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Studies on visualizing frost/freeze damage in subtropical fruit species

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ABSTRACT

Frost is devastating, especially in the northern Sub-Himalayas and adjoining plains. Extent of damage usually comes into knowledge of the growers when winters are over. Early damage assessing techniques are rare and costly. Regional Horticultural and Forestry Research Station, Bhota/ Neri, Himachal Pradesh has conducted studies for standardization of technique for visualization of frost or freeze damage through a cheap and reliable method. It has been observed that drying and rehydration method for visualizing the frost induced damage to the leaves has given excellent visual clarity and reproducibility. The method was compared to the relative ion leakage method (REL) and thereby its acceptance was confirmed. The relative susceptibility of different subtropical species worked out on the basis of this new method has been presented and was found in the close proximity with susceptibility level of these species observed under natural frost conditions.

Key words: Frost susceptibility, low temperature damage, drying-rehydration method.

INTRODUCTION

Frost induced freezing is the severest stress which subtropical plant species face during winters. Frost damage occurs in almost any location, outside the tropical zones, where temperature dips below the melting point of water (0°C). The extent of damage depends primarily upon the crop's sensitivity to freezing at the time of event and the length of time the temperature remains below the critical damage temperature. Time to time, a number of surveys are being carried out to access the level of damage and to draw strategy for protection against frost in the years to come (Kumar *et al.*,11; Sharma and Badiyala, 15). However, most of these studies have been based on isolated and somewhat subjective observations, in areas with different levels of frost intensities, which have led to contradictory findings.

Critical low temperature or low temperature tolerance limits of different subtropical fruit species are rarely available and growers usually remain in fix regarding selection of protection level for ongoing frost conditions. Added to it, to run the protection procedures continuously throughout the winters is a costly affair. Thus, under such circumstances if growers' competence for tracing out the right time by which the effective protection measure should be started would be of great economical significance.

Further, precise information on cold hardiness of subtropical fruit species is also scanty and growers generally adopt similar level of protection for different species which is many a time happens to be insufficient or over expensive. Till date, apart from simply avoiding frost prone sites, use of cold hardy plant material is the most effective indirect method of avoiding losses due to frost. Precise assessment of relative damage in different fruit species under natural conditions is difficult to make as it greatly influenced by topographical, vegetation and hydrological conditions of the plantation site (Soleimani *et al.*, 16). A number of methods have been used to define frost/ freeze injury in different plant species and these methods are primarily based on: measurement of electrical conductance or resistance (Daxter *et al.*, 7; Mancuso, 10); differential thermal analysis, reflecting the presence or absence of exotherms and nuclear magnetic resonance, infrared video thermography also detects exotherms upon a freezing event (Wisniewski *et al.*, 17) vital stain release of phenolic compounds and leaf tissue browning (Roselli *et al.*, 14); plant recovery capacity (Bartolozzi and Fontanazza, 2) and low temperature scanning electron microscopy (Pearce, 12). Such methods have not been always been effective, and even very successful methods tend to be costly and or laborious. The aim of present studies was thus to device a simple, quick, cheap, effective and reproducible method for visualizing the level of damage to the leaf tissues owing to low temperature stress and to work out the relative frost susceptibility of different subtropical fruit species.

MATERIALS AND METHODS

Most significant damaging effect of freezing on subtropical plant species is reported to be the rupturing

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of cell wall and leakage of cellular contents, using Nuclear Magnetic Resonance (NMR) microscopy, it has been studied and demonstrated in number of species by Ishikawa *et al.* (9); Price *et al.* (13) and Ide *et al.* (8). As, the aim of the studies was to visualize the extent of damage to the species under study, but suitable instrumentation required for the purpose happens to be costly hence efforts were made to visualize the damage through alternate method. Mango, litchi, pomegranate, strawberry, papaya and banana were the species selected for the studies. Ten leaves across the periphery were collected in the month of December (in case of papaya and banana portion of leaf 50 cm^2 was taken). Leaf pedicel or cut ends of the leaves were sealed with paraffin wax and then were put at 4°C for ½ hour. After that the leaf surface was wetted with cold water and these were shifted to subzero (-2°C) conditions for 2, 4, 6 hours of freezing (F). After the desired duration of freezing temperature exposure the leaf samples were brought out at 4°C for ½ hours and then gradually to room temperature. In order to fix the leaf injury, these samples were put into oven at 50°C for 48 hours for complete drying. After drying the leaf samples were rehydrated by placing them in between the layers of wet filter paper. The samples were kept in these layers for 4, 8, 12 and 16 h for rehydration (R). After rehydration these samples were held against the light for visualizing the damaged portion. Visual clarity of the damaged leaf area was adjudged by a panel of four observers, visual clarity was ranked as very good (3 points), Good (2 points), Satisfactory (1 point) and Poor (0 points). Repeated running of procedure for 20 samples per speices comprised units of a replication and the experiment was having four replications. Scores assigned by individual judges were pooled and analyzed as per completely randomized design as described by Chandel (6). The damaged leaf area so visualized was photographed through an ordinary digital camera operated in negative mode, which projected damaged area as white, more prominent than ordinary day light mode of photography. This method of visualizing the frost or freeze injury to the plant leaf samples was designated as 'Drying and Rehydration method for visualizing the low temperature damage'. The relative susceptibility of different species to sub-zero temperature damage was worked out on the basis average damage (%), visualized through above said method. The damaged leaf area was calculated by plotting the entire leaf and its damaged portion on simple graph paper. More the damaged leaf area more susceptible the species was designated for low temperature stress.

