Pak. J. Bot., 51(3): 845-854, 2019. DOI: 10.30848/PJB2019-3(15)

EFFECTS OF WATER STRESS AND RECOVERY PERIODS ON TOTAL LIPID AND FATTY ACIDS IN *CUCUMIS MELO* VAR. *FLEXUOSUS* (L.) NAUDIN AND RELATED SPECIES

ŞENER AKINCI[1] AND DOROTHY M. LÖSEL[2]

[1]*Department of Biology, Faculty of Arts and Sciences, Marmara University, 34722 Göztepe-İstanbul-Turkey*
[2]*Department of Animal and Plant Sciences, University of Sheffield, S10 2TN, U.K.*
[*]*Corresponding author's email: akinci@marmara.edu.tr; Tel: 00 90 216 345 11 86 ext. 1211; Fax: 0090 216 347 87 83*

Abstract

Water stress (drought) is one of the most important abiotic factors influencing plant growth parameters, accompanied by changes in biomass ratios and physiological and biochemical alterations depending on the severity and duration of drought exposure, as well as plant species. Total lipids and fatty acids (FA) are major components of the plant cell, and can be used to understand changes in cell lipid concentrations under water stress. In this study, the aim was to investigate the responses of total and fatty acids (FA) being major components of the plant cell, are required to be understood of changes of compositions of cell lipids under two water stress levels and recovery periods by rewatering of Cucurbitaceae members. Total lipid and gas liquid chromatography (GLC) analyses indicated that the species behaved differently with respect to water stress levels and recovery periods. Linolenic (18:3) and eiocosenoic acid (20:1) accounted for the biggest proportion of FA in all species. The major effect of water stress was seen in proportions of 18:3, which increased significantly in leaves of *Cucurbita pepo* under moderate stress (MS) and severe stress (SS) , whereas it remained unchanged in *Cucumis melo* under both treatments, but decreased significantly under SS in *Ecballium elaterium.* During recovery, further changes in FA percentages occurred at different rates in individual species. For the short recovery period determined after 2 days rewatering, the total leaf lipid content increased significantly in previously stressed plants. The effect of the longer recovery period (4d rewatering), was accompanied by a continued increase of total lipid content in all plants in previous MS and SS treatments, and was significant in MS and SS *Ecballium* compared to control plants.

Key words: Water stress, Cucurbitaceae, *Cucumis*, Lipids, Fatty acids, Recovery.

Introduction

Water deficit limits plant growth and productivity on a global basis. It affects the whole plant, starting in the protoplasm where water is crucial for plant cells and the protein, sugar and lipid molecules of the cytoskeleton. Water shortage (drought) causes to changes in cell structure, and ultimately to plant death. (Kramer & Boyer, 1995; Atkinson *et al*., 2000; Massonnet *et al*., 2007; Jaleel *et al*., 2008; Akıncı & Lösel, 2012; Osakabe *et al*., 2014).

Lipids play a vital role in the physiology of higher plants, for instance phospholipids and glycolipids as non-protein components found in cell membranes and triglycerides (fats and oils) as reduced carbonic storage forms particularly in plant seeds (Taiz & Zeiger, 1991). As major components of the membrane, lipid content and composition have been extensively investigated by many workers to assess the impact of different water stress levels on lipid changes in different parts of various plants (Douglas & Paleg, 1981; Pham-Thi *et al.,* 1982; Liljenberg & Kates, 1982; Anh *et al*., 985; Svenningsson & Liljenberg, 1986; Pham-Thi *et al*., 1987; Navari-Izzo *et al*., 1989; Navari-Izzo *et al*., 1990; Navari-Izzo *et al*., 1993; El Kaoua *et al*., 2006; Martins Junior *et al*., 2008; Pandey *et al*., 2010; Moradi *et al*., 2017). Research has showed that plant lipids are related in defense reactions as signal mediators (Wang *et al*., 2006; Munnik and Testerink, 2009; Okazaki and Saito, 2014), as well as in the mitigation processes occuring in plant cells under various stress factors apart from water stress (Nakamura *et al*., 2009; Moellering *et al*., 2010; Gasulla *et al*., 2013).

In the plant cell, the physical state and composition of two layers of *lipid* molecules in which enzymatic proteins are embedded, effect both structural and functional properties of membranes. The membranes selectivity and their functioning range of the types, proportions of lipid and protein components (Navari-Izzo *et al*., 1993; Sanchez-Martin *et al*., 2018). The features and composition of membrane lipids affect enzyme and transport capacity (Gronewald *et al*., 1982; Kuiper, 1985; Whitman & Travis, 1985). Water deficits induce decreasing total lipid content (Gigon *et al*., 2004; Junior *et al*., 2008) and lipid peroxidation index (Irigoyen *et al*., 1992), conformational alterations in membrane proteins (Navari-Izzo *et al*., 2000) and increasing membrane damage (lipid peroxidation) (Esfandiari *et al*., 2007). Pham-Thi *et al*., (1982) and Anh *et al*., (1985) stated that water stress inhibits fatty acid desaturation, resulting in sharply decreased biosynthesis of linoleic and linolenic acids, reduces polar lipid content, and resulted in galactolipid levels much lower than phospholipid content. Svenningsson & Liljenberg, (1986) found that phospholipids and total acyl lipids drastically decreased in rape seedling (*Brassica napus* L. cv. Brink) roots, however individual phospholipid distribution was constant. Wilson *et al*., (1987) found that water deficit caused a significant decline in the relative degree of acyl-unsaturation (i.e. FA-unsaturation) in phospholipids and glycolipids in two different water stress tolerant cotton plants. According to Navari-Izzo *et al*., (1993) water stress causes a significant decrease in glycolipids and diacylglicerol and a reduction of around 24% and 31% in total lipids and phospholipids, but increases in triacylglicerols and free sterols in plasma membranes of sunflower seedlings grown under drought. As a result,

the free sterol to phospholipid molar ratio increased from 0.4 to 0.7 under water deficit conditions. In water-stressed plants the ratio of phosphatidylcholine to phospatidylethanolamine increased and phosphatidic acid rose to 4% of total phospholipids. However, unsaturation level of individual lipid classes was not significantly affected by dehydration.

Major studies have been based on plants under stress conditions, but few studies continued over a recovery period to investigate metabolic changes occurring after relief of stress (Gigon *et al*., 2004). It is clear that a wide range of morphological and physiological changes have been correlated with differences in drought tolerance in various crop plants. Since cucurbits have been less studied in this respect, it is of interest in the present study to examine these aspects in some members of the *Cucurbitaceae*.

In terms of Turkish agricultural products, *Citrullus*, *Cucumis* and *Cucurbita* are widespreadly grown, and the drought-tolerant plant *Ecballium* is very common to Turkey and other Mediterranean countries (Davis, 1972; Janick and Paris, 2006; Paris *et al*., 2006, Mendi *et al*., 2010; Sebastian *et al*., 2010; Paris *et al*., 2011; Paris, 2012; Naz *et al*., 2016).

The aim of the present work was to compare the total and FA (fatty acid) composition of three species of Cucurbitaceae, *Cucumis melo* var. *flexuosus* (L.) Naudin (snake melon or snake cucumber); *Cucurbita pepo* L. (pumpkins, winter and various kinds of summer squash) and *Ecballium elaterium* (L.) A. Rich (squirting cucumber), grown in conditions of normal water supply, and under two different water stress levels. In addition, the changes in leaf lipid content during recovery from stress after rewatering were also investigated.

