tolerance to freezing temperatures. The germplasm consisted of heirlooms, cultivars released or patented in the U.S. in the last five decades, and advanced selections from the CUPBP (Table S1). The material is maintained at the Clemson University Musser Fruit Research Center (Latitude: 34.639038, Longitude: −82.935244) in Seneca, SC, under warm, humid, moderate climate, and standard commercial practices for irrigation, fertilization, and pest-disease control. Average annual rainfall for this location is 942 mm and average annual temperatures is 25.1 °C and 8.8 °C, for summer and winter, respectively. The trees used in the study were 7–10 years old, grafted on Guardian® rootstock and trained as perpendicular V or open-center vase. Chilling requirement (CR) in chill hours (CH), bloom dates in Julian date (JD), and heat requirement (HR) in growing degree hours (GDH) for this material were previously described in Demirel et al. [33] and Atagul et al. [34].

2.2. Phenotyping Fruitlet Freeze Tolerance

Fruitlet freeze tolerance was estimated for 75 and 71 accessions in 2022 and 2023 season, respectively. Fruitlets were sampled during first three weeks of April 2022 and last two weeks of March 2023. The average low temperatures during sampling period were 9.3 °C and 10.0 °C, respectively, and the minimum low temperatures were 2.2 °C and −0.4 °C, respectively (Vantage Pro, Davis Instruments, Hayward, CA, USA). Due to damage from late spring frosts in 2023, fruitlets of peach cultivars FlavrBurst™, Messina®, Rich May and Carored, were not included in the electrolyte leakage study.

One hundred fruitlets per accession were collected at the shuck-off stage, with a diameter not exceeding 13 mm, and transported to the laboratory. Fruitlet freeze tolerance was evaluated using the electrolyte leakage method [10]. Five fruitlets were placed into individual test tubes, with three replicates per accession and temperature, and immersed in ethylene glycol–water refrigerated bath (AP 20R-30, VWR, Radnor, PA, USA). Six treatments using different freezing temperatures: 0 °C, −2 °C, −4 °C, −6 °C, −8 °C, and −10 °C were applied, starting at 0 °C and decreasing 2 degrees every hour. After one hour of exposure to each temperature, the three replicates corresponding to each specific temperature were withdrawn from the water bath, covered with Parafilm® (Bemis, Neenah, WI, USA) and placed in a refrigerator (4 °C) to thaw gradually. After one hour in the refrigerator, 10 mL of deionized water was added to each tube and the tubes were shaken at 200 rpm overnight at room temperature, for at least 12 h. The next day each tube was vortexed for a few seconds and electrical conductivity (EC1) was measured using a conductivity meter (Fisher Scientific Accumet AP85, Thermo Fisher Scientific, Waltham, MA, USA) to determine the amount of solutes from the fruitlets released into the solution. In the final step, the test tubes were autoclaved for 20 min at 121 °C to completely disrupt the cell membranes and release all solutes contained within the cell into the surrounding solution. Following autoclaving, samples were allowed to cool down at room temperature and electrical conductivity was again measured (EC2). Electrolyte leakage was calculated as the ratio of ion leakage from freeze injury: EL = EC1 × 100/EC2 [35]. The ratio of ion leakage at each temperature was used to develop a graphical interpretation of freeze tolerance throughout decreasing temperatures using SigmaPlot 13.0 (Systat, San Jose, CA, USA) and the following equation (Sigmoidal, Sigmoid, 4 Parameter):

$$f = y0 + a/(1 + exp(-(x - x0)/b)),$$

where $x0$ = the temperature at the inflection point (LT50); b = Hill's slope of the curve (i.e., steepness of the curve at $x0$); $y0$ = the lowest electrolyte leakage value (at 0 °C); and a = difference in electrolyte leakage between the minimum and maximum temperature (Figure 1).

