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Root injury detection by impedance loss factor and hydraulic conductance of apple (*Malus domestica*), blackcurrant (*Ribes nigrum*) and blueberry (*Vaccinium corymbosum*) nursery plants

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ABSTRACT

In order to avoid winter damage, freezer storage has become a common practice in production of nursery plants in boreal zones. Previously, winter storage temperature of −3 °C was appropriate for the aboveground parts of apple, blueberry and blackcurrant nursery plants but it remained unknown whether it is optimal for root systems too. Therefore, we examined the effect of different treatment temperatures of 3, −3, −7, −10 °C for five to six weeks on root damage of two cultivars of apple (*Malus domestica*), three cultivars of blackcurrant (*Ribes nigrum*), and three cultivars of blueberry (*Vaccinium corymbosum*). Root damage was assessed by the impedance loss factor (δ) and the reverse-flow hydraulic conductance (K_r) of the roots. In apple, the proper storage temperature for roots during winter storage is −3 °C. However, the appropriate storage temperature for root systems of blueberry and blackcurrant depends on the cultivar. In addition, our results demonstrate the potential of the classification analysis of impedance spectra in identifying the appropriate storage conditions for different species. This approach can provide a rapid way to assess the root damage caused by low temperature and help to optimize the storage conditions for different species and cultivars. Further research is needed to investigate mechanisms underlying the observed effects and to develop practical applications based on these findings.

1. Introduction

The growth of woody horticultural plants in the northern region is possible by synchronization of their annual cycle with the changes in weather conditions during the four seasons (Grace, 1987). The synchronization refers to the proper timing of acclimation and adaptation of the physiological processes of plants to the changes in environmental conditions. One of the traits, i.e., frost hardiness, is one of the major factors limiting the geographical distribution, successful growth and productivity of these plants. Climate change and extreme weather events will exacerbate the situation for perennial plants, such as some woody horticultural plants, growing in their northern distribution areas (Muffler et al., 2016; Venäläinen et al., 2020).

The cultivation of fruit crops has increased rapidly in last decades in the boreal countries. In Finland, the production of fruits, e.g. apple (*Malus domestica* Borkh.) and berries account for 3.5 % and 37.6 % of the total horticultural cultivated area of 18 700 ha, respectively (Horticultural Statistics, 2022). The expanding market demand for new cultivars e.g. for apple, blueberry (*Vaccinium corymbosum*) and blackcurrant (*Ribes nigrum*), that have good acclimation potential to large seasonal variations in environmental conditions. At the same time, they should produce high quality crops with economic benefits (Niemi and Väre, 2019). The basis for the enhanced cultivation is formed by the production of high-quality nursery plants for farmers.

In boreal areas, extreme winter conditions can reduce the quality of the nursery plants. Different species and cultivars respond differently to the drivers of frost hardening and may therefore have different survival capacities. Roots are considered to be the most sensitive organ. Under field conditions, roots can be damaged in winter, particularly in the absence of snow cover (Sakai and Larcher, 1987; Bigras et al., 2001; Drescher and Thomas, 2013; Domisch et al., 2018). Therefore, freezer storage has become a common practice in production, such as in tree nurseries (Landis et al., 2010; Riikonen and Luoranen, 2020). In the freezer storage, it is largely the question of optimization between the

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temperatures to inhibit energy loss by respiration and the avoidance of root injury. The freezer storage temperature that is commonly used for tree seedlings/nursery plants is approximately −3 °C. This was supported in the previous study revealing that the proper storage condition for the development of maximum frost hardiness (FH_{max}) of the aboveground parts in late autumn is three weeks at −3 °C for apple and blueberry, but shorter time is needed for blackcurrant (Wu et al., 2019). It remained unknown whether −3 °C would be the optimum storage temperature for roots too or whether they were damaged in this temperature.

Frost hardiness evaluation of plants is usually based on a controlled freezing test at a series of temperatures, followed by the assessment of cellular damage with various methods (Burr et al., 2001; Wu et al., 2019, Wu et al., 2020). Due to the confounding effects of soil, evaluation of freezing injury in roots is more difficult than in aboveground parts of the plant. For example, relative electrolyte leakage (REL) method may fail since the electrolytes leak from the damaged cells to the soil, and therefore, the threshold temperature for injuries is not possible to assess (Repo and Ryyppö, 2008). In the recent studies, the electrical impedance loss factor at 50 kHz frequency (δ_{50kHz}) and the reverse-flow hydraulic conductance (K_r) of the root system of Scots pine (*Pinus sylvestris* L.) seedlings increased with increasing root damage in controlled freezing tests (Di et al., 2019; Korhonen et al., 2019). Since the damage occurrence, these properties were not stable, but δ_{50kHz} decreased and K_r increased over time however (Repo et al., 2023). Electrical impedance spectra of damaged and undamaged root systems differed too, and they could be separated by the classification analysis using the approach of artificial intelligence. Therefore, it was reason to believe that if there are injuries in apple, blueberry and blackcurrant roots during the freezer storage, they could be observed with these methods. Then it would be possible to assess the proper storage condition from the point of view of physiological condition of roots as well (Repo et al., 2014b; Di et al., 2019).

