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Changes in Leaves Component of Some Mango Cultivars in Relation to Exposure to Low Temperature Degrees

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Abstract: This study was conducted throughout 2008 and 2009 seasons on leaves of Ewais, Sediek, Fagri-Klan and Zebda mango cultivars for studying the changes in electrical conductivity, electrolytes percentage, index of injury and total phenols in relation to exposure to low temperature degrees. The electrical conductivity, electrolytes percentage and index of injury were increased by reducing temperature degree from 4 to-4°C. There was no significant effect for reducing temperature from 4 to-4°C on total phenols in leaves of all studied cultivars. On the other hand, as the period of exposure to low temperature lengthened the values of electrical conductivity, electrolytes percentage and index of injury were increased, this was true when leaves exposed to-4°C, while when they exposed to 4°C, no significant differences were detected between the three periods of exposure (1,2 or 3 h). As for total phenols, it is clear that exposed leaves for 2 h had more effective in increasing total phenols than 1 or 3 h. of exposure. On the other side, the effect of low temperature exposure was differed from cultivar to another, since Fagri-Klan and Zebda cultivars recorded higher electrical conductivity, electrolytes percentage and index of injury when exposed to-4°C comparing with Ewais and Sediek cultivars. While Sediek and Zebda cultivars showed higher values of total phenols than the other cultivars when exposed to 4 or-4°C.

Key words: Mango *(Mangifera indica L.)* · Low temperature degrees · Electrical conductivity · Electrolytes percentage · Index of injury · Total phenols

INTRODUCTION

Mango *(Mangifera indica L.)* is considered one of the most important fruit crops in the world. Mango grows over a wide range of frost-free climates. The trees produce best in climates that have a well defined, relatively cool dry season with high heat accumulation during the flowering and fruit development period. Mango trees show high susceptibility to cold injury where, young trees are damaged by temperature of-5°C and variability among cultivars is apparent after expose to low temperature which is one of the most common environmental stresses and can potentially cause severe losses to major economically important plants. Also, low temperature affecting the growth and development of plants and significantly considered one of the major hazards to agriculture and is an important factor that limits the survival, productivity and geographical distribution of plants in large areas of the world [1]. Conductivity methods have been widely used to estimate frost hardiness in plant tissues [2-4]. Such conductivity measurements have also been used to estimate the passive permeability of membranes to electrolytes [5]. Shawky *et al*. [6] studied winter hardiness of Basrai banana cultivar they reported that electrical conductivity method proved to be suitable for determining cold injury in Banana plants. Zhou Yuping [7] studied the changes in relative electric conductivity (REC) at the period of chilling-stress and recovering of banana leaves. Moreover, Zhang and Willison [8] found that the conductivity method systematically underestimated frost hardiness based on visible injury. The cell membranes are directly involved in cold acclimation and freezing tolerance and plasma membranes are the primary sites of freezing injury [9]. Seasonal variations in the freezing tolerance of many perennial evergreen woody plants are reported [10]. The maximum freezing tolerance of plants is not constitutively expressed, but it is induced in response to low temperature exposure [11]. When a plant is exposed to suboptimal low temperatures, many cellular functions

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are disturbed. The cell membrane is the most direct target of low temperature injury in plants. A major effect is the physical transition of the membrane from a flexible liquid crystalline to a solid gel phase [12]. Almost all of the models that describe how exposure to chilling temperatures can be transduced from a physical into a physiological change involve cellular membranes [13]. In many plant species, a period of exposure to low positive temperatures can result in increased tolerance to subsequent subzero temperatures, by cold acclimation which accompanied by a battery of biochemical, structural and physiological changes [14]. Therefore, knowledge of the physiological events that occur during cold acclimation will allow better understanding of freezing tolerance in evergreen woody plants leaves.

This study was conducted to determine the changes in electrical conductivity, electrolytes percentage, index of injury and total phenols of Ewais, Sediek, Fagri-Klan and Zebda mango leaves in relation to exposure to low temperature degrees.