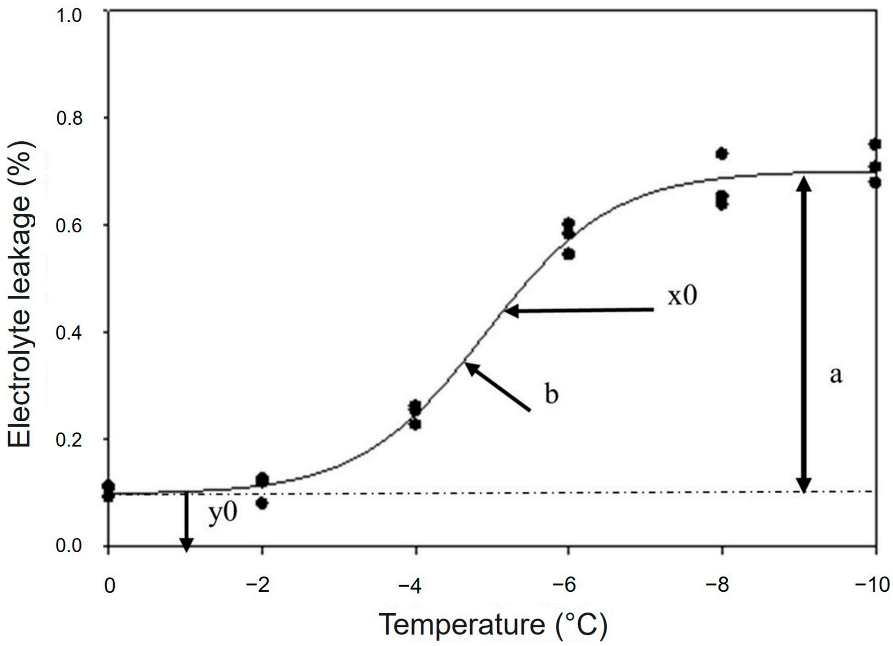

Figure 1. Electrolyte leakage (Y axis) vs. Temperature (X axis), x0, temperature at the inflection point (LT50); b, slope of the curve (steepness of the curve at x0); y0, lowest electrolyte leakage value (at 0 °C); a, difference in electrolyte leakage between minimum and maximum temperature; and b, Hill's slope of the curve (steepness of the curve at x0).

The temperature at which 50% of the ion leakage occurred, termed the inflection point (IP), and the area under the sigmoidal curve (AUC) were recorded. In addition, the material was grouped into three tolerance groups (TG) based on the IP and the AUC. The TG1 (high tolerance), contained material exhibiting no freeze damage at temperatures < −6 °C and with AUC < 25%. The TG2 (intermediate tolerance), contained material exhibiting damage at temperatures from −5 to −6 °C and having AUC between 25 and 35%; and the TG3 (low tolerance), contained material exhibiting damage to temperature > −5 °C and having AUC > 35%.

2.3. Statistical Data Analyses

Statistical analyses were performed in R Studio version 2023.03.1 and SPSS v. 27 (IBM SPSS Statistics for Windows, Version 27.0. Armonk, NY, USA: IBM Corp.).

Data normality and equal variance assumption were determined using the Shapiro–Wilk test. Spearman's correlation coefficient was used to estimate the relationship between two variables ranked on an ordinal scale.

To estimate broad-sense heritability, a mixed linear model (MLM) was fitted using R package 'lme4' [36] with year selected as a random effect:

$$Y_{ij} = \mu + g_i + y_j + gy_{ij} + \varepsilon$$

in which, Y_{ij} is the trait of interest, μ is the overall mean, g_i is the genetic effect of ith genotype, y_j is the effect of the jth year, and gy_{ij} as the interaction effect of ith genotype with jth year, ε is the residual of the model. Broad-sense heritability (H^2) was calculated using the following equation:

$$H^2 = \sigma g^2 / \left(\sigma g^2 + \sigma e^2/\mathrm{n}\right)$$

where σg^2 is the genetic variance, σe is the environmental variance, and n is the number of years.

Data were organized in six datasets using the trait (IP, AUC) values obtained in each experimental season (2022–2023) and average value: IP2022, IP2023, IP_Ave, AUC2022, AUC2023 and AUC_Ave.

3. Results

Diverse peach (66) and nectarine (9) germplasm, consisting of cultivars important for the southeast U.S. peach industry (heirlooms and cultivars patented within the last five decades) and 15 advanced accessions from the Clemson University peach breeding program, was evaluated for fruitlet freeze tolerance (Table S1; Figure 2). The majority of cultivars were released within the last three decades of the 20th century (1971–1999). Some of them are still grown in the southeast U.S. and used as standards in regional trials or represent important breeding parents used in peach breeding programs. This germplasm is adapted to or evaluated for suitability in the southeast U.S. with chilling requirement ranging from 500 to over 1000 CH, majority being in the 600–900 CH range, with bloom time from 62–80 JD and estimated minimum heat requirement from 1362–7039 GDH.