The purpose of this study was to determine the appropriate freezer storage condition for root systems of apple, blueberry and blackcurrant nursery plants. We hypothesized that root damage, as assessed by δ, K_r and classification analysis of the impedance spectra, gradually increase by lowering the freezer storage temperatures between 3 °C and −10 °C.

2. Materials and methods

2.1. Plant material and treatment

The material consisted of two apple (*Malus domestica*) cultivars ('Pirja' and 'Lobo') with 24 nursery plants of each cultivar, three blueberry (*Vaccinium corymbosum*) cultivars ('Aino', 'Alvar' and 'Arto') with 20 nursery plants of each cultivar, and three blackcurrant (*Ribes nigrum*) cultivars with 36, 45 and 44 nursery plants of 'Marski', Ben Tron' and 'Mortti' respectively (Wu et al., 2019). Apple and blueberry plants were two years old and blackcurrant plants were six months old. Blackcurrant plants were raised from cuttings in the greenhouse of the Natural Resources Institute Finland (Luke), Piikkiö unit (60°39 'N, 22°55 'E, 18 m asl). Blueberries were micropropagated in Luke, Laukaa unit (62°32 'N, 25°99 'E) and rooted and grown from microcuttings in a commercial nursery in Raasepori (60°08 'N, 23°40 'E) Finland. The apple cultivars were grafted on 'Antonovka' rootstock and grown at the nursery in Raasepori. Apples were grown in 7.5 L pots and the plant height was 170–180 cm. Blueberries were grown in 2.0 L pots to a height of 20–40 cm and blackcurrants in 1.5 L pots to a height of 10–30 cm.

During the blueberry and apple cultivation in Raasepori, the minimum temperatures were <0 °C for eight nights (minimum −2.9 °C) in late October, before transporting of the plants to Luke, Suonenjoki unit (62°39N, 27°03E, 142 m asl) on October 31, 2017. The blackcurrant plants were exposed to air temperatures below 0 °C (minimum −5 °C) for 10 nights in Piikkiö before transporting to Suonenjoki on November 1, 2017. In Suonenjoki, the plants were maintained at 2 °C for nine days.

On November 9, the plants were transported to Luke, Joensuu Unit (62°61N, 29°74E, 80 m asl) and placed at four storage temperatures (+3, −3, −7, −10 °C) in the dark. The transfer from −3 °C to −7 °C and −10 °C took place gradually after 24 h and 48 h at −3 °C, respectively. Prior to transfer to the storage temperatures, the plants were covered with plastic bags to prevent desiccation. At each storage temperature, the material for assessing the condition of the root systems consisted of six plants for each cultivar of apple, five for each cultivar of blueberry, and nine, 12 and 11 plants of 'Marski', 'Ben Tron' and 'Mortti' of blackcurrant, respectively. After sampling of the aboveground parts for FH testing after three weeks of storage (Wu et al., 2019), the intact root balls were stored for another two to three weeks to be sure that the storage duration would be enough for the roots to acclimatize to a new steady condition. The root balls were then transferred to 3 °C for two to three days to thaw slowly, and then to room temperature (22 °C) for one night prior to measuring electrical impedance spectra (EIS) and hydraulic conductance (K_r) of the root systems.

2.2. Electrical impedance spectra of roots

Electrical impedance spectra (EIS) of the root systems were measured with EIS101 (Simitec Ltd, Joensuu, Finland) at 36 frequencies between 90 Hz and 200 kHz (β-dispersion range, Schwan, 1988). A two-electrode measurement setup was used. The upper stainless-steel needle electrodes (Fig. 1A) were attached at opposite sides of the stem at approximately 5 cm above the root collar. The bottom electrode consisted of stainless-steel rods (2.5 mm) that were protruding from the bottom of the pots to the soil (Fig. 1B). The impedance loss factor of the root system at different frequencies was calculated as

$$\delta = tan^{-1}\left(\frac{Z_{Im}}{Z_{Re}}\right) \quad (1)$$

Based on the previous study, the loss factor at 50 kHz (δ_{50kHz}) was selected to compare root system properties of different species and cultivars among storage temperatures (Di et al., 2019).

The loss factor of EIS of different cultivars of apple, blueberry, and blackcurrant were subjected to Class-Featuring Information Compression (CLAFIC) analysis (Jaaskelainen et al., 1994; Repo et al., 2014a).

2.3. Root hydraulic conductance

Reverse-flow hydraulic conductance (K_r) of roots was measured using high-pressure flow meter (Dynamax Inc., Houston, TX). The stem was cut at approximately10–20 mm above the root collar and the capillary tube of the HPFM device was connected to the stem by means of a coupling set. The root system was then gradually pressurized with water from 0 to 0.55 MPa to obtain the reverse-flow K_r, which was obtained from the linear part of the relationship between water flow and applied pressure (Tyree et al., 1995; Voicu et al., 2008; Di et al., 2019).

