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Environmental alterations in biofuel generating molecules in *Zilla spinosa*

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Abstract: Now days, production of fuels and petrochemicals from renewable lignocellulosic biomass is an indispensable issue to meet the growing energy demand. Meanwhile, the changes in the climate and soil topography influence the growth and development as well as canopy level of the lignocellulosic biomass. In this study, *Zilla spinosa* Turr (Zilla) plants with similar age and size were collected from three main sectors (upstream, midstream, and downstream) of Wadi Hagul during spring (April) and summer (July) seasons. Environmental stresses evoked reduction in the energy trapping pigments concomitant with increments in chlorophyll fluorescence in summer harvested plants particularly at downstream. Furthermore, the biofuels generating compounds including carbohydrate, lignin, and lipid making the plant biomasses are greatly affected by environmental conditions. Greater amount of lignin was estimated in summer harvested *Z. spinosa* shoots particularly at downstream. Moreover, the total oil content which is a promising source of biodiesel was considerably decreased during summer season particularly at downstream. The physical properties of the lipids major constituent fatty acid methyl esters determine the biofuel properties and contribute in the adaptation of plants against environmental stresses. Hence, the analysis of fatty acid profile showed significant modifications under combined drought and heat stress displayed in the summer season. The maximum increase in saturated fatty acid levels including tridecanoic acid (C13:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), and stearic acid (C18:0) were estimated in spring harvested *Z. spinosa* aerial portions particularly at midstream. In spite of the reduction in the total oil content, a marked increase in the value of unsaturated to saturated fatty acids ratio and thereby the unsaturation index were achieved during the dry summer period. Henceforth, these seasonal and spatial variations in fatty acids profiles may contribute in the

acclimatization of *Z. spinosa* plants to soil water scarcity associated with heat stress experienced during summer. In addition, the alterations in the fatty acid profiles may match biofuel requirements. In conclusion, the most adequate growing season (spring) will be decisive for achieving high lipid productivity associated with improved biofuel quality in terms of high saturated fatty acids percentage that improves its cetane number. However, the dry summer season enhanced the accumulation of greater amount of lignin that may enhance the biodiesel quantity.

Keywords: biofuel; carbohydrates; cellulose; fatty acids; heat stress; lignin; lipids; water scarcity; *Zilla spinosa* (Turr).

1 Introduction

The Bio-energy from natural photosynthetic biomass can substitute the fossil fuels in providing clean and reliable renewable energy resources [1]. Meanwhile, the natural green biomass particularly in the desert is regularly face adverse growth conditions, such as drought, salinity, and high temperatures [2]. These stresses can reduce growth and development, productivity, and cause plant death under severe abiotic stress condition [3]. Similarly, environmental stresses can interrupt plant cellular structures and impair the physiological functions [4]. The relative concentrations of energy trapping pigments are known to be altered under abiotic stresses, and therefore, they can be used as indicator for interaction between plants and their environments [5]. Severe drought stress causes changes in chlorophyll content, affecting chlorophyll components, damaging the photosynthetic apparatus, and inhibits the photosynthesis [6]. In addition, the pigment ratio of chlorophylls (a+b)/carotenoids can be used as an indicator for stress. Thus, greater values of chlorophylls (a+b)/carotenoids ratio up to 5–6 indicated intact photosynthetic apparatus. It was deduced that chlorophyll is broken down faster than carotenoids under stress, and the pigment ratio declined to be about 2–3 [7].

Indeed, photosynthesis is one of the most sensitive physiological processes in stressed plants [8], which thereby directly contribute in biomass production. Boughalleb and Hajlaoui [9] postulated that the

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photosynthetic pigments, net photosynthetic rate, stomatal conductance, transpiration rate, the maximal photochemical efficiency of PSII, and the intrinsic efficiency of PSII reaction centers decreased in *Olea europaea* plants exposed to water scarcity. The sensitivity of photosynthesis to heat and drought is related to the damage of photosystem (PS) II components located in the thylakoid membranes [10, 11]. Consequently, the reduction in photosynthesis will reduce the production of metabolites and hence the plant biomass. Indeed, the greatest component of the plant biomass is the cell walls, which comprise lignin and polysaccharides as cellulose and hemicellulose. The utilization of the lignocellulosic plant materials could be considered as a source for the intermediate chemicals and the second generation biofuels [12]. It was recorded that the utilization of lignocellulosic plant residues could provide about 10–20% of the current world energy demand [13]. However, the reduction in lignin is sometimes accompanied by increased cellulose and hemicellulose deposition. Thus, the reduction in lignin biosynthesis was associated with cellulose accumulation and growth in some transgenic trees [14]. The variation in lignin content and components could be used to improve the digestibility of biomass [15]. Lignin provides mechanical support to the xylem cells, plays an important role in plant defense against various biotic and abiotic stresses [16, 17], in seed dispersal and in the formation of an apoplastic diffusion barrier in the roots [18].

Meanwhile, environmental stresses can induce oxidative stress in the plant cell due to overproduction of reactive oxygen species (ROS, [19, 20]). The ROS can directly damage the cellular macromolecules including lipids, metabolic enzymes, and the nucleic acids leading to cell death [21]. Henceforth, the decomposition product of polyunsaturated fatty acids hydroperoxides such as malondialdehyde (MDA) can be used as an evidence for lipid peroxidation extent [22]. Consequently, the change in membranes will usually be reflected by corresponding alterations in plant total lipids content which represents about 80% of the total lipid of leaf tissue [23].

Moreover, the vegetable oil can be used as a renewable biological sources for biodiesel which substitute diesel fuel [24]. Recently, the vegetable oil was transformed into green diesel or renewable biofuel by transesterification [24]. Several researchers reported that vegetable oils are a promising fuel that can substitute petroleum fuels [25]. The vegetable oil composition of arid inhabiting plants is considerably affected by the changes in environmental conditions beside their genetic factors [26]. The nature and the structure of the fatty acid methyl ester determine the biofuel properties. The distribution of fatty acids in the

vegetable oil or fat determines the cetane number of the produced biodiesel. In general, the exposure of various crop species to long periods of water deficits lead to reductions in the levels of phospholipid, glycolipid and linoleic acid contents and increased the triacylglycerol [27, 28]. The relative amounts of the different fatty acid radicals determine the properties of fats.

