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Physiological changes in leaves of some mango cultivars as response to exposure to low temperature degrees

Abstract

This study was conducted throughout 2012 and 2013 seasons on leaves of Alphonso, Balady, Bullock's Heart, Helmand, Hindy Besennara, Mabrouka, Mestekawy, Nabeeh, Ewais, Spates, Taimour and Zebda mango cultivars to evaluate their ability of mango cultivars to acquire cold injury during exposure to low temperature (storage of mango leaves at 5°C and 10°C). The changes in relative water content (RWC), membrane stability index (MSI), electrolytes leakage (EC%) and total free amino acids in relation to exposure to low temperature degrees were studied. The RWC, MSI and TFAA were decreased by reducing temperature degree from 10°C to 5°C. On contrast, EC% was increased. On the other hand, as the period of exposure to low temperature lengthened the values of RWC and MSI were decreased. On contrast, EC% and TFAA were increased. This was true when leaves exposed to 5°C or 10°C between the five periods of exposure (2, 4, 6, 8 or 10 days). It is clear that exposed leaves for 10 days had more effective in decreasing RWC, MSI and TFAA than other days of exposure. On the other side, the effect of low temperature exposure was significantly differed from cultivar to another under the temperature of 5°C and 10°C.

Keywords: Mango *(Mangifera indica L.)*, cultivars, cold tolerance, biochemical changes.

Volume 3 Issue 6 - 2019

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Received: October 17, 2019 | **Published:** November 15, 2019

Introduction

Mango *(Mangifera indica L.)* is a popular, nutrional tropical fruit, which is now one of the most important fruit crops (as evergreen trees) in tropical and subtropical areas of the world.[1] Mango occupies third place in total world production of major tropical fruit crops after citrus and banana.[2] Environmental conditions outside the traditional areas for optimum growth of mango may impose stresses, which can result in physiological changes, reduced growth, or even permanent damage to the trees.[3,4]

Low temperature is acknowledged to be one of the most dominant environmental stresses that affect the growth, productivity and geographical distribution of crops and horticultural plants.[5] Exposure to low, nonfreezing temperatures induces genetic, morphological, metabolic and physiological changes in plants, which result in the development of cold hardiness and acquisition of freezing tolerance.[6] Mango grows over a wide range of the frost-free climates. The trees produce best in climates that have a well-defined, relatively cool dry season with high heat accumulation during flowering and fruit development period.[7] A mango needs an optimum temperature range of 24-26.7°C and minimum threshold temperature is 10-12°C below this temperature, a plant shows chilling injury.[8] Mango trees show high susceptibility to low temperature (0-15°C). Young trees are damaged by low temperature variability among cultivars is apparent after a cold spell, but precise information on this subject is non-existent,[9] nor is it known if mangoes can acclimate to cold.[10] An understanding of the biology of low temperature in mango may provide the basis for potential cold-protection strategies and developing cold tolerant cultivars and may also trigger interest in the responses of other tropical and subtropical plants to cold stress.[11]

Materials and methods

Two experiments were conducted during 2012 and 2013, one of them carried out in a private orchard, Sayleen village, Sennouris district, Fayoum Governorate, Egypt, located at 29° n°. The other one was done in the laboratory of Agricultural Botany Dept., Faculty of Agric., Fayoum University, Egypt. The experiments assess to evaluate the cold tolerance of some mango cultivars grown under Fayoum Governorate condition and check some putative physiological and biochemical events that occur following cold exposure.

Climate of experiment site

At the site of experiment, temperatures fluctuated during the duration of study and five years before the beginning of the study. In this respect, the mean monthly maximum temperature (TMax) ranging from about 17.7°C in January 2008 to about 40.2°C in August 2010 and the fluctuation in mean monthly minimum temperature (TMin) was ranging from about 5.7°C in January 2007/2008 to about 25.2°C in August 2012. An extreme minimum temperature of 0.6°C was recorded in January 2008; however, there were large differences between the selected years (Table 1).

Plant materials

The plant materials comprised 12 mango cultivars which collected from their natural growing location in the period of November 2012 to March 2013. This is approximately the time at which the cold hardiness may be occurring. Mango trees were about 30 years old, planted in a clay soil at 5x5 m apart. The cultivars were used in this study including most of the popular cultivars which grown in Fayoum Governorate. The tested cultivars were, Alphonso, Balady, Bullock's Heart, Helmand, Hindy Besennara, Mabrouka, Mestekawy, Nabeeh, Ewais, Spates, Taimour, and Zebda. In this study, two trials were conducted independently in the field and laboratory.