2.4. Statistical analysis

Differences between the storage temperatures for impedance loss factor and reverse-flow hydraulic conductance were assessed using one-way ANOVA with Duncan's multiple range test (IBM SPSS 25.0, IBM Co., New York, USA). The classification analysis (CLAFIC) of impedance loss factor of electrical impedance spectra (EIS) is based on the principle of artificial intelligence where the testing data are compared with the training data using the LMSSC2 software (Simitec Ltd., Joensuu, Finland). The measure of the similarity of impedance loss factor in different classification groups (i.e., different precondition temperatures) was calculated by comparing the number of impedance loss factor spectra belonging to each group. In the classification, the unknown spectrum is classified by measuring the length of the projection vector in each subspace k, where k considered the fine structure of the impedance

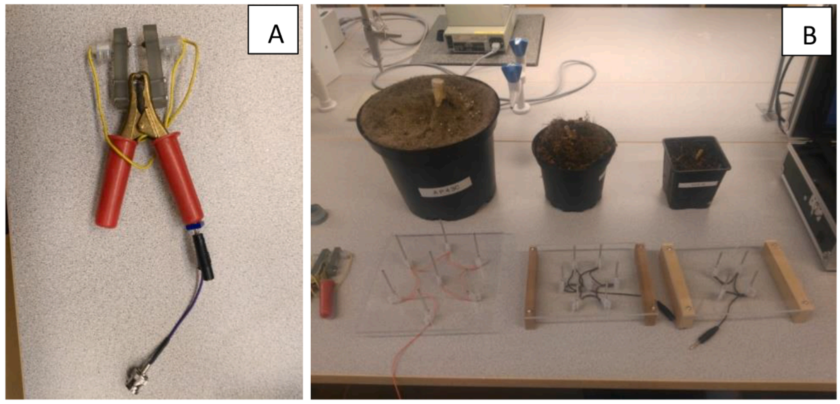

Fig. 1. The electrodes used in the measurements of electrical impedance spectra of root systems of apple, blueberry and blackcurrant. The upper needle electrodes (pliers) (A), the bottom needle electrodes for apple, blueberry and blackcurrant from left to right respectively (B).

loss factor.

3. Results

3.1. Loss factor of impedance spectra

The mean value of loss factor (δ_{mean}) varied from −0.15 to 0.1 rad in apple, from −0.3 to −0.05 rad in blackcurrant, and from −0.6 to 0 rad in blueberry, depending on the frequency and the storage temperature. In apple and blueberry, the loss factor increased with decreasing storage temperature for all cultivars, and more so at higher frequencies (Fig. 2, Table 1). In blackcurrant, there were less differences in the loss factor among storage temperatures (Fig. 2, Table 1).

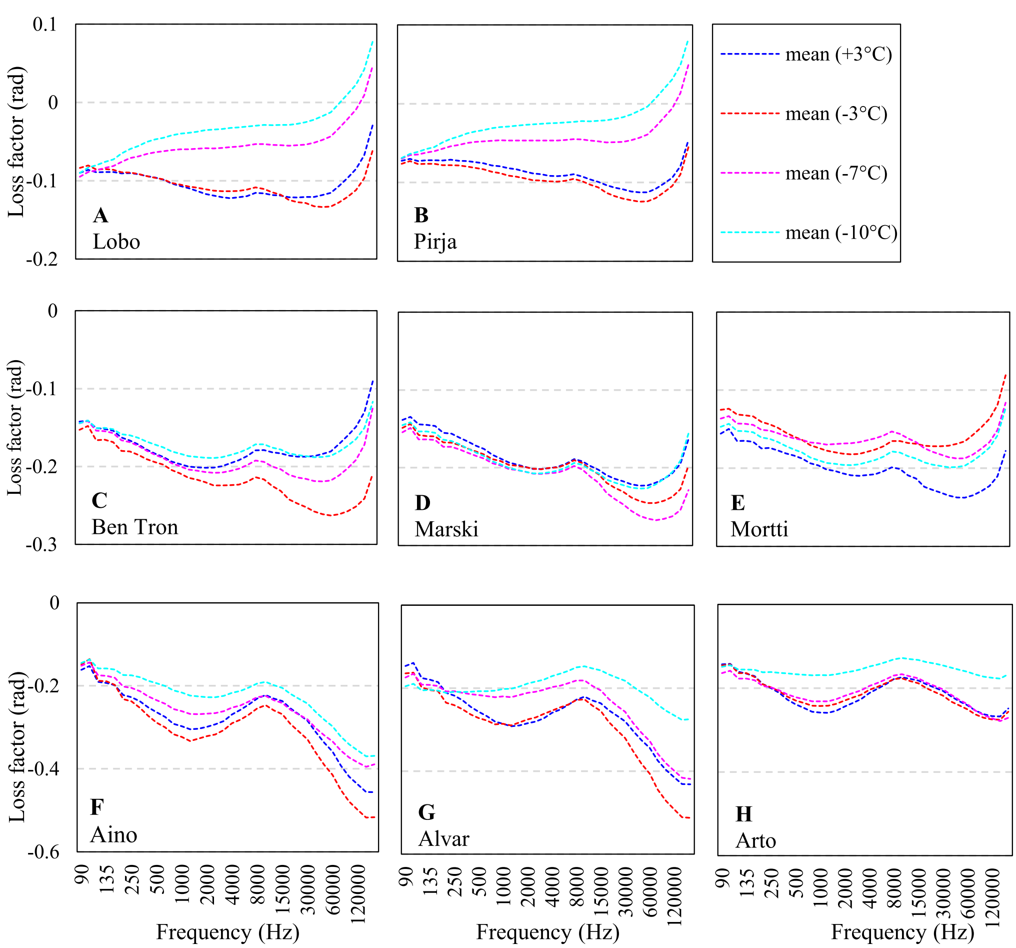

Fig. 2. The frequency (from 90 Hz to 200 kHz) response of the impedance loss factor (δ) of root systems of two apple cultivars, i.e., 'Lobo' (A) and 'Pirja' (B), three blackcurrant cultivars, i.e., 'Ben Tron' (C), 'Marski' (D), and 'Mortti' (E) and three blueberry cultivars, i.e., 'Aino' (F), 'Alvar' (G), and 'Arto' (H) by the storage temperatures (3, −3, −7, −10 °C). The means (dashed line) at different temperatures are indicated by different colors. For the standard deviations (±) of the mean values see from the supplementary file.