Saturated fatty acids including C14:0, myristic acid; C16:0, palmitic acid; and C18:0, stearic acid have higher cetane numbers and are less susceptible to oxidation than unsaturated ones but they tend to crystallize at very high temperatures [29]. A variety of ester-based fatty esters can be used as biodiesel (or biofuel) beside its roles in the adaptation of plants against environmental stresses. Moreover, the adaptive role of lipid modifications evoked by environmental stresses is frequently depend on physical properties of the lipids involved in membrane structure and affected the permeability of biomembranes [23]. So, the knowledge of the lipid composition in plant cells is important issue. The fatty acid composition of all acyl lipids changed during stress in the direction of increased saturation of the fatty acids [30]. It was reported that short chain fatty acids particularly C16 and C18 are non-specific and exist in the plant cell membranes and cuticle or wax [31]. Pham Thi et al. [27] pointed out that water deficits inhibit fatty acid desaturation, resulting in a sharp decrease in linoleic and linolenic acid biosynthesis. However, water stress induces an increase in fatty acid chain length in *Arabidopsis thaliana*, maintains the saturation level of fatty acids through a reduction in 7,10,13-hexadecatrienoic acid, and induces an increase in the proportion of linolenic acid which may help in drought-stress tolerance [32]. It was reported that the unsaturation level of polar lipids decreased in drought-sensitive plants, whereas it persisted unchanged or even increased in drought-resistant plants [33, 34]. The capacity of a plant to maintain (or increase) its polyunsaturated fatty acid contents was related to its resistance to drought stress [32, 34]. In most plants, the five major fatty acids including palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3), forming about 95–98% of the total fatty acids [35]. The proportions of these fatty acids are strongly influenced by high temperatures [36] and drought [37, 38].

Accordingly, wild plants such as *Zilla spinosa* (Brassicaceae or Cruciferae) one of the most common lignocellulosic wild plant species inhabiting deserts has considerable economic importance to local people. Plants acclimate the dry environments by modifying their phenology, morphology, physiology, and metabolism [6, 39]. Hence, studying the physiological and biochemical

changes including bioenergetics molecules as carbohydrates, cellulose, lignin, and lipids may help in better understanding the tolerance strategies against the harsh environmental conditions and well explore the benefits of using *Zilla plants* for producing renewable energy that may be utilized as a second generation biofuel material.

2 Materials and methods

Wadi Hagul situated in the northern portion of the Eastern Desert of Egypt within Cairo-Suez district and is restricted by latitudes $29^{\circ}48'28''$ – $29^{\circ}57'43''$ N and longitudes $32^{\circ}09'32''$ – $32^{\circ}17'27''$ E. Wadi Hagul occupied the valley depression between Gebel Ataq to the north and Gebel Kahaliya to the south. Its main channel extends for about 35 km and collects drainage water on both sides. With reference to the vegetation and geological features of Wadi Hagul, three main sectors may be distinguished, upstream, middle, and downstream (Figure 1). Moreover, the climate of the Wadi area has been described as arid to extremely arid [40].

2.1 Soil sampling

Soil (sand) samples from up-, mid-, and downstream of Wadi Hagul were collected from the surface layer (0–20 cm)

and subsurface layer (20–40 cm) during the flowering (May, spring) and fruiting (July, summer) seasons, air dried, and then large gravels and plant fragments were excluded and made ready for the mechanical and chemical analysis.

Soil–water extract (1:1) was prepared, shaking well for 2 h and leaving overnight. The filtrate was used for measuring the chemical properties of the soil.

2.2 Soil reaction (pH)

Soil reaction (pH) was measured by using a portable pH-meter (Model, ion lab pH level 1) [41].

2.3 Electrical conductivity (E.C.)

The electrical conductivity (E.C.) was measured by using a portable conductivity meter (YSI Model, 35, Yellow Springs instrument, Co. Inc., USA). The results were expressed as dS/m (dS/m = mmhos/cm) [41, 42].

2.4 Plant material

Zilla spinosa plants with similar age and size were collected from the three main sectors upstream, midstream,

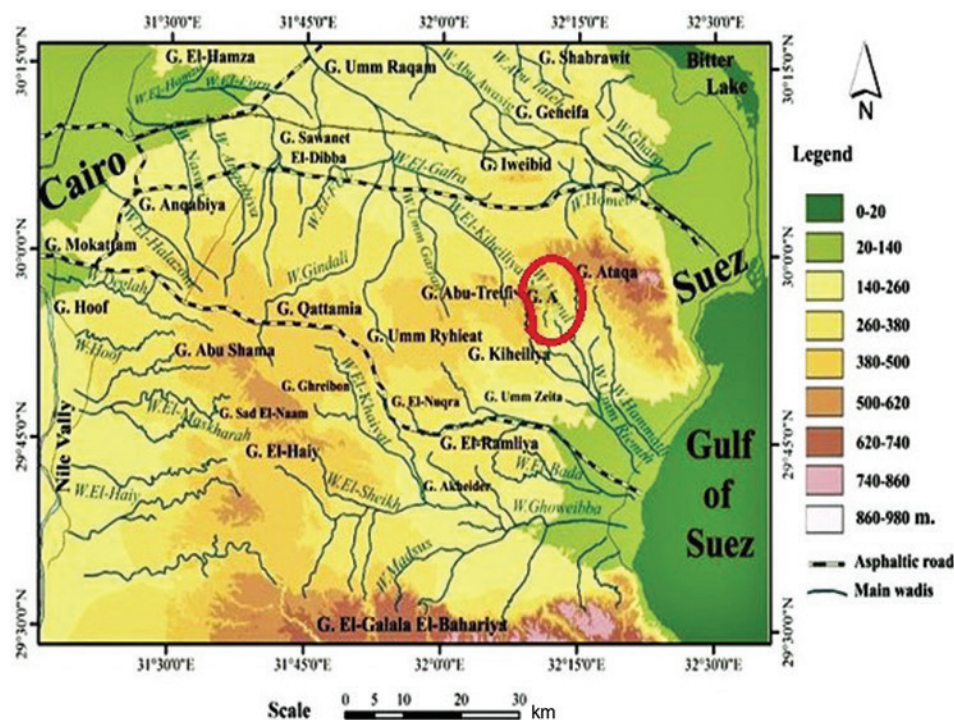


Figure 1: Map of Cairo-Suez road showing the locations of the studied area of Wadi Hagul.

and downstream of Wadi Hagul during spring (April) and summer (July) seasons. The aerial parts of *Zilla spinosa* were dried under shade for 15 days, then ground to a powder and stored in dark bottles until used in the extraction and estimation of total soluble carbohydrates, ligno-cellulosic substances, and total lipids. In addition, the fresh aerial parts were used in the determination of photosynthetic pigments and MDA contents.