Field trial

Thirty-six trees of similar phenotype in the field (size, vigor…etc) with management prehistory were selected for this experiment (no further tests were carried out confirm genetic uniformity). All selected trees were allocated at random with each replicate. The trees were derived from seedlings; named Balady, while the others they had been grafted onto seedling rootstocks. To obtain a complete picture of mango cold tolerance status under natural conditions, measurements must be made at frequent intervals throughout the growth period. Therefore, samples of ten mature terminal fully expanded leaves (similar development ages) were randomized taken from each tree (as a replication) at the end of each month from November to March. Leaves were collected (between 9 to 10 am) from one-year-old shoots of the mango cultivars and used as a plant material. All treatments were applied in a factorial randomized complete block design with three replicates (each of replicate represent of a tree).

Table 1 The Monthly mean of maximum and minimum temperatures (open air-temperature) during the period from January 2006 to March 2013

Month Year	Temp.	2006	2007	2008	2009	2010	2011	2012	2013
January	Max.	19.4	19.4	17.7	20.7	21.9	20.7	20.4	23.0
	Min.	6.6	5.7	5.7	6.7	7.6	8.0	7.2	10.3
February	Max.	22.2	21.4	20.0	22.3	24.4	22.0	22.4	24.3
	Min.	8.4	7.8	6.5	6.4	8.2	9.4	8.2	10.4
March	Max.	26.3	25.0	28.6	23.2	27.5	25.6	25.4	29.0
	Min.	9.7	9.8	11.6	7.9	11.4	9.5	11.8	12.6
April	Max.	30.4	28.6	31.6	30.8	31.8	28.5	29.1	
	Min.	13.3	12.5	13.7	12.5	14.3	13.7	13.6	
May	Max.	33.4	35.2	35.4	32.8	34.1	32.8	34.1	
	Min.	16.9	17.7	18.2	16.7	16.7	17.4	18.3	
June	Max.	36.5	39.3	39.4	38.2	38.4	35.7	38.4	
	Min.	20.0	20.7	22.0	20.4	21.4	20.6	21.8	
July	Max.	37.4	38.9	37.7	38.5	36.3	38.7	39.8	
	Min.	21.3	21.8	22.1	22.7	22.4	21.5	23.6	
August	Max.	38.3	37.8	38.6	37.0	40.2	38.6	38.0	
	Min.	22.1	21.7	22.2	21.8	24.5	22.9	25.2	
September	Max.	34.8	34.3	35.9	35.2	36.2	36.1	35.2	
	Min.	20.3	20.5	20.0	20.7	21.9	22.1	23.4	
October	Max.	31.0	32.1	31.5	31.7	35.9	30.2	34.4	
	Min.	17.2	18.3	17.2	18.1	21.3	17.7	22.2	
November	Max.	23.6	26.7	26.6	25.0	31.3	26.5	27.9	
	Min.	11.1	12.8	13.1	11.7	16.5	12.6	16.4	
December	Max.	19.9	21.3	22.2	22.4	24.1	22.0	21.8	
	Min.	7.5	8.2	9.1	8.9	10.3	9.7	11.0	

*Data applied by Bureau of Meteorology, Agriculture Administration, Fayoum Governorate, Egypt

Laboratory trial (cold storage)

Studying cold tolerance in the field is difficult. Field sites often exhibit either complete survival or complete winter kill. Because of this variability, the laboratory procedures to measure cold tolerance have been developed by a number of investigators.[12–14] The results have usually been in a good agreement with field observations of natural cold injury.[15] At the end of November, the mature fully expanded leaves of each cultivar were collected, cleaned, washed and toweled dry then putted in a vinyl plastic bags and subjected to various low temperature treatments. In this respect, the collected leaves from each cultivar were divided in two batches; each one was composed of 1800 leaves (10 leaves in three replicates for a storage period per cultivar). These batches were stored in a refrigerator at 5°C and 10°C in darkness. Ten leaves were taken from each batch (three replicates) per cultivar every 2 days up to 10 days. The leaves not subjected to storage of low temperature were used as a control sample (zero time). Samples from these leaves were selected for physiological and biochemical studies. All treatments of cold storage were applied in a split-split plot design with three replicates (each of replicate represent of a tree).