Table 1
Mean values of the impedance loss factor (δ_{mean}, rad) at 36 frequencies between 90 Hz and 200 kHz of apple ('Lobo', 'Pirja'), blackcurrant ('Ben Tron', 'Marski', 'Mortti'), and blueberry ('Aino', 'Alvar', 'Arto') at four storage temperatures (3, −3, −7, −10 °C). The lowest value of each cultivar is in bold.

Species	Cultivar	Storage temperature (°C)			
		3	−3	−7	−10
Apple	Lobo	−0.10 ± 0.05	−0.11 ± 0.02	−0.05 ± 0.01	−0.03 ± 0.01
	Pirja	−0.09 ± 0.01	−0.09 ± 0.01	−0.04 ± 0.01	−0.02 ± 0.00
Blackcurrant	Ben Tron	−0.17 ± 0.01	−0.22 ± 0.02	−0.19 ± 0.01	−0.17 ± 0.02
	Marski	−0.19 ± 0.03	−0.20 ± 0.02	−0.21 ± 0.02	−0.19 ± 0.02
	Mortti	−0.20 ± 0.03	−0.16 ± 0.02	−0.17 ± 0.01	−0.18 ± 0.02
Blueberry	Aino	−0.28 ± 0.03	−0.31 ± 0.03	−0.26 ± 0.03	−0.23 ± 0.02
	Alvar	−0.27 ± 0.02	−0.29 ± 0.03	−0.24 ± 0.03	−0.20 ± 0.04
	Arto	−0.21 ± 0.03	−0.21 ± 0.03	−0.21 ± 0.02	−0.16 ± 0.02

Table 2
P-values for the source of variation of the loss factor at 50 kHz frequency (δ_{50kHz}) of different species by the cultivar and the storage temperature (3, −3, −7, −10 °C) with their interactions (*P*-value ≤ 0.05 in bold).

Variation	Apple	Blackcurrant	Blueberry
Cultivar (C)	0.212	**0.007**	**0.005**
Storage temp (T)	**<0.001**	0.305	**0.008**
C * T	0.928	**0.048**	0.481

3.2. The impedance of loss factor at 50 kHz (δ_{50kHz})

In all species, δ_{50kHz} of root varied from −0.01 to −0.40 rad depending on the storage temperature and species. In the apple cultivars, δ_{50kHz} was higher than in the blackcurrant and blueberry cultivars (Fig. 3). In apple, δ_{50kHz} significantly increased with decreasing storage temperature (3, −3, −7, −10 °C) but was not affected by cultivar and their interactions (Table 2). In blackcurrant, δ_{50kHz} was not affected by storage temperatures, but the effect of the cultivar and the interaction with the storage temperature was significant (Table 2). There was no difference among storage temperatures in 'Ben Tron' and 'Marski', but some differences in 'Mortti' (Fig. 3). In blueberry, δ_{50kHz} was significantly affected by the storage temperature and the cultivar, but not by their interaction (Table 2). There was no difference in δ_{50kHz} among the storage temperatures in 'Aino', but in 'Alvar' and 'Arto' δ_{50kHz} was affected by the storage temperature, being the highest at −10 °C.

3.3. CLAFIC analysis of the impedance loss factor

The CLAFIC algorithm was used to classify the impedance loss factor spectra of different species and cultivars between the test set 3 °C and the training sets −3 °C, −7 °C, −10 °C, respectively. In apple, the spectra in 3 °C and −3 °C were similar in both cultivars, and close to similar in 3 °C and −7 °C in 'Lobo', but differed between 3 °C and −10 °C in 'Lobo' and between 3 °C and −7 °C, and between 3 °C and −10 °C in 'Pirja' (Fig. 4A, B). In blackcurrant, the spectra of 'Ben Tron' differed between 3 °C and −3 °C, but did not differ so much between 3 °C and −10 °C (Fig. 4C). The results for 'Marski' look similar as for 'Ben Tron' (Fig. 4D). On the other hand, the spectra of 'Mortti' did not differ between 3 °C and −3 °C as it did between 3 °C and −10 °C (Fig. 4E). In blueberry, the spectra of 'Aino' did not differ very clearly among the comparison groups (Fig. 4F). However, in blueberry, the spectra of 'Alvar' in 3 °C and −3 °C were quite similar, but increasing amount of spectra in −7 °C and −10 °C differed from those in 3 °C (Fig. 4G). Contrasting results were obtained for the blueberry 'Arto' where the spectra in 3 °C differed from those in −3 °C and −7 °C but there were no differences between 3 °C and −10 °C (Fig. 4H).