2.5 Extraction and estimation of photosynthetic pigments

The photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) were extracted from freshly harvested aerial parts of *Z. spinosa* in 80% acetone and determined spectrophotometrically by the method described by Metzner et al. [43].

2.6 Fluorescence spectroscopy

The fluorescence emission spectra analyses were performed using total pigments extracted from one g plant tissue. Fluorescence spectroscopy of acetone extracted pigments was performed in a Perkin Elmer LS 50B spectrofluorometer using the indicated excitation and emission wavelengths [44]. Red fluorescence of chlorophyll *a* was recorded between 650 and 800 nm. The extracts were excited at 435 nm. Blue-green fluorescence emission spectra were recorded between 380 and 600 nm. Moreover, the extracts were excited at 337 nm. The spectral slit widths were set at 3 and 1.5 nm (excitation and emission, respectively).

2.7 Determination of total soluble carbohydrates

Total soluble carbohydrates were extracted following the method of Homme et al. [45] and determined using anthrone reagent according to the method described by Fairbairn [46].

2.8 Determination of cellulose and lignin contents

The cellulose percentage was determined according to the method of Jenkin [47], and the lignin content was determined by the method described by Rittler et al. [48].

2.9 Lipid peroxidation

The lipid peroxidation product MDA was assayed by using thiobarbituric acid (TBA) protocol described by Cakmak and Horst [49]. The amount of MDA was calculated from the absorbance at 532 nm after subtracting the nonspecific absorption at 600 nm. The extinction coefficient 155 mmol/L⁻¹ cm⁻¹ for MDA was used.

2.10 Extraction and determination of lipids and fatty acid profiles

The dried powder (10 g) was extracted with ethyl acetate for 18 h by using Soxhlet apparatus [50]. The extract was then quantitatively transferred to a weighed flask and the solvent was evaporated using an electric fan. The flask was then reweighed and the increase in weight was equivalent to the weight of total lipids.

Methylation was done according to the method described by Metcalfe et al. [51] using boron trifluoride (BF₃)-methanol. Then, the fatty acid esters were extracted from the BF₃-methanol solution by using hexane. The concentrated extract of fatty acid esters was used for gas liquid chromatography (GLC). The GLC analysis was carried out on HP-5 system equipped with a DB-5 fused silica column (30 m × 0.35 mm × 0.88 μm films); oven temperature was 40–240 °C at a rate of 4 °C/min, injector temperature 260 °C, detector temperature 280 °C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, flow rate 1.1 mL/min, rate 4 °C/min, final temperature 260 °C, final time 8 min, run time 30 min, and injected amount 1 μL.

Peaks identification and quantification was carried out by using UP 4810 computing integrator (Perkins Elmer XL, USA). The percentage of each fatty acid was calculated by the following equation:

$$\begin{aligned} \text{\% of fatty acid} &= \\ & \left(\frac{\text{Peak area of each individual fatty acid methyl ester}}{\text{Total peak area of fatty acids methyl esters}} \right) \times 100 \end{aligned}$$

The unsaturation level of all fatty acids was estimated according to Pham Thi et al. [27], the unsaturated index can be calculated from the following equation:

$$\begin{aligned} \text{Unsaturation index} &= (1 \times \%18:1) + (2 \times \%18:2) \\ &+ (3 \times \%18:3) / 100 \end{aligned}$$

where 18:1, 18:2, and 18:3, represent the oleic, linoleic, and linolenic acids, respectively.

2.11 Statistical analysis

Statistical analysis was performed by two-way analysis of variance (ANOVA) using SAS software (SAS Institute, Cary, N.C.) at a significance level of 5%. Duncan's multiple range test was applied to assess the differences between the three sites during the two investigated seasons.

3 Results

3.1 Characteristics of Wadi Hagul habitats

Soil analysis showed a significant decrease in Wadi Hagul soil moisture particularly at the third location (downstream) during the dry season (July), whereas the EC rises compared with the Spring season. However, the pH of the soil remained more or less alkaline throughout the study period and did not seasonally fluctuate (Tables 1 and 2). The high percentage of soil moisture content was more pronounced during the wet period of April which was

related to the fall of rain; however, it significantly reduced in the dry season (July). Soil moisture scarcity in Wadi Hagul was intensified during summer particularly at the edges due to low precipitation concomitant with high temperature (Table 3).

3.2 The alteration in photosynthetic pigments

The values of chlorophylls *a*, *b* and carotenoids are greater in the wet season collected *Zilla spinosa* shoots compared to those of dry season (Table 4). The greatest levels of chlorophylls *a* and *b*, *a/b*, *a/b*, and total carotenoids as well as carotenoids was measured in *Zilla spinosa* shoot tops inhabiting the midstream of Wadi Hagul bed during the wet season followed by that collected during the dry season. Moreover, the minimum values of chlorophylls *a* and *b*, *a/b* ratio, and carotenoids were recorded in plants inhabiting downstream the bed particularly during the dry season.

Measurements of fluorescence emission spectra of chlorophyll *a* have been an early indicator of stress

Table 1: Soil reaction, electrical conductivity (EC), and chemical analysis of soil pastes of Wadi Hagul habitats during summer (Sum) and spring (Spr).