Physiological studies

Some reliable assays to assess physiological changes related to cold tolerance of mango cultivars were done.

Citation: Gadallah FM, El-Yazal MAS, Abdel-Samad GA, et al. Physiological changes in leaves of some mango cultivars as response to exposure to low temperature degrees. *Horticult Int J.* 2019;3(6):266–273. DOI: 10.15406/hij.2019.03.00141

Relative water content (RWC, %) estimation

Leaf relative water content (%) was determined[16] using the entire fully expanded leaves of similar age. Ten leaf samples were taken from each tree (at 8 am) to estimate RWC. Fresh weight (FW), saturated fresh weight (TW), and dry weight (DW) of leaves were determined for each cultivar. After measuring FW of freshly leaves, they were placed into containers (slightly longer than the sample) with distilled water for 24h until a constant weight (TW), (the adhering water of the leaves was bottled with absorbent paper toweling). FW was measured for each sample. DW was obtained after drying these leaves at 70°C in an oven for 72h to a constant weight. RWC (%) was calculated as follows: **RWC (%)** = (FW-DW)/(TW-DW)×100.

Membrane stability index (MSI, %) estimation

A fresh sample of 0.4g (leaf segments) was collected from each cultivar to estimate. Samples were washed three times with distilled water to remove the surface-adhered electrolytes. Plant segments were divided equally and placed in two closed vials containing 20 ml of distilled water. One vial was incubated at 40°C on a water bath for 30 min. The electrical conductivity of the solution (EC1) was determined with conductivity meter (Mod: HI99300). The second vial was boiled placed at 100°C for 10 min. and electrical conductivity of the solution (EC2) was determined with conductivity meter.[17] The MSI was calculated using the following formula:

$$MSI\ (\%) = 1 - (EC1/EC2) \times 100$$

Electrolytes leakage (%) estimation

The total leakage of inorganic ions was estimated[17] using leaf samples that were cut into equal sized pieces (1g/replicate). Each sample was placed in boiling glass bottle (100 ml brown glass bottle) containing 50 ml deionized water. Bottles were then incubated at 25°C in an incubator for 24 h, and then the electrical conductivity (EC1) of the solution was recorded using a conductivity meter (Mod: HI99300). The bottles were then boiled at 100°C for 7 min, and re-incubated at 25°C in an incubator for 24h, and then the electrical conductivity of the solution was recorded (EC2). Electrolyte leakage (EL, %) was calculated using the following formula:

$$EL(\%) = (EC1/EC2) \times 100$$

Total free amino acids

Total free amino acids concentration (mgg^{-1}DW) was determined calorimetrically according to.[18] A ninhydrin solution was prepared by dissolving 2g ninhydrin in 25ml acetone, and then adding 25 ml 0.2M acetate buffer, pH 5.5. The ninhydrin solution was stored in a brown bottle to protect it from light. A standard solution of arginine was prepared by dissolving 5 mg in 10 ml 0.1 M HCl. Ninhydrin reagent (1ml) was added to 1 ml of the amino acid solution in a test tube. Water was added to each tube to a total volume of 4ml. After mixing well, the tube was kept in a boiling water bath for 15min. The contents of the tube were then cooled to room temperature and 1.0ml 50% (v/v) ethanol was added. The pink colour that developed was measured by its absorbance at 550 nm using a spectrophotometer (Spectronic 20, Bausch & Lamb). To determine the content of total free amino acids in each leaf sample (or leakage), the same steps as above were followed for a standard curve based on arginine.

Results

Relative water content (RWC, %)

The pattern of changes in RWC of leaves stored at 5°C and 10°C are shown in Table 2. The results indicate that RWC was significantly differed among the tested cultivars since, the cultivar of Balady gave the minimum value in RWC as compared to the others reached to the maximum value by the cultivar of Alphonso when the leaves were exposed to 5°C while, the exposure of leaves to the temperature of 10°C, the cultivar of Helmand recorded the highest value of RWC (%) and the lowest one was exhibited by the cultivar of Balady. However, when the different mango cultivars were exposed to low temperature of 5°C or 10°C, no clear trend was detected between the periods of storage.

