

VOLATILE OIL PRODUCTION, PROLINE, PROTEIN AND DNA PROFILE IN THREE MEDICINAL PLANTS EXPOSED TO SHORT-TERM DROUGHT STRESS

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Abstract

This present work deals with monitoring some molecular aspects standing beyond the varied production of volatile oil in three medicinal plants (*Ocimum basilicum*, B, *Mentha longifolia*, M and *Origanum majorana*, O) after ten days of exposure to drought stress by using 250 mM mannitol as a drought inducer. The results showed that proline has increased in all drought-stressed plants as compared to the control. Four protein bands (210, 70, 63 and 18 kDa) have disappeared in stressed B-plant, in addition to low molecular weight newly expressed protein bands (16-78 kDa). The protein polymorphism has varied among the species and ranged from 16.66 to 54.54%. RAPD-DNA technique indicated high similarity of genomic-DNA in stressed and unstressed plants. GC-MS screened intensive fluctuations within the pool of phytochemical compounds. The genomic DNA structure was generally stable under drought stress despite of new proteins expression which mediated the induction of chemical compounds with antioxidant property to cope with the drought stress.

Introduction

Drought is the serious agricultural problem been treated as salinity (Ludlow and Muchow 1990). Mannitol-mediates both drought and osmotic stresses and it has been experimentally utilized to find the cause of incident drought stress in plants (Moinuddin *et al.* 2012). Drought sensitivity is a case of both nutrient transport inhibition and reduction of leaf expansion, which subsequently led to reduction of shoot length and root dry weight due to physiological inhibition of cellular elongation and suppression of cell wall carbohydrate synthesis (Dastgheib *et al.* 1990, Neumann 1995 and Ullah *et al.* 2014). The progressive water deficit was found to accumulate proline intensively in ornamental plants (Yamada *et al.* 2005). Only a few reports revealed that protein was not affected in drought-stressed crop plants (Ullah *et al.* 2014), where in others, protein profile under drought stress exhibited synthesis, expression and accumulation of new protein bands (Cheng *et al.* 1993 Kamal *et al.* 2010). However, synthesis and production of volatile oil compounds in subjected-ornamental plants to drought stress are relatively drought-resistant processes (Popp *et al.* 2002).

Unlike fatty acids, essential oils enclose fragrance with unspecific chemical composition. They are industrial compounds used in cosmetics, perfumes, drink and as flavor (Sellar 2001). Drought alters the chemical composition of oil via varying contribution ratio of volatile components in particular genera (Petropoulos *et al.* 2007). Generally, the yield of essential oil decreases under drought stress; however, oil percentage is unaffected or even increased due to the presence of stress antioxidant compounds that assist plants against stress conditions. Essential oil increased under drought stress in *Origanum majorana*, *Origanum majorana* (Rhizopoulou and

Diamantoglou 1991) and in *Salvia officinalis* (Bettaieb *et al.* 2008) and in *Calendula officinalis* L. (Rahmani *et al.* 2019). In critical cases, the essential oil diminishes due to drought effect on reducing shoot, leaf number and /or leaf area (Farahani *et al.* 2008). In addition, the leaf position whether plain or curly has not interfered with oil production under drought stress, although curly leaf in sparsely produces higher amount of essential oil compared to plain leaf. Camphor, B, 1,8-cineole and thujona were the chemical components that increase in ratio during moderate drought stress (Moinuddin *et al.* 2012). The random amplified RAPD-DNA is a powerful, rapid, simple technique and consistent for a particular plant species regardless of its age or origin (Micheli *et al.* 1994). RAPD-DNA elucidates variance of genetic relationship upon plants breeding and considered the molecular marker of phylogenetic analyses of plants species. So far, this substantial protocol had clarified genetic diversity of endangered plants (Liu *et al.* 2007, Zheng *et al.* 2008). In addition, RAPD-DNA had potentially exploited for identification of medicinal and aromatic plants components (Um *et al.* 2001). Previous investigations viewed similarity of DNA sequence between varied mango species, due to the significant similarity of varied species in the primer's amplification sites (Cordeiro *et al.* 2006).