Materials and Methods

Growth of plants and applying water stress: The *Cucurbitaceae* species, *Cucumis melo* var. *flexuosus* (L.) Naudin, *Cucurbita pepo* L. and *Ecballium elaterium* (L.) A Rich. from Turkey were exposed to two different water-stress levels. Seeds of each species were germinated in trays. After 10-17 days, the plantlets were transplanted singly into pots (9 cm diam, 7 cm height) containing compost (85g). Seedlings were grown under controlled conditions in a growth room. Light was provided by fluorescent tubes giving an irradiance of 63-72 µmol. $m^{-2}.sec^{-1}$, with a temperature of 22±2°C and relative humidity of 43-56%. The following investigation examined the effects of water loss on the biomass and morphology of the different parts of plants, based on their fresh and dry weights, according to the methods of Roberts *et al*., (1993) and Beadle (1993). Growth parameters were determined by measurements of different plant parts. Water potential of leaves (Ψl), relative water content (RWC), number of leaves (nl), total leaf area (LA), and fresh and dry weight of leaves indicated the water status of aerial parts of seedlings. Additional measurements including plant height (PH), fresh and dry weights of shoot and root (sfw-sdw and rfw-rdw respectively), and ratios of root/shoot (rfw/sfw, rdw/sdw) were used as growth parameters.

After their first leaves were fully expanded, the plants were exposed to different stress levels, as determined from a previous experiment which was carried out with and without the plant, following the equation of Paquin and Mehuys, (1980):

Water holding capacity= (W1-W2) x /100 where;

W1 is the soil weight when water-saturated, W2 is the soil dry weight
x/100 is the desired level of moisture

Determination water potential and water deficits: Water potentials of leaves were determined by the pressure chamber method and the recommendations of Scholander *et al*., (1965) and Boyer (1967, 1995). Xylem pressure was represented with a negative sign. Values were later converted to MPa (1 MPa = 10 bars).

Water deficit from the three cucumber cultivars was determined according to the methods of Weatherley, (1947, 1950); Barr & Weatherley, (1962); Barrs, (1968); Sanchez-Diaz & Kramer, (1971); Beadle *et al.,* (1993). The fresh weight of 10-15 leaf segments ($1cm^2$) was determined and the tissue immediately put into petri dishes containing distilled water at the room temperature (20°C) for 12 h. The segments were then drained on blotting paper, quickly weighed to determine the turgid weight, finally dried in oven at 75°C for 12 h and kept in a desiccator until weighing for dry weight determination. Relative water content was determined as follows:

$$\text{Relative water content} = \frac{\text{Fresh weight – Dry weight}}{\text{Turgid weight – Dry weight}} \times 100$$

Extraction procedure: Known weights of fresh leaves (0.5 - 1 g) were cut into segments and quickly placed in hot isopropanol (70°C) in McCartney bottles. After 10 minutes at this temperature, the tissue was ground in a mortar, and the extract transferred to a centrifuge tube. The mortar and pestle were washed using a small volume of chloroform: methanol (2:1 v/v), which was transferred into the same tube. After centrifuging for 5 minutes at 3600 rpm, the supernatant was poured into a rotary evaporator flask, then 5 ml chloroform: methanol (2:1) was added to the residue in the centrifuge tube, mixed, centrifuged and the supernatant added to the evaporator flask. The process was repeated once more with the chloroform: methanol 2:1 and further 3 times with chloroform methanol 1:2, the supernatant being added each time to the rotary flask. The extract was evaporated to near dryness and dissolved in 2-3 ml of Folch lower phase (FLP) solvent (chloroform: methanol: water, 18:14:1), and a similar volume of Folch upper phase (FUP) (chloroform:methanol: water-0.74% KCl-, 3: 48:47) was added, in order to remove non-lipid fractions from the lipid extract. After separations of both phases by adding FUP to the solution, the lower phase was evaporated to near dryness in a rotary evaporator and transferred to a 2 ml vial. The content was completely dried with air or nitrogen and then made up to 1mg/ml with chloroform.

Total lipid estimation: Total lipids were determined according the method of Marsh & Weinstein, (1966). Duplicate 10 µl samples from each extract, and standard solutions of 0, 10, 20, 30, 40, 50 mg ml^{-1} of olive oil in chloroform for a calibration curve, were transferred to thick-walled test tubes. After completely drying under a stream of air, 2 ml of concentrated sulphuric acid was added to each tube, which was then placed in a heating block at 200±2°C for 15 min. The tubes were placed in an ice bath for 5 minutes after removal from the heating block. Five ml of distilled water was added to the tubes and mixed thoroughly before they were allowed to cool and settle. The optical density was measured at 375 nm against a blank of 10 µl chloroform which was treated the same way.

Preparing sample for fatty acid analysis: The preparation of fatty acid methyl esters (FAME) for gas liquid chromatography was carried out by the method of Christie, (1989).

Lipid extracts (250 µl) were completely dried in a stream of nitrogen and 1 ml of 1% sulphuric acid in methanol was added and the samples left in the oven at 50°C for 2 hrs for transmethylation. Sodium chloride solution (2 ml 5%) was added, FAME extracted with 2x2 ml hexane, then the upper layer (hexane) was transferred to clean McCartney bottles by means of Pasteur pipettes. The hexane layer was washed with 2 ml 2% potassium bicarbonate and transferred to another McCartney bottle. A small amount of anhydrous sodium sulphate was added to remove traces of water and the extract filtered using filter paper and completely dried under nitrogen. Dried samples were transferred into small injection vials, with the addition of 1 ml hexane, then analysed by GLC along with standard FAME samples. The results obtained show the amount of each individual fatty acid and the percentage of the total fatty acid of the polar lipid fraction.

The unsaturation index (Δ/mol) of leaf polar lipids, during the stress treatments and the recovery periods, was calculated from the following formula:

Δ/mol = 1 x [% of monoenes] + 2 x [% of dienes] + 3 x [% of trienes] + etc.

Statistical analysis: The data from growth parameters and lipid experiments of *Cucumis melo* var. *flexuosus* (L.) Naudin and related species, were subjected to one way analysis of variance (ANOVA) to compare means of replicates. The results were evaluated using the statistical program Minitab 11 for Windows, followed by Tukey's range test 5% to determine significance between means indicated with standard error (±S.E.).

Results

Growth parameters and total lipid changes: The two different water stress levels clearly affected morphological appearances of the species after 3 weeks (4 weeks for *Ecballium*) of withholding water. This was confirmed by measuring ψ_l (leaf water potential) and leaf relative water content (RWC) (Table 1) using young expanded leaves. The seedlings exposed to moderate (MS) and severe (SS) water stress, responded differently to growth parameters. However, all except drought tolerant *Ecballium*, showed clear correlations between different water stress levels and root and shoot growth measurements on harvesting. Under severe stress, leaf water potentials, nl, lfw and LA from all plants were significantly lower than that of control plants, even in *Ecballium*. *Cucumis melo* and *Cucurbita pepo* were more or less similarly affected (Akıncı & Lösel, 2009, 2010). *C. pepo* responded to MS with significant decreases in PH, stfw, stdw and ψ_l, and SS significantly reduced all morphological characters as well as ψ_l. In *C. melo*, the species with the longest stem, and creeping rather than upright habit, MS decreased stfw and stdw but not PH or ψ_l, whereas SS significantly decreased the stem length and other morphological characters as well as ψ_l. The higher rfw/sfw and rdw/sdw ratios of *C. melo* control plants compared with that in the other two species, may be important factors in its drought resistance. *C. pepo* had very small root/shoot ratios, indicating a different response to stress, where rfw/sfw was increased slightly in MS but, rdw/sdw remained unchanged in SS, compared to control plants. *Ecballium* had the lowest rfw/sfw ratio in SS treatments, and only its lfw was affected by SS significantly. The RWC of *C. melo* was not significantly changed by water stress, although the closely similar values in MS and SS leaves appeared to be lower than in unstressed leaves. Similarly, in *C. pepo* and *Ecballium*, no significant changes in RWC were found under water stress, although, unlike the other plants, RWC seemed to rise slightly in *C. pepo* with increasing stress.

