World Applied Sciences Journal 7 (10): 1308-1310, 2009
ISSN 1818-4952


# Ionic Leakage as Indicators of Cold Hardiness in Olive (*Olea europaea* L.)

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**Abstract:** Serious damage caused by cold has frequently been reported from several countries where olives are grown.Temperature is one of the most important climatic factors defining the distribution of plant species. Leaf samples of seven olive cultivars including cvs. 'Roghani', 'Dezfol, 'Roghani riz','Tokhm kabki', 'Drak', 'Dehghan', 'zard'.were taken and exposed to cold temperature at a range of drop of 5 °C per h to -1°C, -5°C, -10°C, -15°C, -20°C. By electrolyte leakage studies the lethal freezing temperature (LT50) of cultivars was found.The ionic leakage technique is appropriate for studing changes in physical properties of membranes and its use in the screening of frost tolerant olive clones is discussed. Whereas results from the electrolytic conductivity indicated that "Dehghan" had the least negative response to freezing, because of their lower ionic leakage contrast.

**Key words:** *Olea europaea* · Artificial Freezing · Electrical conductivity · Frost hardiness · Low temperature

## INTRODUCTION

A strong limiting factor in olive growing is the minimum temperature in winter and early spring [1]. Olive is sub- tropical plant, which grows between 30 and 45 latitiudes in both hemispheres. However, during recent years, the increasing demand for olive oil has expanded the cultivation of olive trees in to geographical zones at higher latitudes than those of the original Mediterranean basin.Moreover, cool autumns which slow down the maturation process, improves the quality of olive oil [2] and this has led to olive trees being cultivated where there is a recurrent danger of frost. Not only below-12° C the trees suffer sever damage [3], but also at -7° C, damage to the aerial parts of the plant, mainly leaf drop and twig desiccation can reduce the productivity and threaten plant life [2]. Selecting frost tolerant genotypes and understanding the mechanism of frost hardiness could greatly improve frost resistance for olive. Recently some new techiniques for identification and classification of frost hardy olive genotypes have been developed [4]. These techniques are based on stomata density, leaf tissue browning and phenolic and ionic leakage. Of these techniques, ionic leakage was found to be the most versatile and to have the greatest sensitivity for acclimation studie.

The aim of the present study was to determine the cold hardiness of some olive cultivars by leaf ionic leakage.

## MATERIAL AND METHODS

The experiment was carried out on olive trees cvs.'Roghani', 'Dezfol, 'Roghani riz','Tokhm kabki', 'Drak', 'Dehghan'and 'zard'.All sampling was taking in late February when air temperature was above 10°C and plants were non acclimated.

Leaf sampling was carried out at late winter, when the olive was in the dehardening phase. The dehardening phase is the periode when the ionic leakage technique is the most effective in discriminating between different genotypes [3] About 15 fully expanded leaves were collected from the mid length of one- year –old shoots from the mother plant of each cultivar and were transferred to a refrigerator at 4 °C.After transfer to a freezing cabinet, they were exposed to low temperatures by 5 °C per h, stages to -1, -5, -10, -15, -20°C. Samples were removed at 5 °C intervals. At each temperature, 10 leaf discs of 10 mm diameter were punched between the major veins and subject to the electrolyte leakage test. Leaf disc were placed in plastic film vials, containing 20 ml of distilled water the vials were then capped with rubber stoppers and shaken for 24 h at 23°C in laboratory.