The present study was aimed at identifying short drought influence on volatile oil production, protein profile and genomic-DNA pattern in three medicinal plants grown for 10 days at the flowering stage with the drought stress induced by D-mannitol treatments.

Materials and Methods

Three aromatic and medicinal plants (65-day-old) at flowering stage, namely *Ocimum basilicum* L. (B), *Mentha longifolia* L. (M), and *Origanum majorana* L. (O) were purchased from local market located at Kafr Hakeem, Giza, Egypt. They were grown in a mixture of sand and clay soil (1:1). The plants were divided into two sets: The first set was left to grow for 10 days using normal irrigation water and the second set of plants (BM, MM, OM) was irrigated for 10 days using D-mannitol solution (M, 250 mM). After the end of the experiment, the vegetative leaves were detached from the plants and used for extraction and estimation of protein and DNA-RAPD. Oven-dried leaves (70°C) were utilized for proline and GC-MS analyses.

Determination of free proline was conducted in dry leaves detached from the normally grown, *Ocimum basilicum* (B), *Mentha longifolia* (M), and *Origanum majorana* (O) or drought-stressed plants using acidic ninhydrin flowing the method described by Bates *et al.* (1973).

For potential identification of the organic components present in the dried leaf powder of the three medicinal plants (basil, mint and origanum) grown normally or under drought stress, GC-MS analysis was performed on HP 6890 Gas Chromatograph fitted with an SGE BPX5 fused-silica capillary column (15 m × 220 µm × 0.25 µm) and coupled to a HP 5973 mass selective quadrupole detector. The derivatized extracts were inoculated through Merlin Microseal™. High pressure Septumin pulsed splitless mode (pulse pressure 17.6 Psi, 325°C). The column oven was programmed to be enhanced with an initial isothermal at 50°C for 2 min. The temperature was increased with 10°C per min reaching 360°C, and then remained at this temperature for 15 min. Helium was a carrier constant gas controlled using 2.0 ml/min flow. The ion source was maintained at 230°C, ionization and fragmentation were accomplished by electron impact (70 eV). The mass filter was scanned between m/z 50 and 700, with a scan rate of 2.29 scans per second. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970), as modified by Studier (1973). Water-soluble proteins were extracted from leaves of the tested plants. Then protein fractionations were performed exclusively on vertical slab gel (19.8 cm × 26.8 cm × 0.2 cm) using the electrophoresis

apparatus manufactured by Cleaver, UK. The images were captured by digital camera (Sony, made in Japan) and transferred directly to the computer.

DNA was isolated from young leaves located at the apex of the shoot of the three medicinal plants: basil, mint and origanum according to the manufacturer's manual of DNA isolation and extraction kit (Promega).

PCR amplification reaction was performed using selected five primers in 0.2 ml PCR Eppendorf tube containing optimized mixture (40 μ l total volume) which consisted of master mix solisprimer (8 μ l), Metabion German (2 μ l) and Template DNA (2 μ l) with the concentration then completed up to 40 μ l by nuclease-free water. Amplification was performed in a programmed thermo cycler as follows: initial denaturation at 94°C for 5 min (one cycle), followed by 35 cycles of annealing at 94°C for 1 min, at 37°C for 45 sec, and extension at 72°C for 45 sec then final extension, 72°C for 7 min (one cycle), and finally holding at 4°C until use. Agarose (1.2%) was utilized for separating PCR products. It was placed in 1X TAE buffer and then boiled in microwave. Ethidium bromide (with the concentration) was added to the melted gel after the temperature became 55°C.

Each experiment was repeated for three times and illustrated data are means of independent replicates from three separate experiments.