In order to validate the findings of drying and rehydration method and subsequent relative susceptibility of the species to freezing temperature stress, Relative Electrolyte Leakage method (REL) was adopted as per procedure described by Soleimani *et al.* (16). For both the methods leaf samples were exposed to sub-zero temperature for four hours and then respective procedures were followed for assessing the low temperature damage. For testing the significance of the discrepancy between theory (drying and rehydration method) and experiment (REL method) 'Chi-square (χ^2) test of goodness of fit was applied as per procedure given by Chandel (6).

RESULTS AND DISCUSSION

Leaf samples when subjected to low temperature stress there was no visual damage observed as such (even after drying the damaged portion was not visible). Under natural conditions also the frost damage to the most number of plants was not visible during the winters but, appeared after a long period when growth started resuming (the frost affected leaves bore burnt appearance). Photographs of leaves damaged due to subzero temperature exposure are presented herewith in Figs. 1 & 2. The leaf area damaged due to low temperature exposure appeared as water soaked lesions, appearing white portion

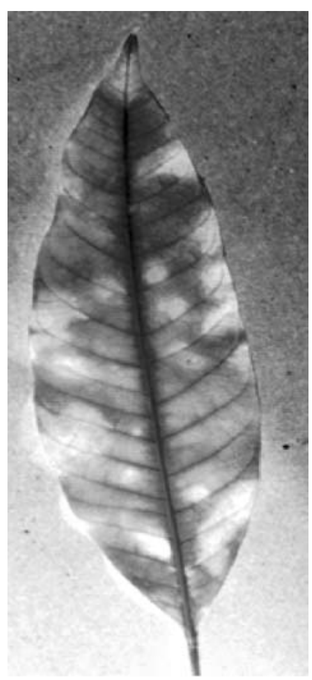

Fig. 1. Rehydrated Freeze damaged leaf viewed through negative mode of camera.

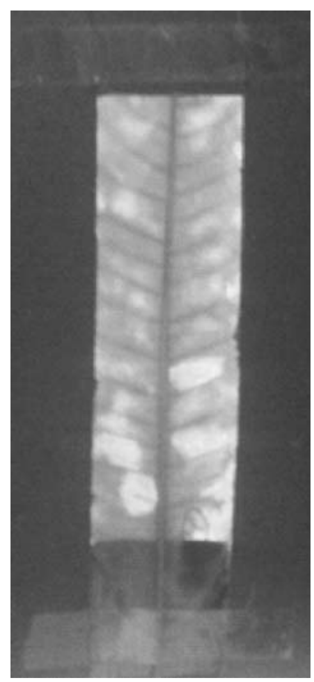

Fig. 2. Rehydrated freeze damaged leaf view against light.