Location	Depth	pH		EC		Soluble anions meq/L (milliequivalents/liter)					
						SO ₄ ⁻²		Cl ⁻		HCO ₃ ⁻	
		Spr	Sum	Spr	Sum	Spr	Sum	Spr	Sum	Spr	Sum
Upstream	0–20	8.5	7.76	1.053	1.379	4.97	3.813	4.1	8.33	0.8	1.6
	20–40	8.5	7.75	0.87	1.118	1.4	3.238	3.3	6.24	0.9	1.66
Midstream	0–20	8.4	7.52	1.80	2.36	9.1	5.588	19.5	14.31	2.1	3.744
	20–40	8.4	7.98	0.198	0.245	2.92	0.728	1.4	0.944	0.5	0.777
Downstream	0–20	8.3	7.96	0.123	0.252	8.2	0.885	0.42	0.95	0.62	0.78
	20–40	8.3	8.09	0.88	0.139	2.65	0.34	0.22	0.65	0.36	0.4

Table 2: The percentage of soil water content and the chemical analysis of Wadi Hagul soil pastes during spring (Spr) and summer (Sum).

Location	Soil water content (%)		Soluble cations (meq/L milliequivalents/liter)								CaCO ₃ (%)	
	Spr	Sum	Mg ²⁺		Na ⁺		Ca ²⁺		K ⁺		Spr	Sum
			Spr	Sum	Spr	Sum	Spr	Sum	Spr	Sum		
Upstream	1.45	0.75	0.86	2.45	2.80	8.10	2.06	2.94	0.214	0.226	7.9	15.5
			0.53	1.56	1.10	6.33	2.42	3.12	0.100	0.133	5.7	8.10
Midstream	2.90	2.75	1.98	4.16	5.43	12.86	5.40	6.24	0.214	0.346	17.3	19.3
			0.12	0.56	0.37	0.95	0.296	0.777	0.100	0.171	13.1	14.9
Downstream	0.90	0.36	0.26	0.60	0.37	0.96	0.41	0.81	0.143	0.154	40.4	41.7
			0.73	0.21	0.10	0.68	0.15	0.295	0.100	0.105	48.7	51.2

Table 3: The meteorological data (climatic condition) of Wadi Hagul at the 4 years 2010–2014.

Months	Wind velocity (km/h)	Relative humidity (%)	Rain fall (mm/month)	Mean minimum temperature (°C)	Mean maximum temperature (°C)
January	7.02	59	3.4	10	20
February	7.65	58	3.7	11	21
March	7.46	54	3.2	13	25
April	8.36	47	17.7	16	28
May	7.46	45	0	19	32
June	8.81	48	0	22	36
July	7.89	52	0.5	24	38
August	7.78	54	0	24	38
September	8.3	56	0	22	34
October	8.56	58	0.2	19	30
November	6.8	60	1.7	15	26
December	6.21	62	3.9	11	22

Table 4: Seasonal changes in pigment levels in *Zilla spinosa* aerial portions collected from different habitats of Wadi Hagul.

Season	Pigment content Location	Chl <i>a</i>	Chl <i>b</i>	Chl <i>a+b</i>	Chl <i>a/b</i>	Total carotenoids	Chl (<i>a+b</i>)/carotenoids
Spring	Upstream	2.23 ± 0.013 ^b	0.60 ± 0.020 ^b	2.83	3.72	1.20 ± 0.013 ^b	2.36
	Midstream	3.00 ± 0.010 ^a	0.8 ± 0.017 ^a	3.80	3.8	1.38 ± 0.010 ^a	2.75
	Downstream	0.92 ± 0.011 ^e	0.42 ± 0.023 ^c	1.33	2.19	0.50 ± 0.0008 ^e	2.66
Summer	Upstream	1.10 ± 0.011 ^d	0.421 ± 0.016 ^c	1.52	2.62	0.57 ± 0.0002 ^d	2.71
	Midstream	1.70 ± 0.009 ^c	0.61 ± 0.010 ^b	2.31	2.79	0.84 ± 0.0004 ^c	2.75
	Downstream	0.81 ± 0.007 ^f	0.41 ± 0.011 ^c	1.22	1.98	0.47 ± 0.0001 ^e	2.60

Each value is a mean of three replicates ± SD. ^{a,b,c,d,e}Changes indicated by similar letters are not significantly different. ^fChange is significantly different.

conditions in plants. Hot dry summer conditions provoke an increase in F675 which is more distinct in downstream inhabiting *Zilla* shoots harvested in dry summer season (Figure 2 A–D). Furthermore, a slight increase in chlorophyll *a* fluorescence emission of upstream and downstream located plants was observed with a red shift at 676 of about 1 nm during Spring (Table 5). Moreover, the maximum chlorophyll *a* fluorescence emission around 675 nm of Summer plants was greater compared to Spring ones at the three studied locations.

It is of interesting to note here that a longer shift at F730 with lengths of 2–4 nm was displayed in summer harvested shoots as compared with those of spring. The increase in the fluorescence emission and the red shift are parallel to the reduction in chlorophyll content. Furthermore, the ratio of F450/F675 was markedly reduced in *Zilla* shoots inhabiting the edges of Wadi Hagul particularly, downstream. In contrary, the ratio of F675/F730 was markedly increased in *Zilla* inhabiting Wadi's edges particularly, in downstream inhabiting shoot tops (Table 5).

3.3 The alteration in total soluble carbohydrates

The greatest level of total soluble carbohydrates accumulation attained in edges of Wadi Hagul inhabiting plants during the wet spring season (Table 6). However, during the dry summer season, the total soluble carbohydrates reached the highest level in *Zilla* inhabiting downstream Wadi Hagul compared to the other locations. The minimum values of total soluble carbohydrates accumulation were estimated in midstream grown plants during the wet and dry seasons as compared with those of the other Wadi's beds.