Results and Discussion

In the present study, three medicinal plants were subjected to short-term drought stress using 250 mM of D-mannitol solution for 10-day-irrigation. The abrupt rise of proline level in all plants upon mannitol treatments (Fig. 1) manifests the generation of stress in these genotypes, as has been also reported by Ashraf and Foolad 2007. Proline biosynthesis occurred via regulation of some enzymes biosynthesis and degradation and via maintenance of membrane stability to sustain stress tolerance (Kishor *et al.*, 2005). The polymorphism that contributed to high and low molecular weight proteins points to the metabolic variations under drought stress (Table 1). In mint and origanum genotypes, six and two new protein bands were expressed, respectively with no missing protein bands. Four of the six protein bands in mint, were of low molecular weight (45, 33, 32 and 16 kDa). In origanum, the low molecular weight expressed proteins were 87 and 18 kDa. In basil, four protein bands have disappeared and two have appeared at low molecular weight (78 and 16 kDa) under stress (Fig. 2). These low molecular weight proteins which are stress proteins, may function to maintain osmotic homeostasis under stress, and are responsible for production of organic antioxidant (Kosakivska *et al.* 2008). Referring to DNA-RAPD technique (Fig. 3), the structure of the genomic DNA under drought stress was stable although undergo some variations upon the gene expression, presumably due to the non-hazardous effect of mannitol, and of the short stress exposure which was not enough to cause mutation or breakdown of the genomic DNA of the medicinal plants. Similar study on tobacco plants showed that the genomic DNA under abiotic stress led to the expression of 30 genes, among which 20 genes were defensive genes modified by methylation in relation to stress (Wada *et al.* 2004). In agreement, the varied chemical compounds pool between control and stressed plants (no illustrated data) confirms the protein banding modification is likely due to gene expression. B-data indicates the presence of 126 compounds at different RTs. Among the most predominant compounds: 2-Propenoic acid (5.2 %), Eucalyptol (3.8%), 2-Bornanone (3.55), acetic acid, hydroxy[(1-oxo-2-propenyl)amino] (3.17%) and 2-isopropyl-5-methyl-9-methylene (1.74%). Alternatively, the BM data detected the appearance of 129 compounds, with the predominant compounds: propenoic acid (5.93%), neophytadiene (3.16%), 9, 12, 15-Octadecatrienoic acid, (Z, Z, Z) - (2.84%), n-hexadecanoic acid (2.26%), Bis (2-ethylhexyl) phthalate (2.6%) and eucalyptol (1.85%). Linalool in B-plants (2.834%) is predominant over BM-plants (0.9311%).

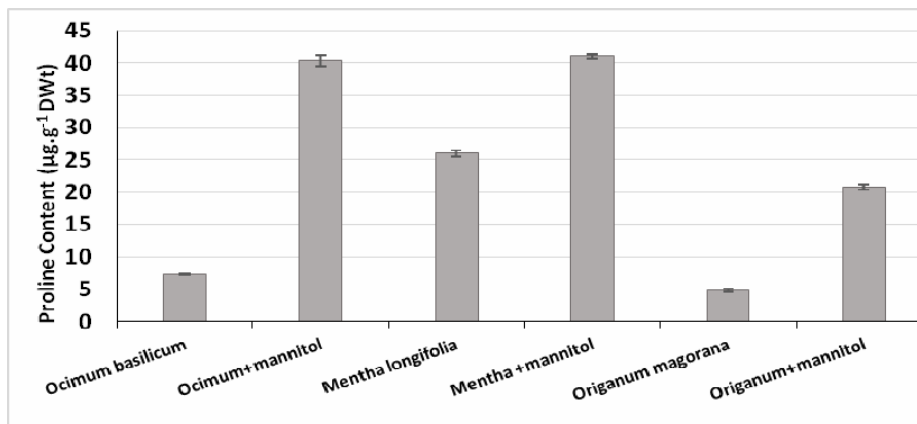


Fig. 1. Effect of D-mannitol treatments on the endogenous proline of the 65-days old medicinal plants (*Ocimum basilicum*, *Mentha longifolia* and *Origanum magorana*). Error bars designate to \pm SD of the mean.