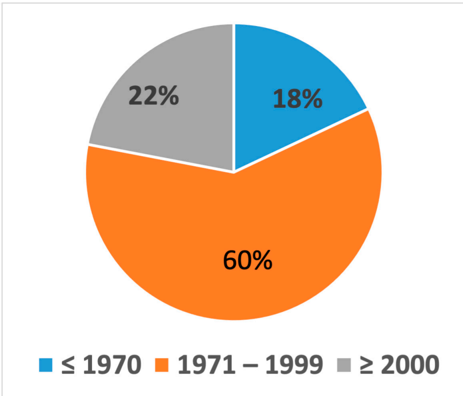

Figure 2. Distribution of peach and nectarine germplasm by release year.

Peach germplasm showed variable fruitlet freeze tolerance, estimated with both inflection point (IP), the temperature at which 50% of the material shows sign of freeze damage, and % of freeze damage, estimated by the area under the sigmoid curve (AUC), in evaluated seasons (Table 1; Figure 3).

Table 1. Descriptive statistics of fruitlet freeze tolerance observed in peach and nectarine germplasm in 2022 (N = 75) and 2023 (N = 71) season for inflection point (IP) and area under curve (AUC).

Trait	Min	Max	Mean	SD	H^2
IP-2022 (°C)	−10.2	−4.0	−5.7	1.1	0.52
IP-2023 (°C)	−7.8	−3.9	−5.8	0.8	
AUC-2022 (%)	18.7	47.8	30.1	6.5	0.85
AUC-2023 (%)	15.8	44.2	28.8	5.9	
IP_Ave (°C)	−9.0	−4.0	−5.7		
AUC_Ave (%)	17.2	46.0	29.5		

N, number of analyzed samples; SD, standard deviation; H^2, broad-sense heritability; Ave; average.

IP distribution was skewed towards lower tolerance in 2022 and did not exhibit normal distribution (Shapiro–Wilk test; W = 0.91135, p-value = 6.484×10^{-5}) (Figures 3 and S1). However, bimodal normal distribution (Shapiro–Wilk test; W = 0.97354, p-value = 0.1385) was observed in IP2023 dataset. Percent of freeze damage, estimated by AUC, was normally distributed in both years (Shapiro–Wilk test; W = 0.96973, p-value = 0.06932 in 2022; 0.98839, p-value = 0.7603 in 2023) with a bimodal distribution (Figure S1) and a higher median observed in 2022 than in 2023 season (Figure 3). The AUC distribution was wider, with a higher degree of variability, than the IP distribution.

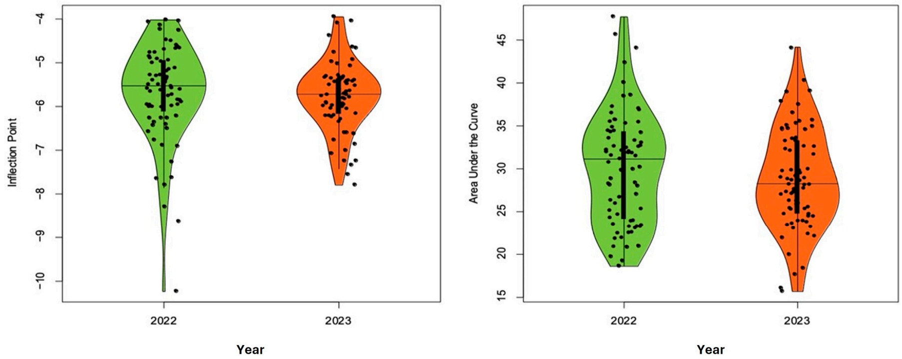

Figure 3. Distribution of fruitlet freeze tolerance estimated with Inflection Point (**left panel**) and Area Under the Curve (**right panel**) in modern peach breeding germplasm in two seasons (2022–2023).

Highly significant positive correlation was observed among all datasets (Figure 4). The lowest correlation was observed for IP between years (r = 0.40), while the highest correlation was observed between AUC datasets (r = 0.80) and IP2022 and AUC2022 (r = 0.66) (Figure 4).