The other growth parameters confirmed that all species showed slight resistance to mild water stress. The drought-tolerant plant *Ecballium*, was hardly affected by MS, like *C. pepo* and *C. melo*, apart from a decrease in water potentials, but had a clearer response to SS. *C. melo* and *C. pepo* were more or less similarly affected. *C. pepo* had very small root/shoot ratios, indicating a different response to stress, where rfw/sfw had increased slightly in MS but rdw/sdw had remained unchanged in SS treatments, where only its lfw was affected significantly by SS (Table 1).

The three species of *Cucurbitaceae* showed different responses to water stress in their total lipid changes. Leaves of *C. melo* showed a slightly different result, with the lipid content in plants under SS remaining equal to that of control plants. The total lipid content appeared lower in leaves from MS *C. melo* but the difference was not significant. In *C. pepo*, the total lipid content of leaves appeared to decrease in proportion to increasing water stress but the differences from the control plants were not significant. Under SS, leaf lipid content was lower in *C. pepo* and *Ecballium* than in the other plants investigated and fell to its lowest level in *Ecballium.* Such findings are in agreement with previous studies where water stress induced a decline in the amount of total lipids (Monteiro de Paula *et al*., 1993; Svenningsson & Liljenberg, 1986; Hubac *et al*., 1989; Dakhma *et al*., 1995; Gigon *et al*., 2004; Moradi *et al*., 2017). On the contrary, the lipid content of leaves of MS was significantly higher than that in controls (Table 2) in the relatively stress-resistant plant *Ecballium.*

Table 1. Growth parameters of *Cucurbitaceae* species under C (control); MS (moderate stress); SS (severe stress) treatments.

Growth parameters	*C. melo*			*C. pepo*			*Ecballium*		
	C	MS	SS	C	MS	SS	C	MS	SS
Plant height (cm)	89.6 ^{a}	65.2 ^{ab}	48.6 ^{b}	59.0 ^{a}	40.4 ^{b}	27.0 ^{b}	42.8 ^{a}	33.0 ^{a}	33.8 ^{a}
No of leaves	16.4 ^{a}	12.5 ^{ab}	7.6 ^{b}	10.8 ^{a}	10.8 ^{a}	7.1 ^{b}	14.5 ^{a}	12.3 ^{ab}	11.0 ^{b}
Leaves fresh weight (g)	8.2 ^{a}	5.3 ^{ab}	1.2 ^{b}	11.6 ^{a}	7.4 ^{ab}	4.1 ^{b}	6.2 ^{a}	4.8 ^{ab}	1.9 ^{b}
Leaves dry weight (g)	0.8 ^{a}	0.6 ^{ab}	0.1 ^{c}	1.4 ^{a}	1.1 ^{ab}	1.0 ^{b}	1.0 ^{a}	0.5 ^{a}	0.2 ^{a}
Stem fresh weight (g)	9.4 ^{a}	5.2 ^{b}	2.5 ^{c}	20.7 ^{a}	10.2 ^{b}	4.1 ^{c}	5.1 ^{a}	5.2 ^{a}	3.3 ^{a}
Stem dry weight (g)	0.8 ^{a}	0.4 ^{b}	0.2 ^{b}	1.5 ^{a}	0.7 ^{b}	0.3 ^{c}	0.8 ^{a}	0.6 ^{a}	0.4 ^{a}
Root fresh weight (g)	6.6 ^{a}	2.1 ^{ab}	0.6 ^{b}	1.7 ^{a}	1.1 ^{ab}	0.6 ^{b}	2.1 ^{a}	1.8 ^{a}	0.6 ^{a}
Root dry weight (g)	0.9 ^{a}	0.4 ^{ab}	0.1 ^{b}	0.3 ^{a}	0.2 ^{ab}	0.1 ^{b}	0.5 ^{a}	0.4 ^{a}	0.2 ^{a}
ψ_l (-MPa)	0.39 ^{a}	0.48 ^{ab}	0.56 ^{b}	0.57 ^{a}	0.63 ^{b}	0.73 ^{c}	0.31 ^{a}	0.54 ^{b}	0.62 ^{b}
Leaf area (cm^2)	41.2 ^{a}	23.6 ^{b}	9.9 ^{b}	64.6 ^{a}	43.9 ^{a}	17.3 ^{b}	12.1 ^{a}	8.8 ^{ab}	6.4 ^{b}
RWC (%)	70.0 ^{a}	58.6 ^{a}	57.3 ^{a}	69.9 ^{a}	76.2 ^{a}	81.9 ^{a}	75.1 ^{a}	75.7 ^{a}	69.9 ^{a}
rfw/sfw	0.37	0.19	0.16	0.05	0.06	0.07	0.19	0.18	0.03
rdw/sdw	0.54	0.39	0.33	0.10	0.11	0.10	0.27	0.38	0.29

No of replicates varied with the parameter measured as indicated below:
PH: *C. melo* >8, *C. pepo* >7, *Ecballium* >5; other parameters: >7 for each; W.p: *C. melo* >5, *C. pepo* >10, *Ecballium* >10; LA: >4 for all plants. ψ_l = 5 replicates; RWC = 2 replicates
Within each cultivar, means with same superscript letter are not significantly different (one way ANOVA Tukey test, $p<0.05$)

Table 2. Total leaf lipid (µg/g dry wt) of *Cucurbitaceae* species under control (C), moderate stress (MS) and severe stress (SS) conditions: (a) under water stress treatments: (b) 2d, (c) 4d, after return to normal water supply (means of 3 replicates ± S.E.).

	Treatment	*C. melo*	*C. pepo*	*Ecballium*
	C	88.7 ± 7.6	102.9 ± 36.4	39.1 ± 4.8
a.	MS	74.2 ± 7.3	74.7 ± 12.9	71.0± 12.2
	SS	92.4 ± 9.8	48.6 ± 5.2	31.9**± 2.4
	C	100.4 ± 9.5	82.7 ± 4.2	38.9 ± 3.8
b.	MS R1	124.8 ± 5.9	99.8 ± 9.9	119.9*± 4.8
	SS R1	84.6** ± 8.7	92.3 ± 10.5	89.5**$^{+}$± 6.0
	C	131.9 ± 28.7	183.7 ± 42.9	71.9 ± 7.0
c.	MS R2	357.8 ± 107.1	146.9 ± 32.5	144.6*± 16.9
	SS R2	278.6 ± 126.7	160.6 ± 25.7	133.4*± 8.7

* = Sig. diff. from control; ** = Sig. diff. from MS; **$^{+}$: Sig. diff. from MS and C ($p>0.05$)

The total leaf lipid content of the three related species two days after rewatering tended to be higher in previously MS exposed plants than in controls, and the increase was significant in *C. melo* and *Ecballium* but not in *C. pepo*. The total lipid content of leaves of *Ecballium* plants previously exposed to either MS or SS was found to be significantly higher than in control leaves, although the values from previously SS plants were significantly lower than in those recovering from MS (Table 2).

After a recovery period of four days of normal water supply, the leaves of *C. melo* which had been exposed to MS had higher contents of total lipids than control and SS plants but the differences were not significant. The total leaf lipid content of previously MS and SS plants of *C. pepo* was similar to that of controls. Both MS and SS *Ecballium* showed clear responses to the longer recovery period with similar, significant increases in leaf lipid content, compared to control plants. The increases are likely to be from renewed synthesis of both membrane lipids, required for growth and some storage lipid (Table 2).