MATERIALS AND METHODS

This study was conducted throughout 2008 and 2009 seasons on Ewais, Sediek, Fagri-Klan and Zebda mango cultivars grown in a private orchard located at Elwadi El-Faregh Cairo- Alexandria desert road, Six[th] of October Governorate, Egypt. Trees were about 12 years old, planted in sandy soil at 5X5 m a part uniform in growth vigor and received the same horticultural practices. A randomized complete blocks design was used where each treatment was replicated three times, each comprising one tree. Samples of fifty mature leaves were taken from each tree during the last week of December in 2008 and 2009 seasons and brought to the laboratory of the Faculty of Agriculture, Cairo University. Leaf samples were cleaned and washed then divided into small segments ($1cm^2$). A sample of leaf segments (two grams) was taken from each replicate and placed in a test tube, then kept at room temperature, at 4°C or at -4°C for 1, 2 or 3 hours. After that 20 cm of distilled water were added to each sample, then left at 25°C for 24 hours and electrical conductivity (EC) of the solution (μ mohs) was measured by using EC Meter Model, HANNA -HI 99331.

After that the samples were boiled for seven minutes to kill the tissue, then cooled and brought back to the initial volume by adding distilled water and held at 25°C for 24 hr. then the electrical conductivity was measured again. According to the values of electrical conductivity both percentage of electrolytes and index of injury were calculated as follows:

$$\text{Electrolytes (\%)} = \frac{\text{EC (μ mohs) before boiling}}{\text{EC (μ mohs) after boiling}} \times 100$$

The index of injury (I_t)

$$I_t = 100\,(L_t - L_0) / (L_k - L_0)$$

Where:

I_t = Index of injury resulting from exposure to temperature 4°C or -4°C

L_t = Electrical conductivity (EC) from sample exposed to temperature 4°C or -4°C.

L_0 = Electrical conductivity (EC) from sample kept at room temperature.

L_k = Electrical conductivity (EC) from sample exposed to temperature 4°C or -4°C and then kept killed.

Two grams of leaf samples were taken from each replicate (after exposure to room temperature, 4°C or -4°C for 1, 2 or 3 hours without boiling) for determining total phenols according to Swain and Hillis [15].

Data were tabulated and statistically analyzed according to Snedecor and Cochran [16] and mean values were compared by Duncan's multiple range test at 5% level of probability [17].

RESULTS AND DISCUSSION

Electrical Conductivity: Data in Table 1 showed that electrical conductivity of different mango cultivar leaves were affected by low temperature either as degree or period of exposure. Generally, it is noticed that electrical conductivity was increased due to the reduce of temperature degree, since exposed mango leaves to-4°C recorded higher significant electrical conductivity values than those recorded when exposed to 4°C. On the other hand, it is clear that as the period of exposure was lengthened, the electrical conductivity value was higher. This was true when the mango leaves were exposed to the lower temperature (-4°C), while when they exposed to 4°C, the lowest period of exposure (1 h) recorded the lowest significant value as compared to 2 or 3 h. This was true in both studied seasons. As for the effect of exposed mango leaves to low temperature on different cultivars, it is clear that Fagri Klan and Zebda cultivars were affected significantly and recorded higher electrical conductivity values when exposed to the lower temperature degree (-4°C) than the other cultivars (Ewais and Sediek). However, when different mango cultivars were exposed to 4°C, no clear trend was detected between the cultivars especially in the first season. While, the second one, only Ewais

Table 1: Electrical conductivity (μ mohs) of mango leaves after exposed to low temperature degrees during 2008 and 2009 seasons