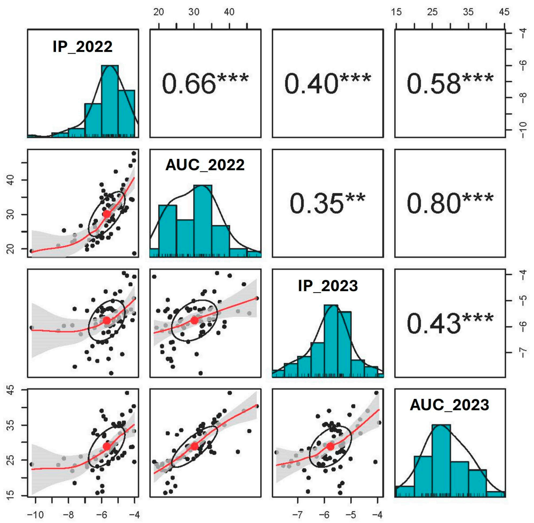

Figure 4. Correlation between inflection point (IP) and area under the curve (AUC) observed in peach and nectarine germplasm among years (2022 to 2023). Asterisks indicate the Spearman correlation coefficient (r) at a significance level of $p < 0.01$ (**) and $p < 0.001$ (***).

IP ranged from −3.9 °C (IP2022) to −10.2 °C (IP2023), with similar overall average IP (IP_Ave = −5.7 °C) observed in both years (Table 1). Significant differences between IP ranges in the two experimental years were observed, with a wider range (−4.0 to −10.2 °C) observed in IP2022 and a narrower range (−3.9 to −7.8 °C) in the IP2023 dataset. The highest negative temperature at which fruitlet damage was observed was −3.9 °C, in peach 'Sweetstar', in IP2023, and the lowest temperature at which the highest fruitlet tolerance was observed was −10.2 °C in nectarine 'Arctic Pride' in IP2022. The remaining accessions fell within the range of freezing temperatures between these two extremes with the majority showing fruitlet tolerance in the −4 to −6 °C range in both datasets (Table S1; Figures S2 and S3).

Overall AUC in all peach and nectarine accessions ranged from 15.8% in 2023 to 47.8% in 2022 (Table 1). The AUC interval was similar in the two experimental years, 29.1% and 28.4% in 2022 and 2023, respectively (Table 1). The lowest AUC was calculated in 2023, with 13.1% less fruitlet damage than the average, while the highest freezing damage was estimated in AUC2022, 17.6% higher than the mean (Table 1). The lowest estimated damage, 15.8%, was observed in 'Glory' in AUC2023, while the highest damage was detected in fruitlets of 'Julyprince' in AUC2022. Commercial peach cultivars Julyprince, June Gold, Rich Joy, and Caroking exhibited high degrees of susceptibility to freeze tolerance, with AUC values of 47.7, 45.6, 44.1, and 40.1%, respectively (Table S1). Interestingly, the same cultivars had the lowest IP range, −4.0 and −4.5 °C.

The highest fruitlet resilience to freezing temperatures according to average IP (<−7 °C) was observed in nectarines 'Arctic Pride' and 'Summerfire' and peaches 'Brightstar', 'Sweet September', and 'Summerprince'. However, the highest fruitlet resilience to freezing temperatures predicted by average AUC was in four peach cultivars Glory, Early Augustprince, Parade, and Redglobe, and four nectarines 'Actic Blaze', 'Silver Gem', 'Arctic Pride', and 'Juneprincess'.

Broad-sense heritability of 0.52 and 0.85, was estimated with IP and AUC data, respectively, from both seasons (Table 1).

Fruitlet freeze tolerance estimated by IP did not accurately represent the divergence and steepness of the sigmoid curve (Figure 5). For example, the same IP of approximately −5.9 °C was observed in 'Glory', 'Fireprince', and 'Julyprince', but the actual temperature when fruitlets started to experience damage differed (Figure 5; Table S1). Fruitlets of 'Julyprince' began to show damage at −2 °C and 'Glory' at −4 °C while the fruitlets of 'Fireprince' did not show any freeze damage until close to −6 °C. The AUC observed in the three cultivars ranged from 22% in 'Glory', 29% in 'Fireprince' to 47% in 'Julyprince', suggesting 'Glory' had the highest fruitlet freeze tolerance and 'Julyprince' the lowest. However, evaluation of the divergence and steepness of the sigmoid curve showed that the electrolyte leakage in fruitlets of 'Fireprince' did not begin until −6 °C, which is two degrees lower than that observed for the fruitlets of 'Glory'.