Table 2 Relative water content (%) in leaves of some mango cultivars after exposure to low temperature degrees (5°C and 10°C) at various storage periods (2 to 10 days)

Cultivars	0-time	Temperature degrees (°C) and storage periods (day)											
		5°C					Mean	10°C					
		2	4	6	8	10		2	4	6	8	10	Mean
Alphonso	125.3	75.9	165.6	99.1	89.4	92.3	104.5	130.4	106.2	102.7	113.2	74.6	105.4
Balady	232.6	78.5	65.0	60.0	57.9	73.6	67.0	86.3	66.2	70.4	97.2	101.0	84.2
Bullock's Heart	118.7	135.7	134.9	62.7	95.7	139.2	113.6	154.3	162.2	81.6	43.1	60.9	100.4
Helmand	124.6	174.1	152.7	114.5	115.1	163.1	143.9	151.8	204.5	105.6	112.5	161.7	147.2
Hindy Besennara	107.5	126.1	95.0	87.3	70.2	119.9	99.7	152.2	148.9	67.9	74.8	112.2	111.2
Mabrouka	135.4	132.5	92.4	76.2	117.2	154.5	114.6	179.3	168.0	55.4	99.7	94.6	119.4
Mestekawy	140.5	119.0	94.2	83.6	130.4	124.8	110.4	172.6	137.2	98.2	69.0	108.9	117.2
Nabeeh	147.0	150.2	161.9	117.1	134.3	179.6	148.6	165.9	170.6	83.5	104.0	105.2	125.9
Ewais	120.7	124.7	122.3	112.9	77.8	153.4	118.2	156.7	152.7	82.3	94.2	148.0	126.8
Spates	110.8	112.4	66.0	76.1	63.8	85.4	80.8	269.0	73.8	106.9	80.0	87.3	123.4
Taimour	143.0	136.6	117.6	126.4	112.4	180.0	134.6	171.5	182.8	100.2	126.6	116.2	139.5
Zebda	151.1	121.4	116.5	109.7	89.7	110.1	109.5	113.6	136.5	104.7	114.6	111.7	116.2

Table Continued

Cultivars	0-time	Temperature degrees (°C) and storage periods (day)											
		5°C					Mean	10°C					
		2	4	6	8	10		2	4	6	8	10	Mean
Mean	138.1	180.9	115.3	93.8	96.2	131.3		158.6	142.5	88.3	94.1	106.9	
	Cultivars (A)							53.9					
	Temprature (C°) (B)							22.0					
	Storage period (day) (C)							34.8					
LSD (5%)	(AxB)							76.3					
	(BxC)							120.6					
	(AxC)							49.2					
	(AxBxC)							170.5					

Membrane stability index (MSI, %)

The results in Table 3 clearly explain that differences were observed during all the periods of storage for the different cultivars. However, the values of MSI were significantly differed among the tested cultivars. In this respect, it is clear that maximum MSI value was recorded by Zebda cultivar and the minimum value was observed by Spates one during the storage at 5°C. while, at 10°C the highest value of MSI was obtained by Nabeeh cultivar and the lowest one was recorded by Balady one. In addition, MSI values of stored leaves was significantly influenced by the interaction effect of temperature regimes and mango cultivars but with no clear trend between the periods of storage.

Table 3 Membrane stability index (%) in leaves of some mango cultivars after exposure to low temperature degrees (5°C and 10°C) at various storage periods (2 to 10 days)

Cultivars	0-time	Temperature degrees (°C) and storage periods (day)											
		5°C					Mean	10°C					
		2	4	6	8	10		2	4	6	8	10	Mean
Alphonso	66.19	70.68	24.29	39.13	43.36	33.97	42.29	65.37	42.51	49.43	58.01	38.84	50.83
Balady	65.07	56.49	52.13	23.44	57.14	19.26	41.69	59.24	28.62	35.25	25.27	15.06	32.69
Bullock's Heart	60.22	51.75	41.05	72.01	36.93	19.39	44.23	68.89	31.98	50.27	58.88	48.88	51.78
Helmand	72.07	45.46	55.30	66.27	55.93	68.33	58.26	76.64	37.48	53.89	65.39	54.27	57.53
Hindy Besennara	66.24	66.46	41.08	61.46	29.53	14.27	42.56	55.13	46.81	38.52	56.61	26.00	44.61
Mabrouka	57.13	50.61	31.63	48.25	48.78	29.24	41.70	69.49	43.89	47.98	50.49	43.48	51.07
Mestekawy	68.71	47.33	41.76	65.59	56.40	53.48	52.91	66.63	44.65	40.31	51.79	32.15	47.10
Nabeeh	74.35	73.57	40.33	65.62	62.93	68.95	62.28	72.66	52.41	58.84	64.54	70.40	63.77
Ewais	54.54	64.51	41.37	52.25	66.91	63.18	57.64	67.96	43.92	41.32	20.97	43.62	43.56
Spates	33.75	44.42	12.41	38.91	16.46	20.59	26.56	66.49	32.84	62.80	43.20	38.84	48.84
Taimour	62.30	50.85	44.83	63.78	38.97	39.66	47.62	66.48	62.82	42.48	52.82	38.67	52.65
Zebda	69.41	72.13	47.01	70.46	59.37	77.21	65.23	48.97	57.44	51.55	30.35	44.51	46.56
Mean	**62.50**	**57.85**	**39.43**	**55.60**	**47.73**	**42.30**		**65.33**	**43.78**	**47.72**	**48.19**	**41.23**	
	Cultivars (A)							4.36					
	Temprature (C°) (B)							1.78					
	Storage period (day) (C)							2.81					
LSD (5%)			(AxB)					6.17					
			(BxC)					9.75					
			(AxC)					3.98					
			(AxBxC)					13.79					