Cultivars	Temperature degrees and time of exposure								
	4°C				-4°C				
	1 h	2 h	3 h	Mean	1 h	2 h	3 h	Mean	Average
				2008 season					
Ewais	5.28 g-i	9.93 e-h	4.99 hi	6.73 DE	12.11 ef	7.97 e-i	8.46 e-i	9.51 D	8.12 C
Sediek	8.12 e-i	10.91 e-h	7.55 e-i	8.86 D	6.45 f-i	12.70 e	44.25 c	21.14 C	15.00 B
Fagri-Klan	2.67 i	6.51 f-i	2.71 i	3.97 E	8.39 e-i	40.79 c	87.53 a	45.57 A	24.77 A
Zebda	4.99 hi	6.21 f-i	8.89 e-h	6.70 DE	11.18 e-g	25.43 d	80.41 b	39.01 B	22.86 A
Mean	5.27 E	8.39 CD	6.04 DE		9.53 C	21.72 B	55.16 A		
Average		6.57 B				28.81 A			
				2009 season					
Ewais	6.54 g-j	11.59 g	10.15 gh	9.43 C	8.67 g-j	5.27 h-j	46.04 c	19.99 B	14.71 B
Sediek	4.05 ij	9.48 g-i	5.44 h-j	6.33 D	10.28 gh	24.20 f	21.05 f	18.51 B	12.42 C
Fagri-Klan	5.15 h-j	5.24 h-j	8.33 g-j	6.24 D	12.02 g	29.32 e	84.79 a	42.05 A	24.14 A
Zebda	4.93 h-j	3.52 j	9.75 gh	6.07 D	33.53 de	35.73 d	57.45 b	42.24 A	24.16 A
Mean	5.17 E	7.46 DE	8.42 D		16.13 C	23.63 B	52.33 A		
Average		7.01 B				30.70 A			

Table 2: Electrolytes (%) of mango leaves after exposed to low temperature degrees during 2008 and 2009 seasons

Cultivars	Temperature degrees and time of exposure								
	4°C				-4°C				
	1 h	2 h	3 h	Mean	1 h	2 h	3 h	Mean	Average
				2008 season					
Ewais	0.0033 e	0.0033 e	0.0035 de	0.0033 BC	0.0038 c-e	0.0038 c-e	0.0040 c-e	0.0039 B	0.0036B
Sediek	0.0030 e	0.0027 e	0.0027 e	0.0028 C	0.0029 e	0.0035 de	0.0059 b	0.0041 B	0.0034B
Fagri-Klan	0.0029 e	0.0027 e	0.0028 e	0.0028 C	0.0036 de	0.0056 bc	0.0090 a	0.0061 A	0.0044 A
Zebda	0.0035 de	0.0040 c-e	0.0029 e	0.0035 BC	0.0040 c-e	0.0054 b-d	0.0083 a	0.0059 A	0.0047 A
Mean	0.0032 C	0.0032 C	0.0030 C		0.0036 C	0.0046 B	0.0068 A		
Average		0.0031 B				0.0050 A			
				2009 season					
Ewais	0.0031 d-f	0.0035 d-f	0.0035 d-f	0.0034 BC	0.0037 d-f	0.0036 d-f	0.0050 cd	0.0041 B	0.0037B
Sediek	0.0026 f	0.0027 ef	0.0027 ef	0.0027 C	0.0029 ef	0.0027 ef	0.0042 c-f	0.0033 BC	0.0030C
Fagri-Klan	0.0029 ef	0.0027 ef	0.0027 ef	0.0028 C	0.0037 d-f	0.0046 c-e	0.0088 a	0.0057 A	0.0042AB
Zebda	0.0038 d-f	0.0035 d-f	0.0034 d-f	0.0035 BC	0.0057 bc	0.0060 bc	0.0071 b	0.0063 A	0.0049 A
Mean	0.0031 C	0.0031 C	0.0031 C		0.0040 B	0.0042 B	0.0063 A		
Average		0.0031 B				0.0048 A			

Table 3: Index of injury of leaves for some mango cultivars after exposed to low temperature degrees during 2008 and 2009 seasons

Cultivars	Temperature degrees and time of exposure								
	4°C				-4°C				
	1 h	2 h	3 h	Mean	1 h	2 h	3 h	Mean	Average
				2008 season					
Ewais	0.033 e	0.085 de	0.033 e	0.050 C	0.101 de	0.080 de	0.058 de	0.080 C	0.065 C
Sediek	0.059 de	0.107 de	0.050 e	0.072 C	0.032 e	0.110 de	0.430 b	0.191 B	0.131 B
Fagri-Klan	0.068 de	0.037 e	0.027 e	0.044 C	0.108 de	0.307 bc	0.872 a	0.429 A	0.236 A
Zebda	0.045 e	0.055 de	0.112 de	0.071 C	0.071 de	0.246 cd	0.775 a	0.364 A	0.217 A
Mean	0.051 C	0.071 C	0.056 C		0.078 C	0.186 B	0.534 A		
Average		0.059 B				0.266 A			
				2009 season					
Ewais	0.046 ef	0.059 ef	0.113 ef	0.072 BC	0.097 ef	0.052 ef	0.455 bc	0.201 B	0.137 B
Sediek	0.031 f	0.087 ef	0.052 ef	0.057 C	0.106 ef	0.211 def	0.202 def	0.173 BC	0.115 B
Fagri-Klan	0.062 ef	0.042 ef	0.068 ef	0.057 C	0.111 ef	0.267 c-f	0.843 a	0.407 A	0.232 A
Zebda	0.053 ef	0.053 ef	0.088 ef	0.064 C	0.298 cde	0.395 bcd	0.577 b	0.423 A	0.244 A
Mean	0.048 C	0.060 C	0.080 C		0.153 BC	0.231 B	0.519 A		
Average		0.063 B				0.301 A			