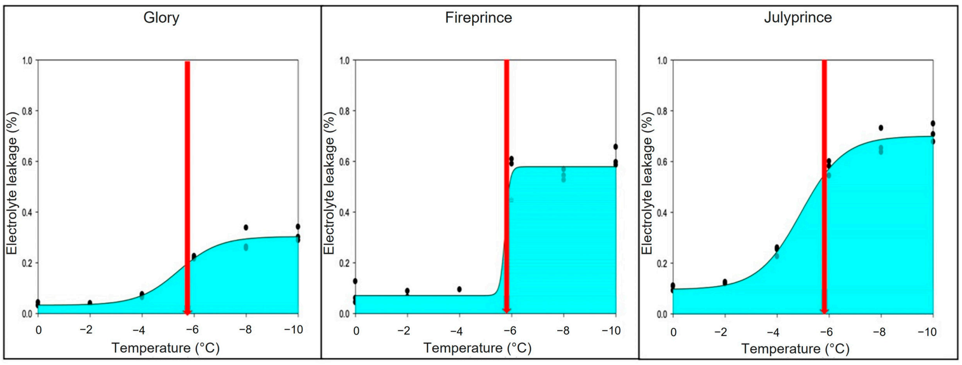

Figure 5. Patterns of fruitlet freeze response to freezing temperatures observed in three peach cultivars in 2023. Sigmoid curve and inflection point (red arrow) showing 50% of ion leakage. Area under the curve is shaded blue.

Grouping the accessions in TGs based on IP classified 26.67% of material as least (TG3) and most tolerant (TG1) and 46.8% in the intermediate group (TG2) in 2022 (Table S1). A similar grouping was observed with IP2023 data with 28% characterized as most tolerant (TG1), 54.6% as intermediate (TG2), and 12% as least tolerant (TG3). While most cultivars and selections maintained the group in both datasets, some discrepancies were observed. The most apparent change was from extreme groups to intermediate, with the most extreme change observed for peach cultivar Sweetstar that moved from TG1 in IP2022 to TG3 in IP2023. The most tolerant group based on the IP2022 data consisted of cultivars released in the last century with few heirlooms such as 'Redrose' and 'Parade' released in 1940 and 1960, respectively, and three newer releases, two nectarines from University of Arkansas, 'Westbrook' and 'Arrington' released in 2002, and one peach from USDA, Byron, GA, 'Augustprince' released in 2006. Interestingly, seven nectarine cultivars, Arctic Belle, Arctic Blaze, Arctic Pride, Arrington, Silver Gem, Summer Fire, and Westbrook, were grouped in the most tolerant group based on IP2022, with five of them (Arctic Belle, Arctic Pride, Silver Gem, Summer Fire, and Westbrook) being classified in the same most tolerant group in 2023. Selections from the CUPBP were mostly grouped in TG2 (75 and 73% in 2022 and 2023, respectively) with SC-12 being the most tolerant in both 2022 and 2023.

Similarly, grouping of peach and nectarine cultivars and accessions based on the AUC values revealed majority of material in intermediate group TG2 (50 and 53% in AUC2022 and AUC2023, respectively), and 28 and 26% of accessions in most tolerant (TG1), and 22 and 17% in the least tolerant (TG3) group in AUC2022 and AUC2023 datasets, respectively (Table S1). Most accessions maintained their group regardless of season with few changing from most ('Arrington' and SC-8) or least ('June Gold', 'Loring', and 'Cresthaven') tolerant to intermediate. Similar to IP grouping, AUC grouping classified all nine nectarines as most tolerant (TG1) in both years, except 'Arrington' which moved to TG2 in 2023 (AUC = 27%), with 5% difference in AUC between the two seasons and only 2% over the group threshold. Two CUPBP selections SC-8 and SC-7 were in the most tolerant group in 2022 with SC-7 in 2023 having AUC of 27%, 2% above the threshold for the TG1. The CUPBP selection SC-12 was grouped as intermediate in both AUC2022 and AUC2023 datasets, with 2 and 0.86%, respectively, above the threshold of the TG1.