3.4. Reverse-flow hydraulic conductance of roots (K_r)

In apple, the reverse-flow K_r of 'Pirja' was significantly affected by storage temperatures, with the lowest K_r (2.06 mg•MPa^{-1}•s^{-1}) being at the highest temperature (3 °C) and the highest K_r (7.13 mg•MPa^{-1}•s^{-1}) at the lowest temperature (−10 °C) (Fig. 5A, Table 3). In the cultivar 'Lobo', there was no difference in K_r among the storage temperatures, however. Between two apple cultivars, K_r differed only at −3 °C. In blueberry, there was no difference in K_r among the storage temperatures of all three cultivars (Fig. 5C, Table 3). Among the cultivars, K_r of 'Arto' was the highest at all storage temperatures. Like in blueberry, there was no difference in K_r of blackcurrant cultivars among the storage temperatures, but there were differences in K_r among the cultivars at 3 °C and −3 °C (Fig. 5B, Table 3).

4. Discussion

The roots of apple, blackcurrant, and blueberry cultivars exhibit negative loss factors, which is consistent with findings in other studies

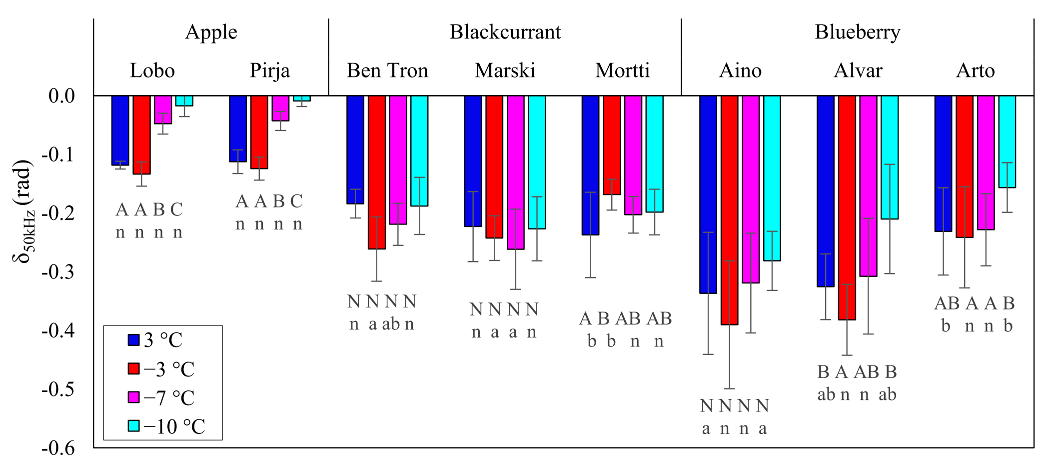

Fig. 3. The impedance loss factor at 50 kHz (δ_{50kHz}) of roots of two apple cultivars ('Lobo' and 'Pirja'), three blackcurrant cultivars ('Ben Tron', 'Marski', and 'Mortti') and three blueberry cultivars ('Aino', 'Alvar', and 'Arto') by storage temperatures (3, −3, −7, −10 °C). Capital letters indicate the differences among the storage temperatures within the same cultivar and the lowercase letters differences between the cultivars of the same species within the same storage temperature ($P < 0.05$). Standard errors of the means are indicated ($n = 6$ for apple, $n = 9$ to 11 for blackcurrant and $n = 5$ for blueberry).