3.4 The alteration in cellulose and lignin

The amount of both cellulose and lignin was significantly increased in *Zilla* shoots inhabiting Wadi Hagul edges (Table 6). Such effect was more pronounced in summer harvested *Zilla* shoots. The maximum increases

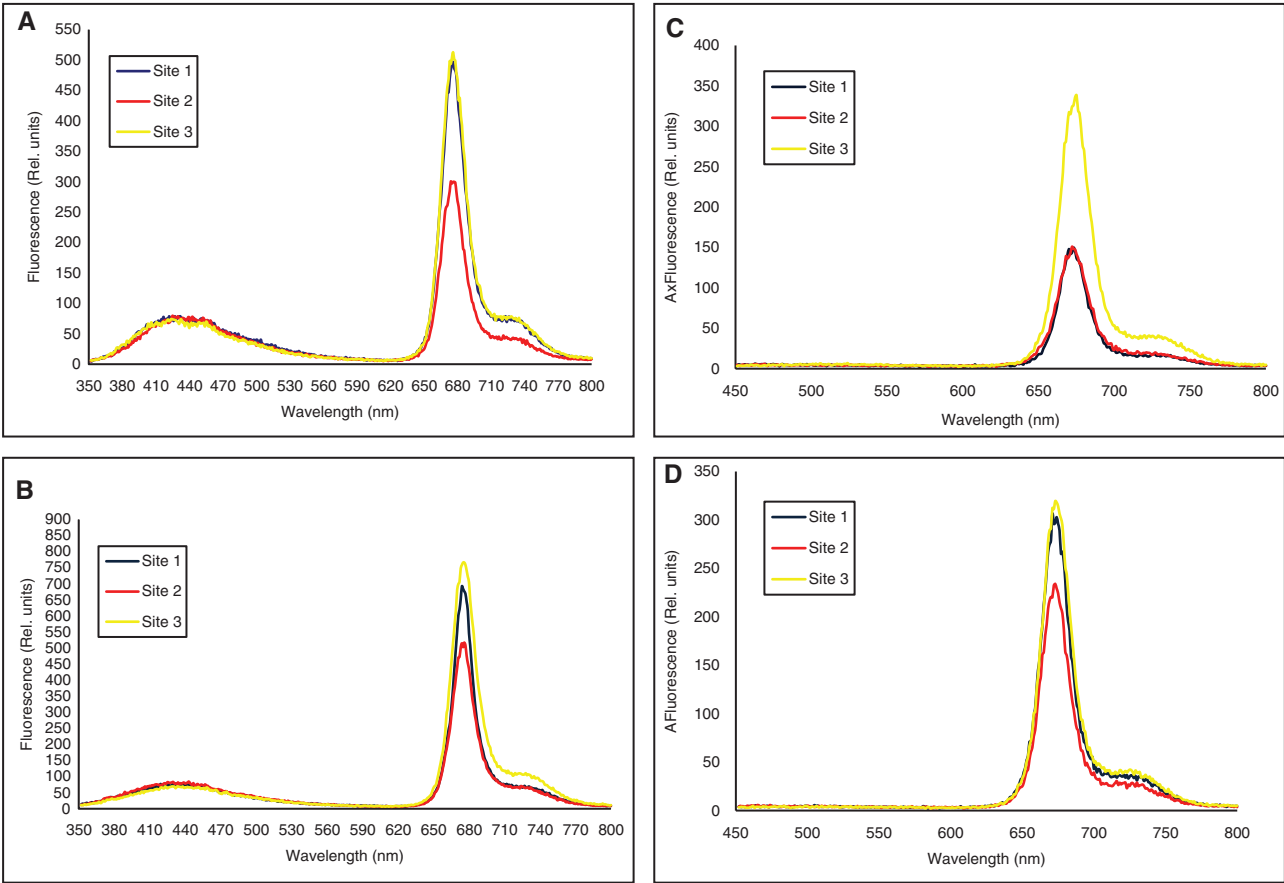


Figure 2: (A and B) Seasonal changes in blue-green fluorescence emission spectra of *Zilla spinosa* green tops inhabiting Wadi Hagul during the spring (A) and summer (B) seasons. $E_x \lambda = 337$ nm (Slit width for both excitation and emission was 3 and 1.5 nm, respectively). Each value is a mean of three replicates. (C and D) Seasonal changes in red fluorescence emission spectra of *Zilla spinosa* green tops inhabiting Wadi Hagul during the spring (C) and summer (D) seasons. $E_x \lambda = 435$ nm (Slit width for both excitation and emission was 3 and 1.5 nm, respectively). Each value is a mean of three replicates.

Table 5: Seasonal changes in the peak position of fluorescence spectra around 450 and 680 nm in *Zilla spinosa* shoot extract grown in different habitats of Wadi Hagul.

Season	Location	Peak position around 675 nm	Peak position around 730 nm	F 450/F 675	F 675/F 730
Spring	Upstream	676	727	0.11	7.3
	Midstream	674	725	0.16	6.9
	Downstream	676	728	0.08	9.7
Summer	Upstream	677	730	0.15	6.6
	Midstream	676	728	0.25	6.5
	Downstream	677	732	0.14	9.9

Values are means of three replicates.

in cellulose (61.6 g/100 g dw) and lignin (66 g/100 g dw) was recorded at the third location during summer season. The lowest amount of either cellulose or lignin was measured in spring harvested *Zilla* inhabits the second location.

3.5 Lipid peroxidation product molondialdehyde (MDA)

The intensification of lipid peroxidation is one of the main reactions for lipid damage by ROS. When the unsaturated

Table 6: Seasonal changes in lipid peroxidation product (malondialdehyde, MDA), total soluble sugars, % of cellulose, % of lignin, and total oil content of *Zilla spinosa* shoots grown in different habitats of Wadi Hagul.