Fatty acids: The major effect of water stress was seen in the proportions of 18:3, which was increased significantly in *C. pepo*, whereas it remained unchanged in *C. melo* under MS and SS, but was decreased significantly under SS in *Ecballium* (Table 3). The percentage of 20:1 increased significantly under both MS and SS in *C. pepo*, as well as in SS leaves of *Ecballium*. The drought tolerant species *Ecballium*, showed significant decreases in the leaf fatty acids 16:0, 18:0, and 18:3 with SS. On the other hand, 20:1 significantly increased in this species. However, 20:1 remained unaffected by stress in leaves of *C. melo*. Palmitic acid, the major saturated FA in all plants, was investigated and showed a tendency to increase under stress in *C. pepo* leaves, whereas 16:0 fell slightly in *C. melo* and decreased significantly in *Ecballium* compared to controls, under both moderate and severe stress. Both MS and SS *C. pepo* showed significantly greater proportions of 16:1 than in leaves of control plants, whereas it remained unchanged in *C. melo* and decreased slightly in *Ecballium*. Under MS and SS the lipid content of *C. pepo* significantly increased for 18:2 and 18:3, but 20:1 significantly decreased. The proportion of 16:3 was below 2% of total FA in all cases and showed no clear response to water stress. No clear effects of stress on 18:0 or 18:1 percentages were found, except in *Ecballium*, where a significant decrease in 18:1 was observed under SS. Percentages of 18:2 significantly higher than in control plants were found both MS and SS *C. pepo*, however, 18:2 appeared to be unaffected by stress in *C. melo* and *Ecballium.*

Table 3. Fatty acid (FA) composition (% of total FA) of polar lipids from *C. melo, C. pepo* and *Ecballium* under control (C), moderate stress (MS) and severe stress (SS) conditions (Means from 6 replicates plants ± S.E.)

	C. melo			*C. pepo*			*Ecballium*		
	C	**MS**	**SS**	**C**	**MS**	**SS**	**C**	**MS**	**SS**
16:0	15.9 ± 1.1	14.5 ± 0.8	12.2 ± 1.4	7.8 ± 1.6	12.9 ± 2.2	12.8 ± 1.1	12.6 ± 0.9	8.8* ± 0.7	7.2* ± 0.4
16:1	3.5 ± 0.6	3.5 ± 0.3	3.6 ± 0.3	1.9 ± 0.5	4.4* ± 0.6	4.9* ± 0.4	5.6 ± 0.7	4.0 ± 0.5	3.3 ± 0.7
16:3	1.7 ± 0.2	1.1 ± 0.1	1.6 ± 0.3	1.4 ± 0.2	2.2 ± 0.3	2.4 ± 0.2	1.6 ± 0.5	0.9 ± 0.1	0.7 ± 0.1
18:0	2.4 ± 0.2	2.7 ± 0.2	3.1 ± 0.6	3.3 ± 2.1	2.7 ± 0.6	2.4 ± 0.5	4.5 ± 0.5	3.8 ± 0.3	2.9* ± 0.1
18:1	2.7 ± 0.5	2.0 ± 0.2	2.5 ± 0.4	2.2 ± 1.5	1.7 ± 0.3	2.7 ± 0.8	1.5 ± 0.3	0.8 ± 0.1	1.1 ± 0.3
18:2	4.7 ± 0.4	5.9 ± 0.7	5.1 ± 0.5	1.2 ± 0.3	2.7* ± 0.3	2.9* ± 0.2	2.6 ± 0.4	1.9 ± 0.2	2.0 ± 0.3
18:3	40.9 ± 4.3	42.6 ± 2.2	39.4 ± 2.5	24.9 ± 5.7	52.4* ± 3.9	52.3* ± 3.3	60.9 ± 4.1	44.4 ± 3.6	35.4* ± 5.6
20:1	28.4 ± 5.3	27.7 ± 3.2	30.7 ± 5.4	57.5 ± 8.7	20.7* ± 7.1	20.1* ± 4.5	19.8 ± 4.9	36.2 ± 3.9	47.2* ± 7.1

* = Significantly different from (C) ($p<0.05$)

Table 4. Fatty acid composition (% of total FA) of leaf polar lipid from *C. melo, C. pepo* and *Ecballium* after 2d of recovery with full watering following control (C), moderate stress (MS) and severe stress (SS). (Means from 3 replicate plants ± S.E.)

	C. melo			*C. pepo*			*Ecballium*		
	C	**MS**	**SS**	**C**	**MS**	**SS**	**C**	**MS**	**SS**
16:0	17.9 ± 0.8	13.8* ± 0.5	10.9* ± 1.1	8.6 ± 0.5	14.9* ± 1.3	13.2* ± 1.2	11.4 ± 0.4	8.8 ± 1.5	9.5 ± 0.7
16:1	4.5 ± 0.2	3.3* ± 0.1	3.1* ± 0.3	2.1 ± 0.2	5.2* ± 0.2	4.6* ± 0.2	5.0 ± 0.5	4.4 ± 0.8	3.9 ± 0.6
16:3	1.6 ± 0.4	2.6 ± 0.5	1.5 ± 0.1	1.1 ± 0.3	3.0* ± 0.3	2.7* ±0.2	1.7 ± 0.4	1.4 ± 0.1	1.1 ± 0.2
18:0	3.1 ± 0.2	3.8 ± 0.5	2.6 ± 0.3	1.3 ± 0.1	2.1* ± 0.1	2.1* ± 0.2	4.6 ± 0.3	3.9 ± 0.8	3.8 ± 0.4
18:1	3.5 ± 1.1	3.8 ± 1.7	2.2 ± 0.1	1.3 ± 0.1	2.3 ± 0.5	3.9* ± 0.5	2.9 ± 0.8	2.6 ± 0.1	7.5 ± 4.2
18:2	7.2 ± 0.5	6.6 ± 1.1	5.6 ± 0.4	2.3 ± 0.3	4.3* ± 0.5	4.6* ± 0.3	2.7 ± 0.5	2.7 ± 0.7	4.6 ± 0.2
18:3	48.6 ± 2.5	38.1 ± 2.4	34.5* ± 3.7	32.4 ± 1.5	62.6* ± 2.3	54.7** ± 1.5	61.6 ± 5.1	49.7 ± 8.7	44.4 ± 5.6
20:1	13.1 ± 2.1	28.3 ± 6.7	39.5* ± 5.2	51.2 ± 1.7	5.6* ± 2.3	14.1* ± 2.8	10.7 ± 7.3	27.9 ± 13.5	24.9 ± 3.8

(***** = Significantly different from C; ****** = Significantly different from MS and C) ($p<0.05$)

Table 5. Fatty acid composition (% of total FA) of leaf polar lipid from *C. melo, C. pepo* and *Ecballium* after 4d of recovery with full watering following control (C), moderate stress (MS) and severe stress (SS). (Means from 3 replicate plants ± S.E.)

	C. melo			*C. pepo*			*Ecballium*		
	C	**MS**	**SS**	**C**	**MS**	**SS**	**C**	**MS**	**SS**
16:0	19.1 ± 2.5	17.9 ± 1.6	17.9 ± 2.1	14.8 ± 0.3	27.2 ± 10.0	28.1 ± 9.1	21.7 ± 5.8	14.6 ± 1.7	16.1 ± 1.4
16:1	6.2 ± 0.5	6.2 ± 0.0	5.1 ± 0.5	6.4 ± 0.6	5.8 ± 0.3	5.7 ± 0.4	4.9 ± 1.0	6.8 ± 0.5	6.4 ± 0.3
16:3	1.1 ± 0.0	2.5 ± 0.8	2.5 ± 0.7	2.4 ± 0.4	5.5 ± 2.0	7.5 ± 2.2	2.9 ± 1.0	1.7 ± 0.1	2.1 ± 0.1
18:0	3.0 ± 0.1	2.5 ±0.0	2.9 ± 0.2	2.3 ± 0.1	4.4 ± 1.4	1.8 ± 0.0	9.8 ± 1.7	7.3 ± 0.6	7.1 ± 0.6
18:1	2.7 ± 0.1	3.1 ± 1.3	2.6 ± 0.4	3.6 ± 1.2	2.3 ± 2.3	1.9 ± 0.2	2.8 ± 0.6	3.7 ± 0.4	4.7 ± 0.6
18:2	5.6 ± 3.1	8.4 ± 1.4	8.1 ± 1.6	6.3 ± 1.3	8.2 ± 2.3	9.0 ± 2.2	5.4 ± 0.9	5.9 ± 0.6	7.7 ± 0.5
18:3	57.9 ± 0.9	54.0 ± 5.8	54.4 ± 5.5	58.7 ± 4.2	42.0 ± 15.2	40.4 ± 10.7	48.7 ± 11.2	55.4 ± 3.0	51.4 ± 2.6
20:1	4.5 ± 1.6	4.9 ± 0.8	6.0 ± 0.7	5.6 ± 2.3	4.6 ± 1.3	7.1 ± 2.5	3.3 ± 1.0	3.8 ± 0.8	2.4 ± 0.4