Electrolytes leakage (EL, %)

The present results in Table 4 exhibit that an increase in EL from the leaves of the most cultivars under cold storage condition reached its maximum level by the cultivar of Mestekawy and the minimum level by the cultivar of Zebda at the temperature of 5°C. at storage of leaves at 10°C, EL level showed a maximum value by the cultivar of Hindy Besennara and the minimal was recorded by Ewais cultivar. In this respect, the EL increased abruptly from 10°C to 5°C and reached a plateau at 5°C in the most of the tested cultivars. When different mango cultivars were exposed to low temperature either 5°C or 10°C, no clear trend in EL was detected between the intervals of the cold storage for the leaves.

Table 4 Electrolytes leakage (%) in leachate of some mango leaves after exposure to low temperature degrees (5°C and 10°C) at various storage periods (2 to 10 days)

Cultivars	0-time	Temperature degrees (°C) and storage periods (day)											
		5°C					Mean	10°C					
		2	4	6	8	10		2	4	6	8	10	Mean
Alphonso	44.00	69.15	92.73	83.82	56.38	68.09	74.03	59.42	80.00	73.77	50.46	52.71	63.27
Balady	49.00	68.45	90.86	81.17	56.76	67.13	72.88	60.30	81.06	68.09	74.26	67.08	70.16
Bullock's Heart	82.00	83.52	75.91	73.45	66.62	61.69	72.24	74.82	75.63	51.65	47.59	48.57	59.65
Helmand	46.00	66.32	49.99	66.06	44.96	48.86	55.24	53.63	65.99	51.78	44.37	45.24	52.20
Hindy Besennara	47.00	73.09	90.60	81.34	49.54	64.00	71.71	78.29	75.54	80.30	61.80	76.81	74.55
Mabrouka	57.00	75.78	61.78	77.27	38.65	47.96	60.29	89.98	70.51	51.33	67.64	78.56	71.60
Mestekawy	63.00	77.87	92.15	84.38	73.46	70.75	79.72	59.26	59.66	56.47	59.94	52.85	57.64
Nabeeh	33.00	56.43	37.38	67.06	55.86	55.97	54.54	51.94	44.88	46.05	50.68	43.92	47.50
Ewais	67.00	84.77	83.21	79.84	69.88	53.91	74.32	58.00	64.87	53.79	37.51	37.59	50.35
Spates	74.00	79.65	83.32	81.68	54.74	43.43	68.57	73.91	65.09	58.33	50.14	61.02	61.70
Taimour	47.00	68.45	68.67	58.64	56.50	52.32	60.92	51.52	75.17	65.07	55.94	69.14	63.37
Zebda	50.00	54.41	50.52	63.44	51.83	49.81	54.00	72.54	68.01	55.15	61.95	57.31	62.99
Mean	**55.00**	**71.49**	**73.09**	**74.85**	**56.26**		**56.99**	**65.3**	**68.87**	**59.32**	**55.19**	**57.57**	
	Cultivars (A)								4.28				
	Temprature (C°) (B)								1.75				
	Storage period (day) (C)								2.76				
LSD (5%)		(AxB)							6.05				
		(BxC)							9.57				
		(AxC)							3.91				
		(AxBxC)							13.54				

Total free amino acids (TFAA)

As it can be seen from the data in Table 5 that, in the leachates of stored leaves, there is an increase in TFAA leakage to reach a maximal level by the cultivar of Mabrouka and a minimal level was recorded by the cultivar of Mestekawy at the temperature of 5°C. The amount of leached TFAA from stored leaves into the imbibing medium recorded the highest value by the cultivar of Bullock´s Heart and the lowest was given by Hindy Besennara at the temperature of 10°C. However, the level of leached TFAA was significantly differed among the tested cultivars. The changes in TFAA along the period of storage have not any trend between the times of storage for all the tested cultivars.