Season	Parameter Location	MAD (mmol)	Total soluble sugars (mg/gfw)	Cellulose (%)	Lignin (%)	Total lipids (%)
Spring	Upstream	3.46 ± 0.13 ^b	14.0 ± 0.20 ^b	52.0 ± 0.020 ^b	43.1 ± 0.020 ^b	3.90 ± 0.013 ^b
	Midstream	1.90 ± 0.10 ^a	6.76 ± 0.17 ^a	50.4 ± 0.017 ^a	41.7 ± 0.017 ^a	7.24 ± 0.010 ^a
	Downstream	5.79 ± 0.11 ^e	10.31 ± 0.23 ^c	56.3 ± 0.023 ^c	42.3 ± 0.023 ^c	3.60 ± 0.0008 ^e
Summer	Upstream	6.820 ± 0.11 ^d	4.19 ± 0.16 ^c	62.1 ± 0.016 ^c	62.1 ± 0.016 ^c	3.20 ± 0.0002 ^d
	Midstream	5.98 ± 0.9 ^c	2.64 ± 0.10 ^b	60.6 ± 0.010 ^b	60.6 ± 0.010 ^b	3.60 ± 0.0004 ^c
	Downstream	7.17 ± 0.7 ^f	6.42 ± 0.11 ^c	66.0 ± 0.011 ^c	66.0 ± 0.011 ^c	2.90 ± 0.0001 ^e

Each value is a mean of three replicates ± SD. ^{a,b,c,d,e}Changes indicated by similar letters are not significantly different. ^fChange is significantly different.

fatty acids are peroxidized, molondialdehyde (MDA) is produced. The increments in MDA levels in *Zilla* tops inhabiting the edges of Wadi Hagul during spring and summer was positively related to the intensity of the soil water scarcity (Table 6).

3.6 The alteration in total lipids contents

The percentage of total lipid content was positively related to soil moisture content along Wadi Hagul (Table 6). The greatest lipid percentage displayed in the areal parts of *Zilla* inhabiting the second location during spring (7.24%) followed by summer (3.6%) season (Table 6). The lowest lipid content (2.9%) displayed by summer harvested *Zilla* inhabiting the third location (downstream).

3.7 The alteration in fatty acid composition in *Zilla* shoots

The GLC analysis of the fatty acid methyl esters resulted in the identification of 12 fatty acids in which palmitic (C16:0) and stearic acids (C18:0) are the main saturated acids and linoleic acid (C18:2) is the main unsaturated acid in *Zilla spinosa* aerial parts inhabiting Wadi Hagul (Table 7). The proportion of tridecanoic acid (C13:0), pentadecanoic (C15:0), palmitic acid (C16:0), and stearic acid (C18:0) were markedly increased in spring harvested *Zilla* aerial parts particularly those inhabiting midstream of Wadi Hagul. The fatty acid profile of *Zilla* characterized also, by a greater amount of unsaturated fatty acids which was detected in summer harvested plants particularly in plants inhabiting downstream Wadi Hagul. The increments in percentage of unsaturated fatty acids such as linoleic acid (C18:2) and linolenic (C18:3) were about 37.33% and 11.1%, respectively, in harvested *Zilla* aerial parts inhibiting downstream Wadi Hagul.

4 Discussion

The geologic alterations impact soil type and hence, the habitat as well as weather condition including fluctuations in seasonal temperature and precipitation crossways the landscape [52]. Moreover, the spatial pattern plays a central role in plant community dynamics, such as succession, adaptation, maintenance of species density, and competition [53].

Indeed, soil moisture scarcity in arid environment affects plant community's occurrence due to low-precipitation-induced drought stress [4]. Soil analysis showed a significant decrease in Wadi Hagul soil moisture content particularly at the third location (downstream) during the dry season (July), whereas the EC rises compared with the spring season. However, the pH of the soil remained more or less alkaline throughout the study period and did not cause significant seasonal alterations. The high percentage of soil moisture content was attained during the wet period of April which was related to the fall of rain. The distinct depletion in soil moisture content at the edges of Wadi Hagul, particularly during summer was attributed to the inclination associated with effects of high temperature combined with low precipitation.

Henceforth, only the arid plant communities such *Zilla spinosa* are able to survive and can either avoid or tolerate drought periods [54]. Meanwhile, *Zilla* inhabiting midstream can survive and tolerate the hot summer under the availability of water (Table 1). Consequently, soil moisture level seems to be the limiting factor for the permanent and continuous growth of *Zilla* populations in Wadi Hagul habitats. Moreover, *Zilla* biomass seems to be more sensitive to water scarcity than heat stress.

Similarly, the environmental stresses alter the metabolic processes in stressed plants particularly, the synthesis of chlorophyll (energy trapping pigment) which was positively related to the availability of moisture in the soil. The values of chlorophylls *a*, *b*, and *a/b* ratios as well as

Table 7: Seasonal changes in composition of lipid content, percentage of saturated and unsaturated fatty acids, unsaturation index, carbon preference index (CPI), and carboxylic acid ratio (CAR) of *Zilla spinosa* shoots grown in different habitats of Wadi Hagul.

Season	Location	C10:0	C11:0	C13:0	C14:0	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C18:3	Unsaturated fatty acids (%)	Saturated fatty acids (%)	Ratio of saturated/unsaturated	Unsaturation index
Spring	Upstream	–	9.55	10.34	1.13	7.02	21.98	1.72	1.26	19.13	7.20	10.96	9.71	29.59	70.41	2.38	0.583
	Midstream	0.25	9.34	13.78	–	10.39	28.46	0.18	6.28	23.16	–	7.98	–	08.16	91.66	11.23	0.161
	Downstream	–	2.44	6.47	–	8.14	23.25	–	3.88	16.29	13.26	16.04	10.10	39.53	60.47	1.53	0.655
Summer	Upstream	–	8.04	6.38	1.08	3.25	21.88	2.31	1.91	14.48	5.33	25.09	10.25	42.98	57.02	1.33	0.853
	Midstream	–	9.24	6.28	1.17	8.23	24.25	1.06	1.78	23.76	–	24.23	–	25.29	74.71	2.95	0.578
	Downstream	–	5.79	4.79	1.11	2.77	17.71	1.41	2.01	9.25	7.00	37.33	11.10	56.57	43.43	0.768	1.150

C10:0, Capric acid; C11:0, Undecylic acid; C13:0, Tridecyllic acid; C14:0, Myristic acid; C15:0, Pentadecanoic acid; C16:0, Palmitic acid; C16:1, Palmitoleic acid; C17:0, Margaric acid; C18:0, Stearic acid; C18:1, Oleic acid; C18:2, Linoleic acid; and C18:3, Linolenic acid.

carotenoids in *Z. spinosa* shoots were markedly increased during the wet season particularly in plants inhabiting midstream. In addition, the higher levels of chlorophylls in *Zilla* shoots at the second location reflected also that the plants did not have much problem to survive in the moist arid habitats that help it to withstand heat stress. However, the reduction in chlorophylls in plants inhabiting the Wadi’s edges particularly during the dry summer season perhaps related to retardation in pigments production and/or increase in their degradation which may be due to the reduction in soil moisture content that comes from the variations in topographic factor and changes in climatic factors, the edaphic factors and organic substances [55, 56], which causes reductions in water use efficiency and plant water potential [57]. However, recently, it was deduced that the reduction in chlorophyll was attributed to the acceleration of chlorophyll breakdown rather than its slow synthesis [58]. Similar seasonal trends were reported for desert shrubs by Aziz [59]. Similarly, it was reported that the altitudinal variation induced changes in pigment content in *Arnica montana* and *Porphyra yezensis* [60, 61].