Two days after rewatering, the percentages of 16:0 and 16:1 in leaves of previously MS and SS plants were significantly lower in *C. melo* and significantly higher *C. pepo* than those in control leaves (Table 3). No significant differences in proportions of these two FA between control and rewatered plants were observed in leaves of the other cucumber cultivars, or of *Ecballium*, at this stage (Table 3). Leaves of *C. pepo*, from plants previously exposed to either moderate or severe stress, also showed significantly higher proportions of 16:3, 18:0, 18:1 (only in previously SS plants), 18:2 and 18:3 than in controls, whereas the other species investigated showed no clear differences between previously stressed and control plants in these FAs. On the other hand, the ratio of 20:1 in rewatered *C. pepo* plant still remanied as lower percentages than that of control leaves. FA proportions of leaves of *C. melo* were found higher significantly in previously SS plants compared to control plants (Table 4).

After a further two-day growth in conditions of full water supply, no significant differences in fatty acid composition of polar lipids could be detected between leaves of plants previously exposed to water stress and unstressed control plants (Table 5).

The unsaturation index rose in *C. pepo* under stress but was higher in MS than in SS. In contrast, Δ/mol of leaf lipids from *C. melo* was little changed from control values by stress treatments, while in *Ecballium*, Δ/mol decreased with each level of stress (Table 6).

Table 6. Unsaturation index of fatty acids under control (C), moderate (MS), severe stress (SS) and after 2d (R1) and 4d (R2) of recovery with full water supply.

	C. melo	*C. pepo*	*Ecballium*
C	1.41	0.83	1.98
MS	1.47	1.72	1.45
SS	1.36	1.43	1.16
C	1.70	1.05	1.97
MS R1	1.37	2.03	1.61
SS R1	1.20	1.81	1.55
C	1.99	1.99	1.72
MS R2	1.92	1.55	1.92
SS R2	1.91	1.52	1.85

(Means of 6 replicates for stress, 3 replicates for R1 and R2 treatments)

C. melo leaves recovering from stress had lower Δ /mol than controls two days after rewatering but, after a further four days, these values had increased to nearly the control level. *C. pepo* showed increases in Δ/mol after two days of return from both SS and MS to normal watering, the values still being higher than in controls, and a slight decrease in Δ/mol after four days of recovery, to a value lower than control leaves. In *Ecballium*, Δ/mol rose slightly in the first recovery phase of plants which had been previously under MS or SS, still remaining below that of controls, and after four days of normal water supply, Δ/mol was slightly higher in both cases than in control plants (Table 6).

Discussion

Many studies on different crop species showed that long term water deficiency causes a decrease in phospholipid, glycolipid and linoleic acid, but an increase in triacylglycerol of leaf tissues exposed to long periods of water deficit (Anh *et al*., 1985; Martin *et al*., 1986; Navari-Izzo *et al*., 1989). Liljenberg (1992) pointed out that there are implications for membranes and membrane lipids and dehydration at cellular level under stress. Nevertheless, the reasons for increasing or decreasing amounts of different lipids in plants exposed to water stress are still unclear and vary by species and stress levels, as well as between the different organs of plants (Pham-Thi *et al.*, 1982; Draper, 1969; Navari-Izzo *et al*., 1993; Douglas & Paleg, 1981; Pham-Thi *et al.*, 1985; Navari-Izzo *et al*., 1990; Svenningsson & Liljenberg, 1986; Benadjaoud *et al*., 2013) and intra-specific varieties showing different degrees of stress resistance appear to respond differently to water deficits, depending on the degree of stress (Monteiro de Paula *et al*., 1990; Akinci & Lösel, 2012; Scotti-Campos & Pham-Thi, 2016).

Three species of *Cucurbitaceae* showed a different response to the water stress, with total lipid changes and leaves of *C. melo* showing only a slight difference, with the lipid content in plants under SS remaining equal to that of control plants. The total lipid content was lower in leaves from MS *C. melo* although not significantly different. In *C. pepo*, the total lipid content of leaves decreased in proportion to increasing water stress, but had insignificant differences compared to the control plants. The total lipid content of *C. pepo* did not change significantly under water stress but *C. pepo* showed reduction in lipid content with each level of stress. *Ecballium* differed from the other species compared here in showing a significant rise in total lipid content under MS. This could indicate either some storage of photosynthate or increased synthesis of membrane lipids. Under SS, leaf lipid content was lower in *C. pepo* and *Ecballium* than that of other plants and fell to its lowest level in *Ecballium*, while the lipid content of leaves of MS plants of *Ecballium* was significantly higher than that in controls. The leaf polar lipid content increased under moderate stress while a sensitive variety of lipid content decreased due to moderate levels of stress in a variety of *Vigna unguiculata* (Monteiro de Paula *et al*., 1990).

In *C. melo*, where previous growth studies have shown it to be relatively drought resistant, the total lipid content of leaves showed no response to stress conditions in the present experiment. In this species, severe stress has appeared to reduce leaf lipid content. This is likely to correspond to the inhibition of photosynthesis, which results, together with reduced transpiration, from closure of stomata, and might indicate the inhibition of homeostasis maintenance, since RWC dropped to 57.3% in severely stressed plants (Gigon *et al*., 2004) (Table 1).

In the first recovery phase of the species, two days after return to full water supply, they appeared to be in a compensating stage, with recovery of most of the lost lipid content to levels similar to those of the controls. At this stage, there were also significant differences between plants recovering from MS and from SS in the most stress-resistant species, *C. melo* and *Ecballium*. Leaves of previously MS *C. melo* and *Ecballium* had increased significantly in lipid content after the two day recovery period and contained significantly more lipid than leaves which had been under SS. Previously SS plants of *C. pepo* and *Ecballium* similarly showed a significant increase in leaf lipid at this stage of recovery, compared to their lipid content while under SS.

In the second recovery phase, the significant increases in lipid seen after two days in *Ecballium* continued to give a significantly higher content than in unstressed controls. Similarly, the leaf lipid content of *C. melo* recovering from MS and SS had risen to very high levels, about three times that of control leaves, four days after rewatering, but with high plant to plant variation, so that the differences were not significant. The higher lipid contents of control plants of all types at this stage may also relate to the maturity of the plants as well as the species ability to withstand, and recover from, dehydration stages (Gigon *et al*., 2004).

The increased levels of lipid found in *C. melo* and *Ecballium* leaves, four days after rewatering, may represent increased photosynthetic production during the recovery period, either already incorporated into membrane lipids or stored as carbon reserve, as growth resumes. Few papers report a decrease of membrane polar lipids as distinct from total lipids (Klein *et al*., 1986; Premachandra *et al.*, 1991;

Zuniga *et al.*, 1990). The changes in proportions of constituent FA, recorded in this study, give some indications of metabolic adjustments taking place at different water stress levels. In all plants examined, 18:3 and 20:1 accounted for the biggest proportion of polar lipid fatty acids and were most affected by water stress. These two FAs, taken together, made up between 70% and 80% of the total polar lipid fatty acids in leaves of control plants of each type. Under water stress, this combined percentage decreased in *C. pepo*, but was unchanged in *C. melo* and *Ecballium*. Since 18:3 is particularly associated with chloroplast membranes and is normally the major FA of leaves, it could be expected to respond to factors affecting the photosynthetic system and thus result from an inhibition of MGDG biosynthesis (Monteiro de Paula *et al*., 1993; Junior *et al*., 2008). MGDG has been suggested to stabilize and maintain the lamellar bilayer structure (Torres-Franklin *et al*., 2007) to maintain photosynthetic energy supply (Gasulla *et al*., 2013). The decrease in polar lipids is often associated with an increase in triacylglycerol level (Douglas & Paleg, 1981; Martin *et al*., 1986) as well as indicating disorganization of cellular membranes, particularly those of chloroplasts (Dakhma *et al*., 1995) and leaf lipid parameters can discriminate between certain cultivars (coconut) with varying protoplasmic tolerance to drought stress (Repellin *et al*., 1997).