4. Discussion

Climate change and increased occurrence of freezing temperature events during spring in the southeastern region of the U.S., demand better understanding of genetic control of all climate resilience traits (chilling and heat requirement, bloom time and fruitlet freeze tolerance) to facilitate development of climate resilient cultivars and ensure sustainability of peach production. Out of all climate resilience traits, fruitlet freeze tolerance is least investigated. Therefore, to support breeding for climate resilience, we have evaluated fruitlet freeze tolerance in the peach germplasm important to the southeast U.S. peach industry, as a steppingstone in understanding the genetic control of this trait. This research also addressed the pressing demand for a standardized phenotyping protocol to assess the freeze tolerance of developing fruitlets, as a necessary tool in obtaining quality phenotypic data needed for downstream genetic studies.

Fruitlet freeze tolerance observed in the peach germplasm evaluated in this study was variable across seasons (2022 and 2023) and traits (IP and AUC), with AUC exhibiting better stability. Commercial peach cultivars Julyprince, June Gold, Rich Joy, and Caroking exhibited high degrees of susceptibility to freeze tolerance, with AUC values of above 40% (Table S1). Furthermore, these cultivars had the lowest IP range, −4.0 and −4.5 °C, close to the critical temperature (~−3 °C) for causing damage to fruitlets [37]. Cultivars Arctic Pride, Rubyprince, Summer Fire, and Sweet September exhibited remarkable tolerance to freezing conditions, as evidenced by their significantly reduced ion leakage in comparison to other cultivars (19–27%) and the temperature at the IP close to −10 °C (Table S1). This suggested that fruitlets of these cultivars have a high tolerance to low temperatures which is in agreement with previous reports [10].

Lower correlation between IP datasets posed difficulty in comparing data from different studies. However, the AUC values, calculated for Melgar et al. [10], were in agreement for the common cultivars.

The outcomes of this study indicate that fruitlet freeze tolerance in peach germplasm shows a diverse range of responses, with IP values ranging from −3.9 °C to −10.2 °C. Furthermore, the accessions exhibited varying degrees of vulnerability to freeze tolerance, with AUC values ranging from 16% to 48%. While both approaches were effective in distinguishing differences in fruitlet freeze tolerance, it is worth noting that the IP value was most variable and vulnerable to methodological error. The observed difference in IP values between the two seasons could be attributed to variation in fruitlet sampling due to slight differences in fruitlet size and or timing between sampling and analysis that could affect healing of the scar after fruitlet detachment from the branch, thus increasing electrolyte leakage and skewing the results. Similar discrepancy in IP seasonal values were reported by Melgar et al. [10]. When combining Melgar et al. [10] data from two seasons (2019 and 2021) with this study for the common cultivars, the differences in observed

IP values were still present, suggesting that care in sample collection and experiment preparation might be more important than the repetition of the experiment.

Another important point is the steepness of the curve. When the slope is highly inclined, most fruits are damaged simultaneously. In opposition, on a gentler slope, some fruits may be damaged sooner, while others may be affected later. The timing of damage to specific fruits on a less-steep slope can also be crucial in determining overall crop success. A difference of just one degree in tolerance in peach production could mean having the peach crop or not, so it is important to further describe the fruitlet freeze tolerance. Even though the AUC was obtained from sigmoid curve developed using IP data, this study showed that the AUC approach reduces data variability and, therefore, might be more adequate for predicting a cultivar's response to freezing temperatures. This is also supported by the tolerance group assignment, as fewer group assignment mismatches were observed for AUC than for IP values. Furthermore, the mismatches observed in the AUC group assignment were on the borderline of the TG threshold.

Interestingly, nectarine fruitlets showed high tolerance in both seasons, suggesting that the nectarine fruitlet morphology or absence of pubescence might influence fruitlets' susceptibility/tolerance to freezing temperatures. A single gene mutation from pubescent (*G*-) to glabrous (*gg*) skin on chromosome 5 is the difference between the peach and nectarine phenotype [38,39]. Other subtle differences in the flesh density and texture between peach and nectarine are suggested and speculated to be attributed to the pleiotropic effect of this single gene mutation, but the research documenting them is lacking. Increased tolerance to freezing temperatures in nectarines observed in this study might be explained by the morphological characteristics of the fruit tissues, e.g., lower water content in the nectarine fruit tissue than in peach [40]. Formation of both intercellular or intracellular ice crystals can lead to cell death. However, a grape study on freeze damage during spring suggested that damage was caused by the formation of intercellular rather than intracellular ice [41]. Generation of a water vapor gradient between the interior and exterior of cells directly contributes to intercellular ice crystal formation. Furthermore, cells become dehydrated as water moves from the interior to the exterior of the cell and accumulates on intercellular ice crystals, causing a loss of turgor [42]. To confirm if this contributed to the higher tolerance predicted in nectarine fruitlets, further studies are needed.