Discussion

More than half of the 350000 plant species on earth are grown in the tropics and subtropics. In the course of evolution, they could not develop the ability to withstand low temperatures.[19] Most of these are damaged during storage or exposing at temperatures above the freezing point of tissue, but lower than 15°C (chilling temperatures). This damage is called chilling injury as opposed to damage during freezing (freezing injury).[20] Thus, chilling injury is damage to chilling sensitive plant species during exposure to chilling temperatures. Chilling temperatures lead to numerous physiological disturbances in the cells of chilling-sensitive plant and result in chilling injury and death of tropical and subtropical.[19] Obviously, the problem of plant resistance to chilling temperature, which often occurs in spring and autumn in many countries, is important for practical plant breeding. A change in the Egyptian climate has already been observed during the last decades, and this is expected to continue throughout this century. This change in climate affects the behavior of fruit trees and their productivity. In 2008, Egypt was exposed to a wave of frost that caused severe damage to mango trees; Hence, there is a need to know which cultivars are resistant to winter cold.[21] As a result of this sensitivity, low temperature produce a range of physiological and metabolic disorders that lead to serious losses. The various dysfunctions that arise under low temperature conditions result in various physical and metabolic changes that are easily scored and which can therefore be used to assess the degree of chilling injury. The effect of low temperature on RWC in mango cultivars leaves in Table 2 show that the high content of RWC in the cultivars of Mabrouka; Alphonso as well as Helmand under field and laboratory studies respectively is commonly related to the increased cold hardiness. The water status may also have an indirect effect on cold hardiness mediated by a decreased respiratory consumption of cryoprotective sugars in dehydration tissues.[22] The changes in both the amount and physical state of water are perhaps dependent on the accumulation of hydrophilic substances such as proteins and sugars. Plants may develop the capacity not only to retain water in tissues but also to protect cell structure against desiccation by accumulating substances that bind water molecules, which leads to a reduction in free water and to an increase in bond water.[23,24] Additionally, lowering the temperature results in increased viscosity of water and membranes, hydraulic conductivity for water uptake may be reduced, regardless whether uptake is induced by cell water deficit. Reduced temperatures also affect cell metabolism, including photosynthesis associated to these processes. Availability of solutes is also an important of cell expansion and photosynthesis and respiration are potential sources of such solutes. Thus, temperature effects on cell metabolism may also reflects on the water uptake. So, because low-temperature may affect water uptake associated to both changes in hydraulic conductivity and cell metabolism, measurements of RWC may vary greatly with temperature in rapidly expanding tissues, regardless the imbibition period.[25] Cell membrane is the first barrier that separates cells from their environment and also is a primary target for damage under environmental stress. Dysfunction of cell membrane at low temperature is considered to be the primary molecular events ultimately leading to the development of chilling injury symptoms.[26] Maintenance of membrane stability at low temperature is important for plant resistance to cold stress.[27] Membrane lipids from chill-resistant plants often have a great proportion of unsaturated fatty acids than those from chill-sensitive plants.[28] This rising in lipids (unsaturated fatty acids) allows membranes to remain fluid by lowering the temperature at which the membrane lipids begin a gradual phase change from fluid to semicrystalline. Thus, desaturation of fatty acid provides some protection against damage from chilling. In cold tolerant plants, the modified membranes are better able to remain fluid and function at lower temperature.[29] In this study, MSI is an important indicator for chilling tolerance, as was illustrated by which mango leaves under field conditions (Table 3), the cultivar of Helmand recorded the highest value of MSI and the lowest by Spates one. But, Zebda and Spates stored at 5◦C as well as Nabeeh and Balady at 10◦C show relatively higher and lower tolerant to low temperature in terms of MSI, respectively (Table 3). Moreover, electrolytes leakage is widely used to measure chilling damage as well as to quantity species resistance to cold and chilling injury in conifer[30] and in apple.[31] Low temperature alters the physical properties of cell membranes. Chilling of sensitive plants leads to multiple changes in their membranes, which enhance permeable properties of cell membrane.[19] These changes in the state of membranes may lead to secondary of irreversible reactions, depending on temperature, exposure duration and sensitivity of the species (or cultivars). After a prolonged chilling, these changes will cause loss of membrane integrity and comport mentation, the leakage of solutes, decrease of oxidative activity of mitochondria, increase of the activation energy of membrane-bond enzymes including H^{+-}ATPase, reduce the rate of photosynthesis, cause disruption and imbalance of metabolism, the accumulation of toxic substances and the symptoms of chilling injury.[32,33] In addition, A number of species of tropical have the lateral phase separation temperature some higher (15ºC) than in plants from temperate zones (6-8ºC) suggesting that plants reduce the freezing point of membranes with the distance from zone of tropical origin.[34] However, the increased leakage of electrolytes from the cultivar of Hindy Besennara (Tables 4) and Mestekawy cultivar was interpreted as resulting from deteriorative changes in membranes and corresponds to the presence of released inorganic and organic ions. Similar results were obtained by Loay[35] and Chinnusamy et al.[36]