The large percentage of 20:1, which was found in even higher proportions than 18:3 in leaves in *C. pepo*, was unexpected, since this FA is less often recorded and no information on its location within the cell has been found in the literature examined so far. The significant increase of 18:3 in *C. pepo* under water stress was accompanied by significant decreases in 20:1 in the same plant. This observation strongly suggests that 20:1 may contribute to the observed increases in 18:3. A greater decrease in polar lipid content without major changes in fatty acid composition has been reported for stress sensitive varieties when compared with stress-resistant ones (Hubac *et al*., 1989).

The more stress-resistant *C. melo* and *Ecballium*, showed different responses from *C. pepo.* In *C. melo*, the percentages of 18:3 and 20:1 remained unchanged by water stress. In SS *Ecballium*, 18:3 was significantly lower than in controls while the proportion of 20:1 doubled, although this difference was not significant. The non-significant decrease in 18:3, and increase of 20:1, seen under MS in *Ecballium,* both lay between their respective values in control and SS conditions. Under MS and SS, *Ecballium* also showed significant decreases in 16:0, the only other FA which generally formed over 10% of the polar lipid fatty acids in these plants. Under SS, 18:0 also decreased in this species. It is possible that this decrease could have contributed to the synthesis of the longer chain FA 20:1. The increases in both 16:1 and 18:2 in *C. pepo,* under water stress, are likely to indicate other stress-induced changes in lipid metabolism. These changes might be related to the limited phosphate availability from the soil, as the roots stop absorption, causing phospholipid biosynthesis to decrease (Monteiro de Paula *et al*., 1993).

After the first recovery stage, polar lipids of previously stressed plants of *C .pepo* contained significantly higher percentages of 16:0, 16:1, 16:3, 18:0, 18:1, 18:2, 18:3 and significantly lower proportions of C20:1, than in controls. This may indicate active synthesis of fatty acids taking place at this stage of recovery, with less accumulation of photosynthate in 20:1, than in unstressed plants. As discussed above, active lipid metabolism, including synthesis of polar lipid fatty acids at this stage of recovery, would be expected from the increases in total lipid content in leaves of *C. melo*, *C. pepo* and *Ecballium* which were recorded in previously stressed plants, during their return to normal water supply.

Liljenberg (1992) suggested that, when stress intensity is high, physiological ageing might be accelerated by injuries arising from by water stress. During recovery following rewatering, further changes in FA percentages occurred at different rates in individual cultivars and species. The changes in degree of unsaturation accompanying these responses to water supply may indicate some alterations affecting membrane permeability and stress tolerance of the plants in these experiments.

Conclusion

In the present study, results indicate a number of differences in the ways in which lipid metabolism may respond to water stress in the related species examined. The two parameters concerned are:

1. Total lipid changes, giving some idea of the effects of stress on the partitioning of photosynthate into this fraction;

2. The fatty acid composition of the leaf polar lipids, which should indicate the major effects of water stress on membrane composition, including chloroplast membranes.

Some previous reports state that polar lipid decreased in sensitive plants but remained unchanged, moreover, increased in resistant varieties under drought stress (Monteiro de Paula *et al*., 1990; Repellin *et al*., 1997; Gigon *et al*., 2004). High levels of polyunsaturated FAs are reported to enhance tolerance to desiccation (Li *et al*., 2014), and to improve recovery capacity following re-hydration (Liu *et al*., 2013).

Results from unsaturation index suggested that the changes taking place with increasing water stress could increase membrane permeability in *C. pepo*, but at the same time might decrease permeability in *Ecballium.* On the other hand, in *C. melo*, the other stress-resistant species, and the results could show no indication of altered membrane lipid unsaturation. Relatively drought tolerant *C. melo* showed no significant responses in polar lipid composition under these treatments. This seems to correspond to the general tolerance of water stress in this species. Unsaturation index has often been reported to remain constant during stress and help plants maintain membrane integrity, (Toumi *et al*., 2008) or may decrease in the later stages of exposure to stress, when stress intensity is high (Liljenberg, 1992). The changes in fatty acid unsaturation indices of the leaf polar lipids probably mainly reflect the changes in 18:3 and 20:1, since these accounts for such a high proportion of the total polar lipid

FA. The Δ/mol values calculated would suggest changes with increasing water stress which could increase membrane permeability in *C.pepo*, but would decrease permeability in *Ecballium*, whereas *C. melo*, the other stress resistant species, showed no indications of altered membrane lipid unsaturation.

As a conclusion, the tendency for proportions of 18:3, in some plants to increase under stress, relative to other FA, may indicate protection of the photosynthetic apparatus. It would be of interest to examine this apparently reciprocal relationship of 20:1 and 18:3 in more detail.

Acknowledgement

We wish special thanks to Dr. Ian MILNE for English corrections of the text.

References

Akıncı, S. and D.M. Lösel. 2009. The soluble sugars determination in *Cucurbitaceae* species under water stress and recovery periods. *Adv. Environ. Biol.,* 3(2): 175-183.

Akinci, S. and D.M. Lösel. 2010. The effects of water stress and recovery periods on soluble sugars and starch content in cucumber cultivars. *Fresen. Environ. Bull.,* 19(2): 164-171.

Akinci, S. and D.M. Lösel. 2012. Plant water-stress response mechanisms. In: *Water Stress* (Eds.): Md, I., M. Rahman & H. Hasegawa, pp. 15-42. In Tech, Rijeka, Croatia.

Anh, T.P.T., C. Borrel-Flood, J. Vieira da Silva, A. Marie Justin and P. Mazliak. 1985. Effects of water stress on lipid metabolism in cotton leaves. *Phytochem.,* 24: 723-727.

Atkinson, C.J., M. Policarpo, A. D. Webster and G. Kingswell. 2000. Drought tolerance of clonal *Malus* determined from measurements of stomatal conductance and leaf water potential. *Tree Physiol.,* 20(8): 557-563.

Barrs, H.D. 1968. Determination of water deficits in plant tissues. Water Deficits and Plant Growth, (Ed.): Kozlowski, T.T. Academic Press, New York, pp: 235-368.

Barrs, H.D. and P.E. Weatherley. 1962. A re-examination of the relative turgidity technique for estimating water deficit in leaves. *Aust. J. Biol. Sci*., 15: 413-428.

Beadle, C.L. 1993. Growth Analysis. Photosynthesis and Production in a Changing Environment: A Field and Laboratory Manual, (Eds.): Hall, D.O., J.M.O. Scurlock, H.R. Boolhar-Nordenkampf, R.C. Leegood and S.P. Long. Chapman and Hall, London, pp: 22-46.

Beadle, C.L., M.M. Ludlow and J.L. Honeysett. 1993. Water Relations. Photosynthesis and Production in a Changing Environment: A Field and Laboratory Manual, (Eds.): Hall, D.O. J.M.O. Scurlock, H.R. Boolhar-Nordenkampf, R.C. Leegood and S.P. Long Chapman and Hall, London, pp: 113-128.

Benadjaoud, A., G. Benhassaine-Kesri, A. Zachowski and F. Aid. 2013. Effects of dehydration and rehydration on the leaf lipids and lipid metabolism in *Parkinsonia aculeata* (Caesalpiniaceae). *Botany,* 91: 505-513.