Absence of fuzz or trichomes might be another reason for nectarine fruitlets exhibiting higher tolerance to low temperatures. However, studies mostly focused on the trichome morphology and differences between peach and nectarine but never on other aspects of fruit morphology [43,44]. It would be interesting to explore this line of thought and determine the cause of higher tolerance to freeze in nectarine fruitlets in laboratory experiments, especially since our field observations do not support this difference between peach and nectarine fruitlet tolerance to freeze, nor it was reported in the literature [22,45]. The lab data suggest significant differences between peach and nectarines, thus, expanding the fruitlet freeze evaluation to include more nectarine cultivars might help explain observations made here. In addition, it is important to emphasize that electrolyte leakage method, used in this study, is only a way to compare the accessions for their tolerance to low temperatures and do not necessarily reflect temperatures in field. Field freeze tolerance data for the same material will be needed to validate the accuracy of the lab prediction.

However, the ability to characterize peach and nectarine cultivars for potential of their fruitlets to tolerate low temperature in spring and assign them to tolerance groups outweighs the shortcomings of this assessment. Tolerance group assignment, based on either the IP or AUC, could be valuable information for advising growers about cultivars' young fruit ability to tolerate low temperatures. This information can be included when choosing the cultivars to plant in areas more prone to freezing, or consulted for arranging freeze protection (e.g., wind machines, irrigation) especially when limited resources to reduce the impact of late spring (radiation) frosts are available. Change in just a few degrees could mean a difference between full production or a total crop loss. Thus, results of this study may provide crucial pieces of information on climate resilience in peach and nectarine cultivars for growers and county agents that contribute to minimizing possible economic damage due to low spring temperatures. This information can also be useful to breeders that are developing climate resilience as an important trait.

The observed variation in IP among cultivars suggested that both IP and AUC would be beneficial for estimating fruitlet freeze tolerance through multiple conditions or time. Increased frequency in the occurrence of spring frosts all over the world is putting emphasis on adding this trait in breeding efforts for climate resilience. It is crucial to conduct more in-depth investigations into the influence of climate characteristics of plants. Peach crop losses the southeast U.S. industry endured in the last two decades indicate that current breeding goals of lowering chill requirements to ensure its satisfaction and increasing heat requirements to delay bloom are no longer enough to ensure the sustainability of peach production in the changing climate. Thus, fruitlet freeze tolerance should be included in the suite of traits when breeding for climate resilience. Furthermore, broad-sense heritability estimated in this study supports genetic control of the fruitlet freeze tolerance in peach and nectarine germplasm and potential for improvement of this trait via breeding. This is the first extensive study into the peach fruitlet freeze tolerance that lays the foundation for further investigation into the genetic control of this trait.

5. Conclusions

This study showed that peach germplasm is variable in fruit freeze tolerance. The wide diversity of fruitlet freeze tolerance, and broad-sense heritability (H^2) estimates of 0.52 and 0.85 for infection point and the area under the curve, respectively, observed in peach germplasm suggest that this trait is genetically controlled and has the potential to be used in breeding. Consequently, the results of this study lay the groundwork for future investigation into the regions of the peach genome responsible for controlling this trait.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14020302/s1, Figure S1: Frequency distribution of Inflection Point (IP) and area under the curve (AUC) of fruitlet freeze tolerance within the peach and nectarine germplasm in 2022 and 2023 season; Figures S2 and S3: Fruitlet freeze response to freezing temperatures observed in peach and nectarine accessions listed by release year in 2022 and 2023, respectively; Table S1: Characteristics of the U.S. modern peach breeding germplasm used in this study, including fruitlet freeze tolerance and classification in freeze tolerance groups based on IP (inflection point, °C) and AUC (area under the curve, %). Fruit type—FT; origin—country (state); CR—chilling requirement in chill hours (CH); HR—heat requirement in growing degree hours (GDH); BD—bloom date (BLUP) in Julian Days (JD).

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