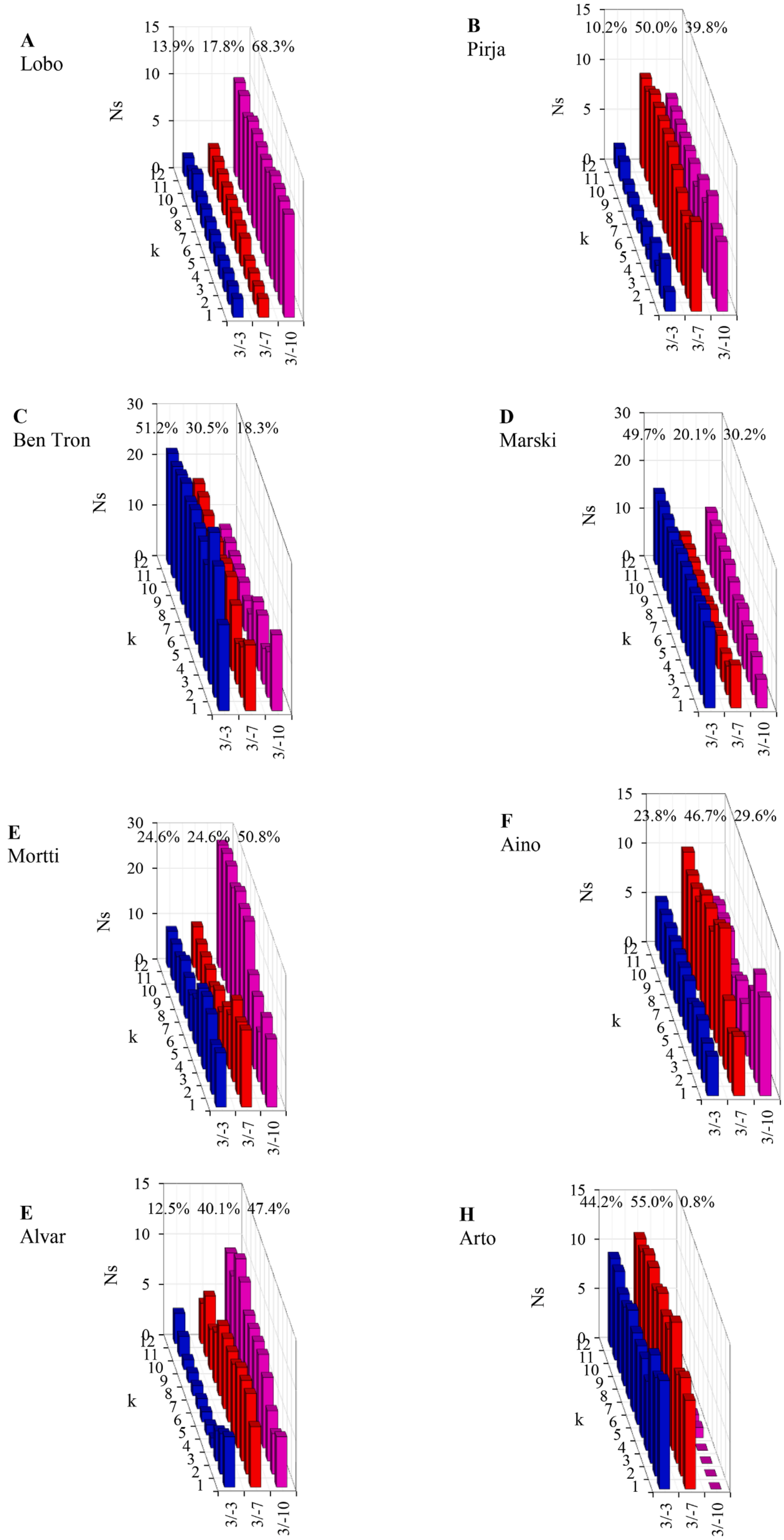

Fig. 4. CLAFIC analysis of the impedance loss factor spectra (data from 90 Hz to 200 kHz) for the roots of apple ('Lobo' (A) and 'Pirja' (B)), blackcurrant ('Ben Tron' (C), 'Marski' (D), and 'Mortti' (E)) and blueberry ('Aino' (F), 'Alvar' (G), and 'Arto' (H)). The storage temperature at 3 °C was used as test set and the storage temperature at −3 °C, −7 °C and −10 °C as the training set, i.e., the groups were 3/−3, 3/−7, 3/−10. N_s indicates the number of impedance loss factor spectra classified into each group based on k-values. The percentages shown on the (k, N_s) plane represent the proportion of the impedance loss factor in each comparison group, as averaged over the k-values.