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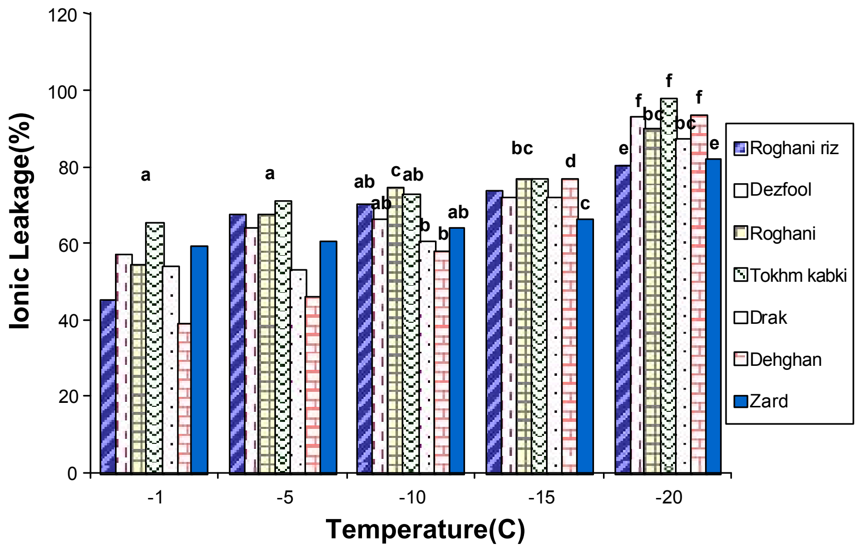


Fig. 1: Ionic leakage from leaf tissue exposed to different temperature

Electrical conductivity was measured with a digital conductivity meter (HI 8633 conductivity meter), in order to kill the tissues completely the solution and samples were then autoclaved, after thawing, the solution was cooled and then conductance was again measured.

Ionic Leakage was expressed as the percentage of the final reading.LT50 the lowest temperature causing 50% damage, was determined by plotting conductivity data against temperature, using a logistic regression model by means of the statistic program [5]. Electrolyte leakage data was subjected to an analysis of variance and to Tukey Multiple comparison test (HSD)

## RESULTS

With decreasing temperature, a clear increase in electrolyte leakage was observed in leaves of all cultivars (Fig. 1).

Leakage release from mature leaves of cv. 'Dehghan' displaying the lowest electrolytic leakage ( below 50 %) at -1 °C and -5 °C, but reached 93 % of total ions at -20 °C, while in other cultivars "Roghani",'Zard', 'Tokhm kabki','Dezfol', 'Roghani riz',' Drak' displaying the highest electrolytic leakage, there was a significant different between cultivars at -1 °C and -5°C with -10 °C, -15 °C and -20°C based on the ionic leakage. Not only at -20°C could distinguish between 'Dezfol', ' Zard', 'Dehghan' with other cultivars (Fig 1) but also at -15 °C was a significant different between cultivars. In 'Roghani' and ' Roghani riz', LT50 were -8± 0/3 °C and in 'Zard' was -8.7± 0/5 °C and in 'Dehghan' was -12.3± 0/5 °C and 'Drak', 'Tokhm kabki', 'Dezfol' were between the two amount.

## DISCUSSION

Trees have great difficulty in absorbing water when the temperature drops below 0°C. [6]. Winslow and Havis [7] found that in Ilex, water movement stops in the stem if the temperature falls within a temperature range of -1.1 °C and -1.4 °C. However, water continues to evaporate from the aerial part and most water is lost through stomata. Based on ionic leakage, 'Dehghan' was considered a frost hardy cultivar. But also the other cultivars will be more sensitive than ' Dehghan'. Ionic leakage technique seems a valuble tool since it can detect changes in the physical properties of the plasma membrane that occure during de-hardening phase [8].

Since frost resistance is a highly complex and not well- known subject in olive, further studies also on the compounds in this mechanism are needed. In fact, many physiological and biological changes are apparently involved in hardiness regulation including carbohydrate metabolism, protein synthesis, gene expression and alteration of membranes. Moreover, selected frost-resistant genotypes must be tested to verify their agronomic behaviour [1].

## CONCLUSIONS

In order to estimate frost damage, we believe that the entire plant should be used instead of single plant parts and also to receive more reliable results in screening of cultivars based on ionic leakage, study of plants at different phases of their growth will be needed. The results presented in this work should be considered as preliminary observation, but there is need for more investigation before any reliable recommendation can be made.

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