Table 5 Total free amino acids concentration (mg $100g^{-1}$ FW) in leachate of some mango cultivars leaves after exposure to low temperature degrees (5ºC and 10ºC) at various storage periods (2 to 10 days)

Cultivars	0-time	Temperature d storage periods (day)											
		5ºC					Mean	10ºC					
		2	4	6	8	10		2	4	6	8	10	Mean
Alphonso	13.33	28.09	29.52	49.04	34.76	27.62	33.81	26.66	34.28	23.81	15.24	25.24	25.04
Balady	19.05	37.14	17.14	40.95	26.19	15.71	27.43	46.66	36.66	34.28	19.52	23.81	32.19
Bullock's Heart	12.86	37.61	27.14	36.19	35.23	20.00	31.23	51.90	50.95	24.76	16.66	22.38	33.33
Helmand	14.76	30.00	21.90	28.09	24.76	20.95	25.14	30.47	34.28	22.38	32.85	39.04	31.81
Hindy Besennara	20.00	20.47	25.71	30.00	27.62	23.19	25.40	21.43	20.47	18.09	20.95	22.38	20.66
Mabrouka	15.71	34.28	32.85	49.04	31.90	28.57	35.33	46.66	29.04	22.85	17.14	25.24	28.19
Mestekawy	17.62	25.71	20.00	20.00	18.57	18.09	20.47	31.42	30.95	21.43	21.90	21.43	25.43
Nabeeh	16.19	25.24	26.66	26.19	17.14	26.66	24.38	23.33	15.24	20.95	28.09	26.66	22.85
Ewais	13.81	16.66	20.95	24.28	26.66	19.52	21.62	21.43	30.95	18.09	19.52	20.00	22.00
Spates	14.28	28.09	32.85	28.57	33.33	26.19	29.81	23.33	25.24	25.24	21.90	19.05	22.95
Taimour	13.81	35.71	42.38	33.33	21.90	29.04	32.47	43.80	27.14	19.05	14.28	18.57	24.57

Table Continued

Cultivars	0-time	Temperature d storage periods (day) 5°C					Mean	10°C					Mean
		2	4	6	8	10		2	4	6	8	10	
Zebda	10.95	18.57	18.09	39.04	26.66	20.95	24.66	24.28	18.09	15.71	19.05	32.85	22.00
Mean	**15.20**	**28.13**	**26.27**	**33.73**	**27.06**	**23.04**		**32.62**	**29.44**	**22.22**	**20.59**	**24.72**	
LSD (5%)	Cultivars (A)								3.36				
	Temprature (C°) (B)								1.37				
	Storage period (day) (C)								2.17				
	(AxB)								4.76				
	(BxC)								7.52				
	(AxC)								3.07				
	(AxBxC)								10.64				

Alternations in the level of total free amino acids TFAA content in the leaves have been shown when plants are subjected to artificial conditions **(**laboratory study, Table**)**, the cultivar of Alphonso (field conditions) and Balady one (storage of at 5°C and 10°C) recorded the highest values of TFAA. Accumulation of substantial amounts of compatible TFAA play a role in protection of cells from cold injury.[37,38] In previous study, cold treated plants were shown to have relatively increased amounts of free amino acids.[39]

Acknowledgments

None.

Conflicts of interests

Authors declare no conflict of interest exists.

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