Values followed by the same letter (s) are not significantly different at 5% level of probability.

Table 4: Total phenols (mg/100 g F.W) in leaves of some mango cultivars after exposed to low temperature degrees during 2008 and 2009 seasons

	Temperature degrees and time of exposure								
	4°C				-4°C				
Cultivars	1 h	2 h	3 h	Mean	1 h	2 h	3 h	Mean	Average
				2008 season					
Ewais	270.4 d	279.4 d	254.4 d	268.1 C	250.5 d	276.7 d	260.0 d	262.4 C	265.2 B
Sediek	589.5 ab	598.4 ab	467.0 c	551.6 AB	590.6 ab	488.8 bc	549.4 a-c	542.9 B	547.2 A
Fagri-Klan	283.3 d	280.0 d	290.6 d	284.6 C	256.6 d	273.9 d	280.0 d	270.2 C	277.4 B
Zebda	581.2 ab	646.7 a	585.6 ab	604.5 A	483.9 bc	558.9 a-c	582.8 ab	541.9 B	573.2 A
Mean	431.1A	451.1 A	399.4 A		395.4 A	399.5 A	418.1 A		
Average		427.2 A				404.3 A			
				2009 season					
Ewais	277.9 gh	263.0 h	245.3 h	262.0 C	266.5 gh	280.4 gh	264.3 h	270.4 C	266.2 B
Sediek	590.8 ab	615.3 a	461.5 f	555.9 A	515.6 c-f	527.9 c-e	521.4 c-e	521.6 B	538.7 A
Fagri-Klan	286.5 gh	275.1 gh	286.6 gh	282.7 C	251.7 h	287.9 gh	325.9 d	288.5 C	285.6 B
Zebda	508.4d-f	564.9 a-d	570.0 a-c	547.8 AB	492.2 ef	551.3 b-e	551.4 b-e	531.7 AB	539.7 A
Mean	415.9AB	429.6 A	390.8 BC		381.5 D	411.9 A-C	415.7 A-C		
Average		412.1 A				403.0 A			

Values followed by the same letter (s) are not significantly different at 5% level of probability.

cultivar gave a higher significant value for the electrical conductivity compared with the other cultivars.

Electrolytes (%): As the result showed in Table 2 it is clear that exposing mango cultivars to low temperature had a significant effect on electrolytes percentage of their leaves. In this respect, mango leaves exposed to the lower temperature degree (-4°C) gave higher significant electrolytes values comparing with the higher one (4°C), this was true in both studied seasons. However, the period of exposing mango leaves to low temperature had no effect on electrolytes percentage when the leaves were exposed to 4°C. In this concern, neither 1 hr nor 2 or 3 h as an exposure period had any effect on electrolytes percentage in the first and second seasons of the study. While the period of exposure to lower temperature degree (-4°C) significantly affected the electrolytes values. Since the determination was higher when the leaves exposed to 3 h compared with 1 or 2 h of exposure, this result was detected in the first and second seasons. On the other hand, the electrolytes percentage was significantly differed among the cultivars. Since Fagri Klan and Zebda cultivars gave the higher values in comparing with Ewais and Sediek cultivars especially when leaves exposed to-4°C. This was true in the two seasons of study.