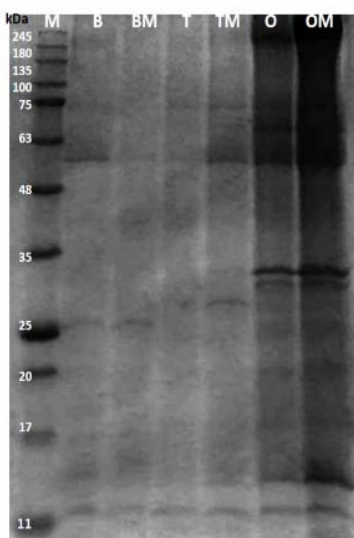


Fig 2. SDS-PAGE electrophoresis of the soluble protein extracted from apical leaves of some medicinal plants : basil (B), mint (T) and origanum (O) plants grown for 10 days either under normal irrigation condition using water or with irrigation using 250 mM mannitol-induced drought stress (BM, TM and OM). M: protein marker 245, 180, 135, 100, 75, 63, 48, 35, 25, 20, 17 and 11 kDa).

GC-MS data of normal growing M-plants determined 146 chemical compounds, the most predominant compounds: (Z)6, (Z) 9-pentadecadien-1-ol (6.05%), methyl 8,11,14-heptadecatrienoate (3.75%), hydroxyl [(1-oxo-2-propenyl)amino]-(3.26%), n-hexadecanoic acid (3.05%), D-carvone (2.5%) and nonacosane (2.17%). On the other hand, GC-MS data of mannitol-treated *Mentha longifolia* (MM) revealed the presence of 129 compounds, some of them exist with relatively high ratios : 1,2-propanediamine (6.26%), n-hexadecanoic acid (4.9%), 12-octadecadienoic acid (Z,Z)- (4.3%) and D-carvone (3.3%).

Table 1. Percentage of polymorphism of extracted soluble protein from leaves of drought stressed medicinal plants (Basil + M, Mint + M and origanum + M) using mannitol (250 mM) for 10-day irrigation compared to the control plants (Basil, Mint and Origanum).

Band No.	Rf	MWt	Medicinal plants					
			Basil	Basil+M	Mint	Mint + M	Origanum	Origanim + M
1	0.043	230	-	-	+	+	-	-
2	0.052	210	+	-	-	-	-	-
3	0.058	190	-	-	-	+	-	-
4	0.104	119	-	-	-	+	-	-
5	0.115	110	-	-	-	-	+	+
6	0.124	105	-	-	+	+	-	-
7	0.147	87	-	-	+	+	-	+
8	0.160	78	-	+	-	-	-	-
9	0.175	70	+	-	+	+	+	+
10	0.241	63	+	-	+	+	+	+
11	0.271	60	+	+	+	+	+	+
12	0.311	52	-	-	+	+	-	-
13	0.329	49	-	-	-	-	+	+
14	0.352	45	-	-	-	+	+	+
15	0.372	42	-	-	-	-	+	+
16	0.390	40	+	+	+	+	+	+
17	0.408	38	-	-	-	-	+	+
18	0.444	35	-	-	-	-	+	+
19	0.481	33	-	-	-	+	+	+
20	0.503	32	-	-	-	+	+	+
21	0.546	29	-	-	+	+	+	+
22	0.600	25	+	+	+	+	+	+
23	0.629	23	-	-	-	-	-	+
24	0.674	20	+	+	+	+	+	+
25	0.702	19	-	-	+	+	-	-
26	0.732	18	+	-	+	+	-	+
27	0.816	17	+	+	+	+	+	+
28	0.856	16	-	+	-	+	-	-
29	0.881	15	+	+	+	+	+	+
30	0.937	12	+	+	+	+	+	+
Band number			11	9	16	22	18	21
% polymorphism			-	54.54	-	37.50	-	16.66