Similarly, hot dry summer induced reduction in chlorophyll *a/b* ratio particularly at the edges of the Wadi. It was postulated that chlorophyll *a/b* ratio slightly increased in drought tolerant wheat cultivars and significantly decreased in the susceptible ones under water deficit conditions [62]. Such differences could be due to a shift in an occurrence of photosynthetic systems toward a lower ratio of photosystem (PS) II to PSI [63] or/and reduction in Chl biosynthesis as reported for several plant species [64–66].

Similarly, the ratio of chlorophylls (*a+b*) to carotenoids decreased in *Zilla* inhabiting the edges particularly during the dry season. Such effect was negatively related to soil moisture and stress intensity and could be used as a stress indicator [7]. The substantial reduction in chlorophyll content assayed in *Zilla* inhabiting the edges particularly during the dry summer season suggested a possible influence of drought stress on the reduction of stomatal conductance and photosynthetic rates during the dry periods [57, 67] and thereby the biomass.

Furthermore, the spectroscopic methods have been used to characterize the physiological state of plant as an early stress indicator. The relationships between the intensity of fluorescence emission bands or band ratios to plant health and stress condition were investigated by Buschmann [68] and Lichtenthaler et al. [69]. The intensity and the form of the fluorescence emission spectra are influenced by environmental conditions [70]. The chlorophyll fluorescence emission of far red values and ratio of

F680 to F730 and blue to red (F450/F680) are very suitable to describe the seasonal and spatial variation in photosynthetic activity in *Zilla* shoot tops. The fluorescence emission reflects the intactness of the internal photosynthetic activity [71]. The greatest increase in the chlorophyll *a* fluorescence emission and the ratio of F675/F730 associated with the decline in F450/F675 of upstream and downstream collected *Zilla* during dry seasons was positively related to the reduction in the level of chlorophylls which displayed by the variation in the intensity of soil water deficit and the EC of the soil. Moreover, the observed increase in red fluorescence emission particularly in downstream inhabiting plants during summer and spring occurs at the expense of the photosynthetic conversion of the absorbed light and is related to damage of PSII and light harvesting system complex (LHC) [71]. Furthermore, excitation at 337 nm shows the presence of blue fluorescence with an emission near 425 nm, smaller shoulder around 520 nm region (green fluorescence), and the red chlorophyll fluorescence with emission near 675 nm. The increase in the intensity of red fluorescence emission near the 680 nm region was attributed to the reduction in chlorophyll content, which might be results from the injuries of antenna and reaction centres of chlorophyll *a* in PSII [72]. The increase in the fluorescence emission and the red shift due to varying chlorophyll content was reported also by Krause and Weis [73] and Kancheva et al. [74].

The plants response to water stress depends mainly on the severity and duration of the stress and growth stage of the plant [75]. Thus, different physiological and biochemical processes are altered by stress such as water relation [76], gas exchange, photosynthesis [77], and carbohydrates, protein, amino acids, and other organic compounds metabolism [78], which may contribute in stress tolerance and thereby biomass production. The total soluble carbohydrates were quite different among *Zilla* shoots grown in different habitats during the wet and dry seasons. Higher values of soluble carbohydrates were measured in *Zilla* shoots during the wet season particularly in upstream inhabiting plants. The greater accumulation of soluble sugars attained in spring collected *Zilla* shoots was positively related to chlorophyll content and chlorophyll molecules efficiency [79]. The accumulation of carbohydrates depends on the plant species, soil topography, and moisture content. Some plants as palms and some leguminous species accumulated high carbohydrate content during the wet season as to reinstate the growth of new tissues [80, 81] and survive the dry season characterized by low soil moisture content [82]. Subsequently, the accumulated carbohydrates involved in the adaptation of arid deciduous species, for maintenance of metabolism

under long drought period [83]. Soluble sugars can serve as osmoprotectant and a carbon source for biomass production [84].

On the other hand, the decline in soluble carbohydrates in summer collected *Zilla* shoots might be due the decrease in chlorophyll content which may result in lower rate of photosynthesis and minimal metabolic activity under extreme conditions [85, 86] or allocation to the underground roots [87] to promote root growth to search of water [88].

Furthermore, under the harsh environmental stressful conditions, the woody plants tend to spend large amounts of carbon in the production of lignified support tissues [89]. The greatest amounts of lignin displayed in *Zilla* shoots inhabiting downstream the Wadi particularly during summer was concomitant with the increases in total phenols particularly P-coumaric and caffeic acids and the activities of PAL and peroxidases which involved in lignin biosynthesis parallel with reduction in ferulic acid (Khattab et al., Unpublished). Such biochemical alterations induced lignin deposition and cell wall stiffness as well as reduced cell wall extensibility and consequently enhanced plant stress tolerance [90]. The accumulation of lignin in response to biotic and abiotic stresses is an important defence mechanism in plants [91] to cope with the severe stresses. It confers stability to xylem vessels required for efficient water transport [92]. In addition, the carbohydrate polymers including cellulose, hemicellulose, and lignin forms the largest portion of “lignocellulosic” plant materials which have recently been utilized as a source of feedstock for bioenergy production [93, 94]. Similarly, the accumulation of lignin in response to biotic and abiotic stress was recorded in many plants [91, 95].