Boyer, J.S. 1967. Leaf water potentials measured with a pressure chamber. *Plant Physiol.,* 42: 133-137.

Boyer, J.S. 1995. Measuring the Water Status of Plants and Soils. Academic Press, N.Y.

Christie, W.W. 1989. Gas Chromatography and Lipids: A Practical Guide. The Oily Press, Ayr, Scotland.

Dakhma, W.S., M. Zarrouk and A.K. Cherif. 1995. Effects of drought-stress on lipids in rape leaves. *Phytochem.,* 40: 1383-1386.

Davis, P.H. 1972. The Flora of Turkey and the East Aegean Islands. Vol. 4, Edinburgh University Press, Edinburgh, UK., pp: 200-208.

Douglas, T.J. and L.G. Paleg. 1981. Lipid composition of *Zea mays* seedlings and water stress-induced changes. *J. Exp. Bot.,* 32: 499-508.

Draper, S.R. 1969. Lipid changes in senescing cucumber cotyledons. *Phytochem.,* 8: 1641-1647.

El Kaoua, M., R. Serraj, M. Benichou and D. Hsissou. 2006. Comparative sensitivity of two Moroccan wheat varieties to water stress: the relationship between fatty acids and proline accumulation. *Bot. Stud*., 47: 51-60.

Esfandiari, E.O., M.R. Shakiba, S.A. Mahboob, H. Alyari and M. Toorchi. 2007. Water stress, antioxidant enzyme activity and lipid peroxidation in wheat seedling. *J. Food Agri. Environ.,* 5: 149-153.

Gasulla, F., K. Vom Dorp, I. Dombrink, U. Zähringer, N. Gisch, P. Dörmann and D. Bartels. 2013. The role of lipid metabolism in the acquisition of desiccation tolerance in *Craterostigma plantagineum*: a comparative approach. *Plant J.,* 75: 726-741.

Gigon, A., A.R. Matos, D. Laffray, Y. Zuily-Fodil and A.T. Pham-Thi. 2004. Effect of drought stress on lipid metabolism in the leaves of *Arabidopsis thaliana* (Ecotype Columbia). *Ann. Bot.,* 94: 345-351.

Gronewald, J.W., W. Abou-Khalil, E.J. Weber and J.B. Hanson. 1982. Lipid composition of a plasma membrane enriched fraction of maize roots. *Phytochem.,* 21: 859-862.

Hubac, C., D. Guerrier, J. Ferran and A. Tremolieres. 1989. Change of leaf lipid composition during water stress in two genotypes of *Lupinus albus* resistant or susceptible to drought. *Plant Physiol. Biochem.,* 54: 484-486.

Irigoyen, J.J., D.W. Einerich and M. Sanchez-Diaz. 1992. Water stress induced changes in concentrations of proline and total soluble sugar in nodulated alfalfa (*Medicago sativa*) plants. *Physiol. Plant.,* 84: 55-60.

Jaleel, C.A., P. Manivannan, G.M.A. Lakshmanan, M. Gomathinayagam and R. Panneerselvam. 2008. Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits, *Colloids and Surfaces B: Biointerfaces*, 61: 298-303.

Janick, J. and H.S. Paris. 2006. The cucurbit images (1515-1518) of the Villa Farnesina, Rome. *Ann. Bot.,* 97: 165-176.

Junior, R.R.M., M.S.C. Oliveira, M.A. Baccache and F.M. de Paula. 2008. Effect of water deficit and rehydration on the polar lipid and membrane resistance leaves of *Phaseolus vulgaris* L. cv. Perola. *Brazilian Arch. Biol. Technol*., 5: 361-367.

Klein, R.R., J.J. Burke and R.F. Wilson. 1986. Effect of osmotic stress on ion transport processes and phospholipid composition of wheat (*Triticum aestivum* L.) mitochondria. *Plant Physiol.,* 82: 936-941.

Kramer, P.J. and J.S. Boyer. 1995. Water relations of plants and soils, Academic Press, San Diego

Kuiper, P.J.C. 1985. Environmental changes and lipid metabolism of higher plants. *Plant Pysiol.,* 64: 118-122.

Li, A., D. Wang, Y. Buzhu, Y. Xiaomei and L. Weigi. 2014. Maintenance or collapse: Responses of extraplastidic membrane lipid composition to desiccation in the resurrection plant *Paraisometrum mileense*. *PLoS ONE,* 9, e103430.

Liljenberg, C. 1992. The effect of water deficit stress on plant membrane lipids. *Prog. Lipid Res.,* 31: 335-343.

Liljenberg, C. and M. Kates. 1982. Effect of water stress on lipid composition of oat seedling root cell membranes. *In: Biochemistry and Metabolism of Plant Lipids*, (Eds.): Wintermans, J.F.G.M. and P.J.C. Kuiper. Elsevier Biomedical Press, Amsterdam, pp: 441-444.

Liu, X., S. Zhai, Y. Zhao, B. Sun, C. Liu, A. Yang and J. Zhang. 2013. Overexpression of the phosphatidylinositol synthase gene (ZmPIS) conferring drought stress tolerance by altering membrane lipid composition and increasing ABA synthesis in maize. *Plant Cell Environ*. 36: 1037-1055.

Marsh, J.B. and D.B. Weinstein. 1966. Simple charring method for determination of lipids. *J. Lipid Res.,* 7: 574-576.

Martin, B.A., J.B. Schoper and R.W. Rinne. 1986. Changes in soybean (*Glycine max* [L.] Merr.) Glycerolipids in response to water stress. *Plant Physiol.,* 81: 798-801.

Martins, J., R.R. Oliveira, M.S. Costa, B.M. Ary and P.F. Monteiro de. 2008. Effects of water deficit and rehydration on the polar lipid and membranes resistance leaves of *Phaseolus vulgaris* L. cv. Perola. *Braz. Arch. Biol. Techn.,* 51(2): 361-367.

Massonnet, C., E. Costes, S. Rambal, E. Dreyer and J.L. Regnard. 2007. Stomatal regulation of photosynthesis in apple leaves: evidence for different water-use strategies between two cultivars, *Ann. Bot.,* 100: 1347-135.

Mendi, Y.Y., S. Eldogan, R. Gutakev, M. Ipek, P. Curuk and S. Cetiner. 2010. Regeneration and histological analysis of snake melon (*Cucumis melo* var. *flexuosus* (L.) Naudin) by direct organogenesis. *Turk. J. Agric. For.,* 34: 309-317.

Moellering, E.R., B. Muthan and C. Benning. 2010. Freezing tolerance in plants requires lipid remodeling at the outer chloroplast membrane. *Science,* 330: 226-228.

Monteiro, de Paula, F., A.T. Pham-Thi, Y. Zuily Fodil, R. Ferrari-Iliou, J. Vieira da Silva and P. Mazliak. 1993. Effects of water stress on biosynthesis and degradation of polyunsatured lipid molecular species in leaves of *Vigna unguiculata*. *Plant Physiol. Biochem.,* 31: 707-715.

Monteiro, de Paula, F., A.T. Pham-Thi., J. Vieira da Silva, A.M. Justin, C. Demandre and P. Mazliak. 1990. Effects of water stress on the molecular species composition of polar lipids from *Vigna unguiculata* L. leaves. *Plant Sci.,* 66: 185-193.

Moradi, P., A. Mahdavi, M. Khoshkam and M. Iriti. 2017. Lipidomics unravels the role of leaf lipids in thyme plant response to drought stress. *Int. J. Mol. Sci.,* 18: 2067.

Munnik, T. and C. Testerink. 2009. Plant phospholipid signaling: "in a nutshell". *J. Lipid Res.,* 50 (Suppl): 260-265.

Nakamura, Y., R. Koizumi, G. Shui, M. Shimojima, M.R. Wenk, T. Ito and H. Ohta. 2009. *Arabidopsis* lipins mediate eukaryotic pathway of lipid metabolism and cope critically with phosphate starvation. *Proc. Natl Acad. Sci*., 106: 20978-20983.