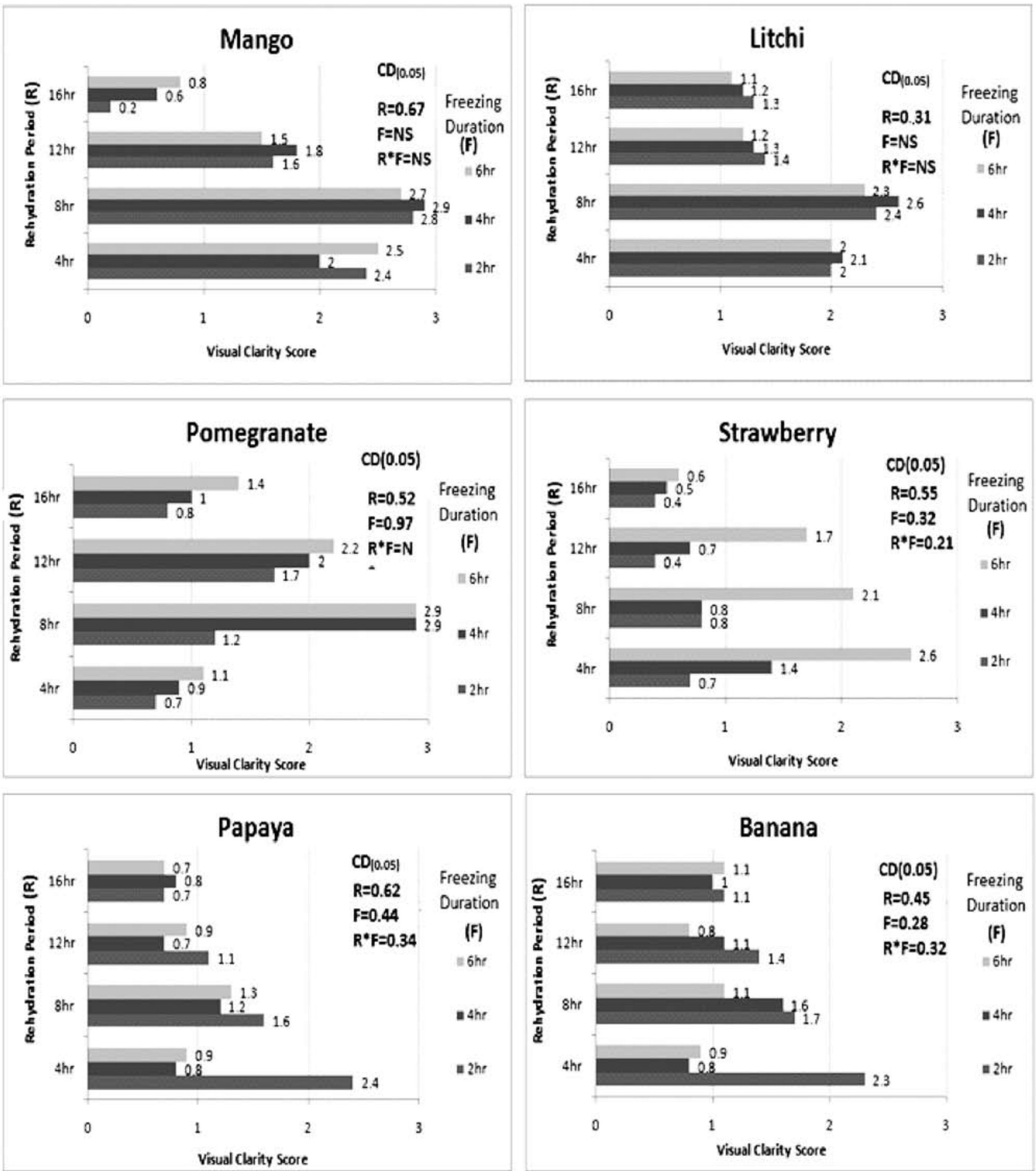

Fig. 3. Visual clarity in leaf samples for frezee damaged area after variable freezing and rehydration periods.

when shot in negative mode (Fig. 1) and translucent when viewed against light (Fig. 2). It appeared as the damaged portion contained only fluid. It might be attributed to the disorganization of the cellular array due to rupturing of cell wall on freezing. Contrarily, the leaf portion which was not damaged to lethal limits maintained its cellular integrity even upon drying and rehydration and thus appeared normal after rehydration. In an experiment with freezing of potted barley plants. Pearce (12) obtained almost similar type of false colour images of leaf samples through infrared video thermography.