In addition, plant nutrient deficiency induced by different stressors during summer may result in a considerable reduction in total lipid content. The total lipids content of *Zilla spinosa* inhabiting different habitats of Wadi Hagul was positively related to the soil moisture level. On the other hand, the reduction in the total lipids content in *Zilla* inhabiting the edges particularly during summer might be attributed to the inhibition of lipid biosynthesis [96] and/or stimulation of lipolytic and peroxidative activities [97, 98], concomitant with decline in membrane lipid content [99]. Similarly, the total lipid content generally exhibits a decline in response to either drought or temperature stress in various plant species [32, 100–103].

Indeed, the fatty acid pattern which composes plants lipids depends mainly on the temperature and water availability [104, 105]. Meanwhile, the alteration of fatty acid composition particularly in membrane lipids is critical for plant adaptation against drought stress [100, 106].

The fatty acid profile analysis showed the occurrence of the saturated fatty acids including undecylic acid (C11:0), trideclic acid (C13:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), margaric acid (C17:0), stearic acid (C18:0), and unsaturated fatty acids as linoleic acid (C18:2) in *Zilla* aerial portions inhabiting Wadi Hagul. However, oleic acid (C18:1) and linolenic acid (C18:3) were absent in midstream inhabiting plants during the two investigated seasons. Hence, the greatest levels of total saturated fatty acids have been exhibited in *Zilla* aerial portions during the spring growing season; however, the total amount of unsaturated fatty acids was attained during the dry summer period concomitant with increments in the proportions of oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) and decline in saturated fatty acids palmitic acid (C16:0) and stearic acid (C18:0). The downstream inhabiting plants exhibited the greatest level of unsaturation index (DBI) during the dry summer period (Table 7). The stress-induced changes in unsaturated fatty acids may play a role in the defense mechanism and it reflects the deleterious effects in *Zilla* plants. Such increments in the unsaturated fatty acids and the double bond index (DBI) might be due to the effect of heat and drought stresses which may speed up the kinetic energy and molecules movement across membranes, thus cause loosening of chemical bonds inside molecules of biological membranes. Such effect increases the membrane fluidity by either denaturation of proteins or an increase in unsaturated fatty acids [107]. Meanwhile, the extent of fatty acids unsaturation varies by the plant species and the drought intensity [108]. It was reported that the unsaturation level of lipids decreased in sensitive plants, whereas it stayed unchanged or even increased in resistant cultivars under drought stress conditions [33, 34]. Therefore, the specific adjustments in the fatty acid composition and unsaturated lipid level under drought stress could help plant maintain membrane integrity [108, 109] and plant dehydration tolerance [110, 111]. Henceforth, the greatest increase in the unsaturated level and DBI in *Zilla* inhabiting downstream seems to be concomitant with the superior extent of stresses which stimulate the activities of desaturases enzymes and thus the production of unsaturated fatty acids. The increases in desaturases activities improved drought and salt stress tolerance in transgenic tobacco and mutants *Synechocystis* [112, 113], which suggest that drought and salt tolerance of plants depends on the levels of unsaturated fatty acids [113, 114]. Similarly, Sui et al [115] and Sui and Han [116] showed that the increments in unsaturated fatty acids in halophytes were concomitant with the increased tolerance of the photosystem to salt stress.

In addition, many reports pointed out that environmental stresses such as heat, drought, and salt induce changes in FA composition, mainly in the content of linolenic acid (18:3) [34, 117]. In the present investigation, the observed predominant accumulation of free linolenic acid (C18:3) in summer growing *Zilla* inhabiting Wadi Hagul edges particularly at the third location may serve as a stress signal and precursor for phyto-oxylipin biosynthesis [118] and involved in formation of cellular membranes, suberin and cutin waxes which act as protectors against stressful environmental conditions [119]. Furthermore, such fatty acids could reduce the structural and functional damages of cellular membranes induced by stresses [99, 120]. It was reported that the increase in C18:3 fatty acids was associated with enhanced plants tolerance against abiotic stresses which dependent on the inherent level of fatty acid unsaturation and/or the ability to maintain or adjust fatty acid unsaturation [114, 115]. Similarly, the greater unsaturation level induced by drought stress was reported in *Arabidopsis thaliana* [32] and kentucky bluegrass [121].

On the other hand, both salt and drought stress were found to reduce the amount of 18:3, in rape leaves, cruciferous herbs (*Crambe* sp.), pea (*Pisum sativum*), the legume *Pachyrhizus ahipa*, and in salt-tolerant but not salt-sensitive citrus cells [122–124].

Similarly, the integrity and functions of cell membranes are sensitive to stresses such as drought and heat. Membranes are the main targets of degradative processes induced by drought and it has been shown that water scarcity decreases membrane lipid content [27, 33] concomitant with inhibition of lipid biosynthesis [98] and stimulation of lipolytic and peroxidative activities [97, 125]. Hence for, the reduction in the total lipid content is concomitant with increments in MDA (a lipid peroxidation product) levels in *Zilla* tops inhabiting the edges of Wadi Hagul particularly during hot–dry summer season. Moreover, the substantial greatest increase in the MDA suggests more ROS in summer collected *Zilla* shoots, reflected the greater membranes damages and decreased cell membrane stability which serves as an indirect measure of stress tolerance in diverse plant species [126–129].

5 Conclusion

The hot dry summer associated with water scarcity in the arid habitats of Wadi Hagul markedly modified the quantity and composition of infochemicals which in turn influence the occurrence and density of *Zilla* population and

thereby biomass production, meanwhile contribute in plant tolerance. Thus, deficiency in soil water content and plant nutrient induced by different stressors during dry hot summer season evoked a considerable increase in the lignin content concomitant with reduction in total oil level in *Zilla* aerial portions inhabiting different habitats of Wadi Hagul. The environmental stressors not only limited resources of vegetable oil but also modulate the oil comprises fatty acids in favor of increasing the percentage of unsaturated fatty acids particularly in downstream inhabiting plants which is undesirable for biodiesel generation (greater cetane number). *Zilla spinosa* is a lignocellulosic woody shrub with promising biofuel sources including lignocellulosic compounds beside the vegetable oil content. Finally, the biomass and chemical composition of *Zilla spinosa* are greatly affected by water scarcity compared to the upraised temperatures displayed in summer season.

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