The Index of Injury: Data in Table 3 showed the effect of exposed mango leaves to low temperature degree on index of injury. Values of index of injury take the same trend of electrolytes percentage since the lower temperature (-4°C) recorded higher values comparing with 4°C. As for the period of exposure, no differences were detected between the three periods of exposure when leaves exposed to 4°C, while as when leaves exposed to-4°C, the longer exposure period, the higher value of injury index. Regarding to the effect of low temperature on injury index of different cultivars, it is noticed that Fagri Klan and Zebda cultivars recorded the higher index of injury when exposed to-4°C as compared with Ewais and Sediek cultivars. The previous results were true in both studied seasons.

Total Phenols: Data in Table 4 showed that exposed mango leaves to low temperature had a little effect on total phenols in the leaves. However, neither 4°C nor-4°C showed significant effect in the first and second seasons. On the other side, exposed mango leaves to low temperature slightly increased total phenols which recorded a highest value when the leaves exposed for 2 h of low temperature either 4°C or-4°C. Concerning the effect of low temperature on different mango cultivars, it is clear that Sediek and Zebda cultivars showed the higher values of total phenols when exposed to 4°C or-4°C than the other cultivars; this was true in both studied seasons.

From the above mentioned results it could be concluded that, the electrical conductivity, electrolytes percentage and index of injury were increased by exposing leaves of Ewais, Sediek, Fagri-Klan and Zebda mango cultivars to -4°C. However there was no significant effect of reducing temperature from 4 to-4°C on total phenols in the leaves of all studied cultivars. The effect of low temperature exposure was differed from cultivar to another since, Fagri-Klan and Zebda cultivars recorded higher electrical conductivity, electrolytes percentage and index of injury when exposed to-4°C comparing with

Ewais and Sediek cultivars. While Sediek and Zebda cultivars showed higher values of total phenols than the other cultivars when exposed to 4°C or-4°C. The changes in the electrical conductivity, electrolytes percentage and index of injury in relation to exposure to low temperature could be explained due to the results obtained by Shawky *et al.* [6] who reported that, a significant reduction in the index of injury was accompanied by a significant increase in bound water and significant reduction in free water. Also, Shawky *et al.* [18] reported that the low temperature released some electrolytes from the tissues. Moreover, the longer the period of cold treatment the, more electrolytes were released. They added that the degree of injury increased as the period of exposure increased. Also they added that the percentage of electrolytes and the index of cold injury showed parallel trends. Mango trees show a high susceptibility to cold injury where, the young trees are damaged by temperatures of-0.5°C and the variability among cultivars is apparent after cold spell [19]. Moreover, Mckellar and Buchanan [20] reported that the effect of low temperature was differed according to the cultivar and the planted area of Avocado trees. Shawky *et al.* [21] reported that the increment in cell sap concentration was related to the decrease in leaf injury index. They added that there was a strong relationship between the cell sap concentration and cold hardiness of Basrai banana leaf. They added that the only factor which seems to be of a close relationship with cold hardiness is the cell sap concentration. When the cell sap concentration was not changed plants were susceptible to cold injury. Moreover, the increase in glucose, sucrose and total sugars are not closely correlated with cold hardiness as measured by injury index. Wilner [22] stated that "because permeability is related to injury and cold tolerance and electrolytic conductance is could be taken as suitable method for measuring cold injury of plants". The electrolytes leakage method is based on freezing damage to cell membranes being indicated by an increase in the conductivity of water extracts of excised plant tissues after freezing stress. Shawky *et al.* [23] reported that cold temperatures damaged Basrai banana leaves through disrupting the cloroplastides in the palisade tissue. Young and Mann [24] reported that leaves of banana frozen at -3.3°C and -6.7°C had loss of chloroplast structure. Zhang and Willison [25] found that tissue frost-hardened by maintenance at 2°C leaked more electrolytes than the relatively frost-sensitive tissue grown at 20°C. Levitt [26] reported that membranes are the cell structures most sensitive to freezing stress. Glerum [27] and Repo [28] found that, injured cells are unable to maintain the chemical composition of their contents and release electrolytes through damaged membranes. The electrical impedance of plant tissue has been found to decrease with cell damage and the membranes are directly involved in cold acclimation and freezing tolerance, plasma membranes are the primary sites of freezing injury [9]. Saltveit [13] Show that, the increase in membrane permeability and rates of ion leakage are associated with chilling of sensitive tissue. Further studies are needed for testing cold hardiness of different mango cultivars and employed the result of our study under different conditions.

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