The data pertaining to visual clarity of the damaged leaf samples at different levels of sub-zero temperature exposure and variable rehydration periods are presented in Fig. 3. It is evident from the data that visual clarity of damaged leaf area was not influenced by duration of low temperature exposure in case of mango. It has been observed to increased with increase in time period of rehydration and was highest when the samples were rehydrated for eight hours. Beyond eight hours of rehydration the distinction between the damaged and undamaged portion of leaf got intermingled and clarity decreased. Similar level of clarity of damaged leaf area was observed in case of litchi and pomegranate at eight hours of sample rehydration. In pomegranate, very minute spots of damage were observed at two hours of exposure to low temperature, significant damage to the leaves was observed only after four hours of exposure to low temperature. In strawberry six hours of exposure and four hours of rehydration produced most clear picture of damaged leaf area. In papaya and banana also four hours of rehydration resulted in best clarity, of the damaged leaf area. In these species clear damaged area was depicted only in two hours of low temperature exposure, at higher exposure levels complete loss of cellular integrity could not produce better clarity of the damaged area. The appropriateness of eight hours of rehydration for development of clear symptoms in case of mango, litchi and pomegranate and four hours for strawberry, papaya and banana may be attributed to the anatomical features of leaves of woody and herbaceous features. Presence of cutical layer on the leaves of woody species rendered the leaves lesser permeable to water in comparison to the leaves of herbaceous species (Becker *et al.*, 3). Initially it was difficult to clarify the variable impact of time period of low temperature exposure on development of clear images of damaged leaf area but through the later parts of the studies it became clear that medium frost sensitive species might got affected significantly upon four hours of subzero temperature exposure whereas the sensitive species took lesser time period for getting damaged significantly. The less sensitive species like strawberry may take longer time than the medium sensitive species for getting damaged significantly at low temperature.

Further, the data pertaining to percent damage to the leaves of different fruit species exposed to different duration of sub-zero temperature are presented in Table 1. It is evident from the data that as the duration of freezing increased the damage level increased significantly in different species. In mango and litchi damage level was near to hundred percent after six hours of exposure to sub-zero temperature. In case of papaya and banana this damage was near to hundred percent with in four hours of such exposure. In case of banana though the leaf area wise the damage was not hundred percent but it was rated on the basis of damage to the mid rib or main vein. Heavy damage to mid rib prior to leaf lamina may be attributed to the high water content which prevails inside the mid rib in comparison to leaf lamina (Barr and Weatherley, 1).

In pomegranate and strawberry only 51 and 34 percent damage was observed respectively, to the leaf area after six hours of exposure to sub-zero temperature. Statistically Mango, litchi and papaya, banana and pomegranate and strawberry experienced similar level of damage. Relative frost susceptibility of different species may be ranked on the basis of mean value of percent damaged leaf area in increasing order as: strawberry≈<Pomegranate <Litchi≈<Mango <Banana≈<Papaya.

The validity of the above findings as confirmed through REL method. The findings of REL method in comparison to the drying and rehydration method are presented in Table 2. The findings have revealed that relative order of damage to different species was almost similar through both the methods. Further, Percent damage to the leaves owing to sub-zero temperature exposure was also almost similar. χ2-test revealed that calculated value of χ2 was lesser than the tabulated value and hence led to the acceptance

Table 1. Visualized damage (%) to leaves of different subtropical fruit species following freezing-drying-rehydration procedure.

Fruit species (S)	Mango	Litchi	Pomegranate	Strawberry	Papaya	Banana	Mean	$CD_{(0.05)}$
Hours of exposure to sub-zero temp (H)								
2	7.43	4.45	2.71	1.82	70.2	64.2	25.1	S = 18.7
4	64.8	61.6	15.7	15.4	100	100	59.6	H = 12.2
6	97.7	97.0	51.3	34.3	100	100	80.1	S*H = 16.3
Mean	56.6	54.4	23.2	17.2	90.1	88.1		

Table 2. Comparison of drying-Rehydration method with REL method for accessing the low temperature damage to subtropical fruit species.

Fruit	Per cent damage estimated through Drying and Rehydration method	Per cent damage estimated REL method	χ^2 - value	Significance at 5% level
Mango	64.8	74.3	12.4	NS
Litchi	61.6	60.2	0.3	NS
Pomegranate	15.7	20.1	9.6	NS
Strawberry	15.4	9.71	33.3	NS
Papaya	100	97.0	0.9	NS
Banana	100	93.2	0.5	NS

of null hypothesis, *i.e.* acceptance of the drying and rehydration method of accessing the damage in low temperature exposed leaf samples. The findings of these methods have also been found in close conformity with those of Sharma and Badiyala (15). Under natural frost conditions of low hill and valley region of Himachal Pradesh they reported almost similar order of damage to different subtropical fruit species.

From the above described findings it can be concluded that foliar damage in subtropical fruit species due frost induced freezing can be visualized with sufficient clarity with drying and rehydration method. This visualization of damage can serve as a guide for appropriate selection of protection method against the frost. The findings on relative susceptibility of different species presented here can help in selection of species for a particular site and avoiding frost damage.

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