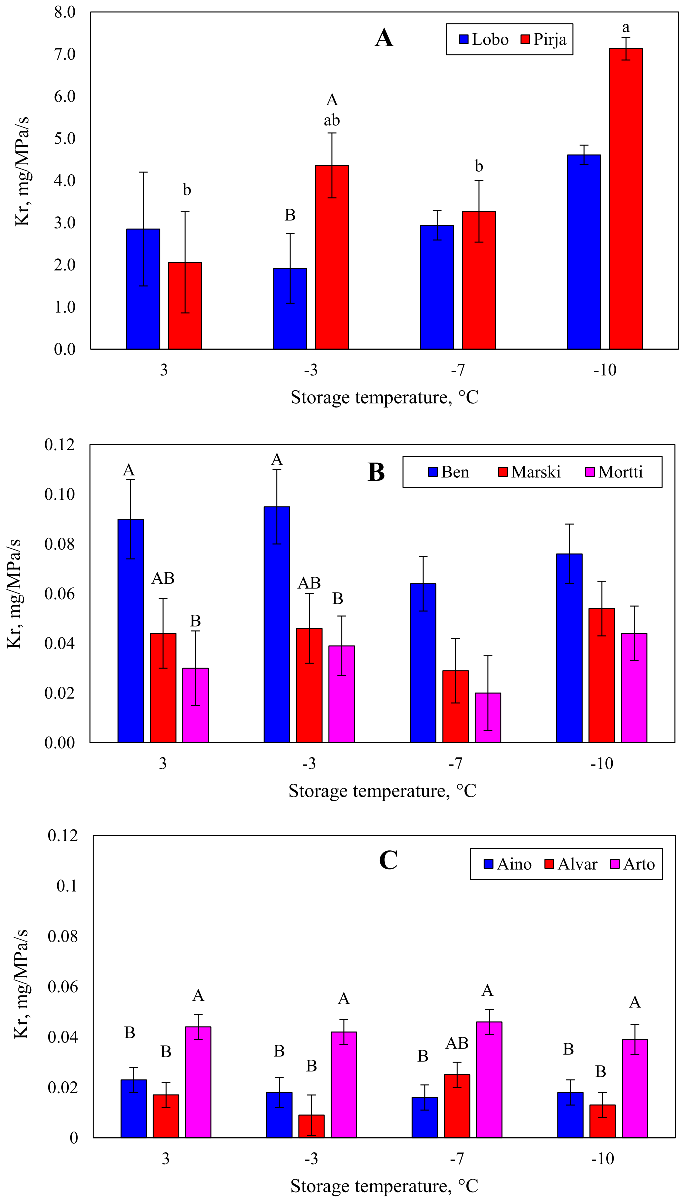

Fig. 5. The reverse-flow hydraulic conductance of roots (K_r) of two apple cultivars ('Lobo', 'Pirja') (A), three blackcurrant cultivars ('Ben Tron', 'Marski', 'Mortti') (B) and three blueberry cultivars ('Aino', 'Alvar', 'Arto') (C), which were stored at four storage temperatures (3, −3, −7 °C – 10 °C) for five to six weeks. The different capital letters indicate the differences between the cultivars within the same storage temperature, and the small case letters the differences between different storage temperatures within the same cultivars. No letter means no difference. Bars indicate standard errors. Note the different scales in different figures.

Table 3
P-values for the source of variation in the root hydraulic conductance (K_r) as analyzed for the cultivars, the storage temperatures (+3, −3, −7, −10 °C) and their interactions (*P*-value ≤ 0.05 in bold).

Variation	Apple	Blackcurrant	Blueberry
Cultivar (C)	0.082	<0.001	<0.001
Storage temp (T)	0.003	0.138	0.467
C*T	0.208	0.930	0.852

on Scots pine seedlings (Di et al., 2019). Negative loss factors indicate that the ions responsible for the alternating current passing through the plant tissue are strongly bound by the structural components of the plant tissue, such as the integrity of cell membranes and cell wall (van den Driessche and Cheung, 1979; Yamada et al., 2002; Takahashi et al., 2019). Intact cell membranes act as a barrier that prevents the leakage of symplastic ions to the apoplastic space, maintaining a controlled internal environment and proper ion balance in cells (Tyree and Hammel, 1972; Schwan, 1988; Steudle, 2000). However, under certain conditions, such as low temperatures, cell membranes can be damaged. When cell membranes are damaged, symplastic electrolytes, including ions and other solutes, can leak into the apoplastic space (Ryyppö et al., 1998). The impedance loss factor (δ) can be used to detect and quantify this leakage. An increase in δ indicates a decrease in low-frequency resistance and suggests that symplastic electrolytes are leaking into the apoplastic space due to cell membrane damage (Di et al., 2019).

The findings of our study revealed differences in the behavior of apple, blackcurrant, and blueberry cultivars concerning the impedance loss factor (δ) in their root tissues under varying storage temperatures. Specifically, in apple roots, the impedance loss factor increased as the storage temperature decreased. This observation suggests that a higher loss factor corresponds to a greater amount of electrolyte liquid seeping or leaking into the apoplastic space. In contrast, blackcurrant and blueberry cultivars did not exhibit such pronounced behavior in the impedance loss factor, although noticeable differences were still observed among these cultivars. These differences indicate that there might be specific physiological or biochemical characteristics unique to each species and even cultivar, influencing their response to changing storage temperatures, such as cell wall properties, degree or lignification, cell maturation, and embolism (Stuart et al., 2007; Ishikawa et al., 2009; Arias et al., 2015; Zhang et al., 2016; Wu et al., 2019). One important difference among the species and cultivars is their ability to increase the concentration of solutes, such as sugars and proteins, within the cells during cold acclimation (Liang et al., 2022). These solutes act as cryoprotectants, lowering the freezing point of the cell sap and reducing the formation of ice crystals, which can cause cellular damage. This adaptive mechanism allows the cells to remain viable even at sub-freezing temperatures (Zhang et al., 2016; Takahashi et al., 2019). In addition to cryoprotectants, woody plants may also undergo other physiological changes in response to cold temperatures. For example, they may adjust the fluidity of cell membranes or increase the production of antifreeze proteins, or even change their xylem structure, all of which may affect loss factor and further enhance their frost tolerance (Kazemi-Shahandashti and Maali-Amiri, 2018).

Apart from inherent physiological differences among plants, the external factors also significantly influence the observed results. Studies conducted by van Beem et al. (1998); Preston et al., (2004), and Ozier-Lafontaine and Bajazet (2005) have indicated that the distance and depth of the soil electrode do not affect capacitance and resistance. However, impedance measurements and data interpretation can become complex due to variations in soil moisture and soil air content, as they can alter the electric field distribution (Rajkai et al., 2005). Additionally, the roots and soil possess different electrical properties compared to aboveground parts (Ehosioke et al., 2020). Consequently, this non-uniform electric field distribution within the root system can result in variations in the measured impedance values and deviate from idealized assumptions of the measurement setup. Factors such as soil electrical conductivity and capacitance, soil moisture, the presence of air gaps or heterogeneities in the soil structure, as well as the varying conductivities and geometries of roots, can introduce artifacts and alter the impedance measurements. Accurate interpretation of impedance data requires accounting for these factors and considering the potential impact of the electric field distribution due to the counteracting effects of roots and soil.

The significant differences in the impedance loss factor at 50 kHz (δ_{50kHz}) of apple were observed within the temperature range of −3 °C to −7 °C. The results suggest increased ion leakage from cells between these temperatures due to freezing damage in roots similarly as observed previously for excised fine root segments (Ryyppö et al., 1998) and whole root systems (Di et al., 2019) after exposure to frost temperatures. Our findings contribute to determine the optimum storage temperature of −3 °C for roots of apple nursery plants for winter storage, which aligns with our earlier study on the aboveground parts of the same plants as here (Wu et al., 2019). However, for blackcurrant and blueberry, the appropriate storage temperature appears to be more dependent on the specific cultivar.