Navari-Izzo, F., M.F. Quartacci, C. Pinzino, N. Rascio, C. Vazzana and C.L.M. Sgherri. 2000. Protein dynamics in thylakoids of the desiccation-tolerant plant *Boea hygroscopica* during dehydration and rehydration. *Plant Physiol.,* 124: 1427-1436.

Navari-Izzo, F., M.F. Quartacci, D. Melfi and R. Izzo. 1993. Lipid composition of plasma membranes isolated from sunflower seedlings grown under water-stress. *Physiol. Plant.,* 87: 508-514.

Navari-Izzo, F., N. Angioni and M.F. Quartacci. 1990. Lipids of soybean and sunflower seedlings grown under drought conditions. *Phytochem.,* 29: 2119-2123.

Navari-Izzo, R., M.F. Quartacci and R. Izzo. 1989. Lipid changes in maize seedlings in response to field water deficits. *J. Exp. Bot.,* 40: 675-680.

Naz, H., N.A. Akram and M. Ashraf. 2016 Impact of ascorbic acid on growth and physiology of cucumber (*Cucumis sativus*) plants under water-deficit condition. *Pak. J. Bot*., 48: 877-883.

Okazaki, Y. and K. Saito. 2014. Roles of lipids as signaling molecules and mitigators during stress response in plants. *Plant J*., 79, 584-596.

Osakabe, Y., K. Osakabe, K. Shinozaki and L.S. Tran. 2014. Response of plants to water stress. *Front. Plant Sci*., 5: 86

Pandey, H.C., M.J. Baig, A. Chandra and R.K. Bhatt. 2010. Drought stress induced changes in lipid peroxidation and antioxidant system in genus *Avena*. *J. Environ. Biol.,* 31: 435-440.

Paquin, R. and G.R. Mehuys. 1980. Influence of soil moisture on cold tolerance of Alfalfa. *Can. J. Plant Sci.,* 60: 139-147.

Paris, H.S. 2012. Semitic-language records of snake melons (*Cucumis melo*, Cucurbitaceae) in the medieval period and the "piqqus" of the "faqqous". *Genet. Resour. Crop Evol*. 59: 31-38.

Paris, H.S., J. Janick and M.C. Daunay. 2011. Medieval herbal iconography and lexicography of *Cucumis* (cucumber and melon, Cucurbitaceae) in the Occident, 1300-1458. *Ann. Bot.,* 108: 471-484.

Paris, H.S., M.C. Daunay, M. Pitrat and J. Janick. 2006. First known image of *Cucurbita* in Europe. 1503-1508. *Ann. Bot.,* 98(1): 41-47.

Pham-Thi, A.T., C. Borrel-Flood, J. Vieira da Silva, A.M. Justin and P. Mazliak. 1985. Effects of water stress on lipid metabolism in cotton leaves. *Phytochem.,* 24: 723- 727.

Pham-Thi, A.T., C. Borrel-Flood, J. Vieira da Silva, A.M. Justin and P. Mazliak. 1987. Effects of drought on [1-14C]-oleic and [1-14C]-linoleic acid desaturation in cotton leaves. *Physiol. Plant.,* 69: 147-150.

Pham-Thi, A.T., C. Flood and J.V. da Silva. 1982. Effects of water stress on lipid and fatty acid composition of cotton leaves. In: Biochemistry and Metabolism of Plant Lipids: Proceedings of the 5th International Symposium on the Biochemistry and Metabolism of Plant Lipids, (Eds.): Wintermans, J.F G.M. and P.J.C. Kuiper. Elsevier Biomedical Press, Amsterdam, pp: 451-454.

Premachandra, G.S., H. Saneoka, M. Kanaya and S. Ogata. 1991. Cell membrane stability and leaf surface wax content as affected by increasing water deficits in maize. *J. Exp. Bot.,* 42: 167-171.

Repellin, A., A.T. Pham-Thi, A. Tashakorie, Y. Sahsah, C. Daniel and Y. Zuily Fodil. 1997. Leaf membrane lipids and drought tolerance in young coconut palms (*Cocos nucifera* L.). *Eur. J. Agron.,* 6: 25-33.

Roberts, M.J., S.P. Long, L.L. Tieszen and C.L. Beadle. 1993. Measurement of plant biomass and net primary production of herbaceous vegetation. Photosynthesis and Production in a Changing Environment: A Field and Laboratory Manual, (Eds.): Hall, D.O., J.M.O. Scurlock, H.R. Boolhar-Nordenkampf, R.C. Leegood and S.P. Long. Chapman and Hall, London, pp: 1-21.

Sanchez-Diaz, M.F. and P.J. Kramer. 1971. Behavior of corn and sorghum under water stress and during recovery. *Plant Physiol.,* 48: 613-616.

Sánchez-Martín, J., F.J Canales, J. Tweed, M. Lee, D. Rubiales, A .Gómez-Cadenas, V. Arbona, L.A.J. Mur and E. Prats. 2018. Fatty acid profile changes during gradual soil water depletion in oats suggests a role for jasmonates in coping with drought. *Front. Plant Sci.,* 9: 1077.

Scholander, P.F., E.D. Bradstreet, E.A. Hemmingsen and H.T. Hammel. 1965. Sap pressure in vascular plants. *Science,* 148: 339-346.

Scotti-Campos, P. and A. Pham-Thi. 2016. Correlation between total lipids, linolenic acid and membrane injury under PEG-induced dehydration in leaves of *Vigna* genotypes differing in drought resistance. *Emir. J. Food. Agri*., 28(7): 485-492.

Sebastian, P., H. Schaefer, I.R.H. Telford and S.S. Renner. 2010. Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. *Proc. Natl. Acad. Sci*. USA, 107: 14269-14273.

Svenningsson, M. and C. Liljenberg. 1986. Changes in cuticular transpiration rate and cuticular lipids of oat (*Avena sativa*) seedlings induced by water stress. *Physiol Plant.,* 66: 9-14.

Taiz, L. and E. Zeiger. 1991. Plant Physiology. The Benjamin/Cummings Publishing Co., California, pp: 265-291.

Torres-Franklin, M.L., A. Gigon, D.F. de Melo, Y. Zuily-Fodil and A.T. Pham-Thi. 2007. Drought stress and rehydration affect the balance between MGDG and DGDG synthesis in cowpea leaves. *Physiol. Plant.,* 131: 201-210.

Toumi, I., M. Gargouri, I. Nouairi, P. N. Moschou, A. B. Salem-Fnayou, A. Mliki, M. Zarrouk and A. Ghorbel. 2008. Water stress induced changes in the leaf lipid composition of four grapevine genotypes with different drought tolerance. *Biol. Plant*., 52: 161-164.

Wang, X., S.P. Devaiah, W. Zhang and R. Welti. 2006. Signaling functions of phosphatidic acid. *Prog. Lipid Res.,* 45: 250-278.

Weatherley, P.E. 1947. Note on the diurnal fluctuations in water content of floating leaf disks. *New Phytol.,* 46: 276-278.

Weatherley, P.E. 1950. Studies in the water relations of the cotton plant I. The field measurement of water deficits in leaves. *New Phytol.,* 49: 81-97.

Whitman, C.E. and R.L. Travis. 1985. Phospholipid composition of a plasma membrane-enriched fraction from developing soybean roots. *Plant Physiol.,* 79: 494-498.

Wilson, R.F., J.J. Burke and J.E. Quisenberry. 1987. Plant morphological and biochemical responses to field water deficits. II. Responses of leaf glycerolipid composition in cotton. *Plant Physiol.,* 84: 251-254.

Zuniga, G.E., J. Fernadez, R. Cristi, M. Alberdi and L.J. Corcuera. 1990. Lipid changes in barley seedlings subjected to water and cold stress. *Phytochem.,* 29: 3087-3090.

(Received for publication 22 March 2018)