When comparing the effects of different storage temperatures, we observed that both the loss factor (δ) and K_r exhibited responses to freezing stress in most cases. It is presumed that the passage of both electric current and water might have been facilitated by cellular damage, although they followed slightly different pathways. Cellular damage, such as membrane disruption or structural changes, can lead to increased electric conductivity in the apoplastic space. This increase in conductivity may occur due to ions leaking from the symplastic space, where the ions are normally contained within the cells. Additionally, this cellular damage could create openings or permeable areas that allow the movement of electric current and water. Electric current may flow through damaged cell membranes. In contrast, intact root tips work like stoppers for tubes formed of water conducting xylem cells, restricting the flow of water. However, the routes for electric current are not so much dependent on such openings and probably it may pass from root to soil along the root surfaces in contact with soil (Repo and Ryyppö, 2008). Previous studies on EIS have primarily focused on aboveground organs and excised samples, providing valuable insights into their electrical properties (Repo et al., 1994, Repo et al., 2000) but much less is known about the properties of roots. Recent studies highlight the importance of understanding the electrical properties of root tissues and their response to environmental stressors.

The classification and comparison of impedance loss factor spectra among different species and cultivars at different storage temperatures indicate that the spectra exhibit varying degrees of similarity and difference depending on the species, cultivar, and storage temperature. Specifically, in apples, the spectra at 3 °C and −3 °C were similar, while differences were observed between 3 °C and −10 °C in the two cultivars. In blackcurrants, the spectra showed differences between 3 °C and −3 °C, but less pronounced differences between 3 °C and -10 °C. Blueberries displayed varied patterns, with some cultivars showing similar spectra between 3 °C and −3 °C but increasing differences at −7 °C and −10 °C, while others exhibited differences between 3 °C and −3 °C but no differences at −10 °C. These results highlight the importance of storage temperature in affecting the impedance loss factor spectra of different species and cultivars. It suggests that the electrical properties of roots can vary under different temperature conditions, potentially indicating variations in root physiology or metabolic activity. The findings from the CLAFIC analyses on changes in the EIS of intact root systems suggested that with enough training data, it is possible to monitor the FH of the root systems of woody horticultural plants by repeating measurements at intervals (Repo et al., 2014b; Di et al., 2019). However, for nursery applications such as assessing appropriate storage temperature, it is necessary to define criteria for relevant changes in spectra. This could involve, for example, averaging the k-value of the percentages of the spectra in the classification groups, as was done in this study.

The reverse-flow conductance (K_r) of apple roots increased with the decrease of the storage temperature. This observation is similar with the previous studies on Scots pine and Norway spruce seedlings, which showed an increase in K_r as the exposure temperature decreased (Korhonen et al., 2018; Di et al., 2019; Repo et al., 2023). The increase in K_r was accompanied by an increase in δ_{mean} and δ_{50kHz}, suggesting that K_r and δ are associated with freezing injuries in roots. It is likely that low-temperature stress causes cellular damage, resulting in a decline in the hydraulic conductivity of plants. Specifically, the roots tips may be vulnerable to damage, which can impact the measurement of hydraulic conductance when water is pressurized into the roots from the stem (Tyree and Hammel, 1972; Tyree et al., 1995; Steudle and Peterson, 1998; Zwieniecki et al., 2003). In contrast, the reverse-flow conductance of blackcurrant and blueberry were significantly affected by the cultivars rather than storage temperature. This suggests that different cultivars exhibit varying sensitivities to storage temperatures, indicating differences in their tolerance to cold temperatures as well. Additionally, there were notable variations in Kr levels between species, which may be attributed to differences in their morphological and anatomical characteristics (Tyree and Ewers, 1991; Huang and Eissenstat, 2000; Luis et al., 2006; Rodríguez-Gamir et al., 2010). It is important to note that this study was conducted on nursery plants, and it is possible that different results could be obtained with mature plants.

5. Conclusion

In conclusion, our results indicated that the proper storage temperature for roots of apple is −3 °C. However, the appropriate storage temperature for root systems of blueberry and blackcurrant seedlings depends on the cultivar. In addition, our results demonstrated the potential of the classification analysis of impedance spectra in identifying the appropriate storage conditions for different species. This approach can provide a rapid way to assess the root damage caused by low temperature and help to optimize the storage conditions for different species and cultivars. Further research is needed to investigate mechanisms underlying the observed effects and to develop practical applications based on these findings.

Availability of data and material

The data of this study are available from the corresponding author (DW) on request.

Code availability

Not applicable.

CRediT authorship contribution statement

Dongxia Wu: Conceptualization, Methodology, Data curation, Writing – original draft, Writing – review & editing. **Jian Sun:** Methodology, Data curation, Writing – original draft, Writing – review & editing. **Raimo Silvennoinen:** Methodology, Data curation, Writing – original draft, Writing – review & editing. **Tapani Repo:** Conceptualization, Methodology, Data curation, Writing – original draft, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2024.112864.

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