GC-MS data analysis of chemical composition of normal growing *Origanum majorana* (O) elicited the presence of 114 compounds at different RTs. Some of these compounds are detected in high proportions: Hydroquinone (3.77%), octadecanamide (3.28%), hexacosane (2.7%), n-hexadecanoic acid (2.68%) and 9, 12, 15-octadecatrienoic acid, (Z, Z, Z) - (2.55%). On the other hand, the chemical composition obtained from GC-MS analysis of mannitol-treated origanum plants (OM) shows 118 compounds where 9,12,15-Octadecatrienoic acid, (Z, Z, Z) - (4.7%), cyclobutanol (3.3%), hydroquinone (2.87%), 9-hexacosene (2.6%) and n-hexadecanoic acid (2.58%) were predominating.

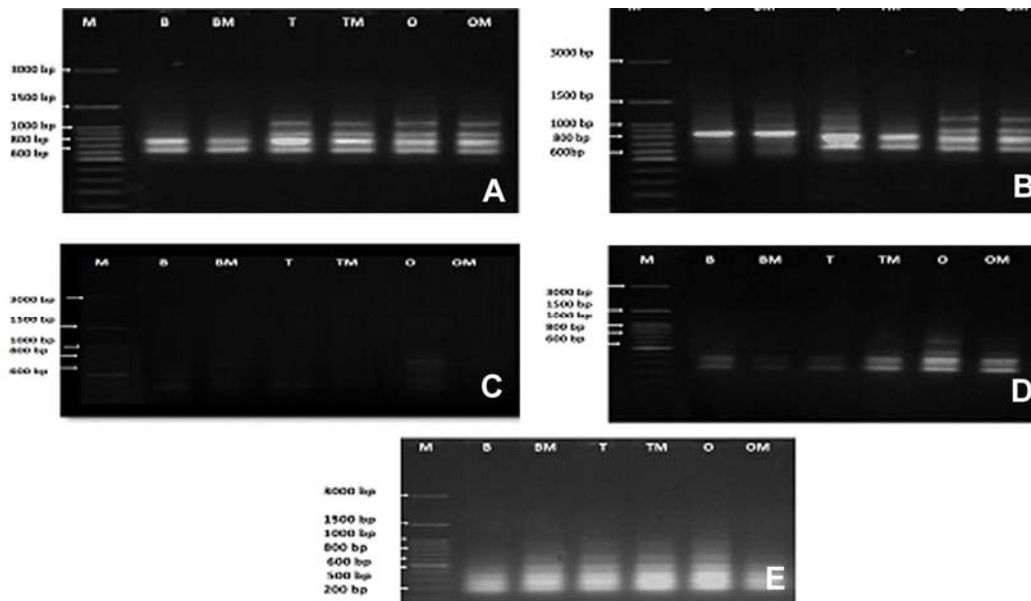


Fig 3. Agarose gel electrophoresis of RAPD-PCR amplification fragments as products obtained using five primers: (A; OPA-1, B; OPA-4, C; OPA-11, D; OPB-3 (not successful), E; OPB-10). The primers introduced to isolated DNA from medicinal plants grown for 10-day with or without drought stress: basil (B), stressed basil (BM), mint (T), stressed mint (TM), origanum (O) and stressed origanum (OM). M; DNA size marker (100-3000 b).

The biological activities of the detected chemical substances are summarized as follows: Ethylene oxide is an insecticide and antimicrobial compound used for disinfection (Lin *et al.* 2017). Cyclobutanol is an inhibitory substance to mushroom tyrosinase (Xie *et al.* 2016). Dimethyl aminomethyl and methylamine are famous corrosive substances to eye and skin (Kennedy 2014). Furfural is the collagen stabilizer (Lakra *et al.* 2014). Linalool is antifungal and insecticidal natural product (Nyasembe *et al.* 2012). Acetic acid possesses antibacterial and antifungal properties and acts effectively as inhibitor of carbohydrate metabolism and on solubilization of cell membrane lipid causing subsequent death (Kim *et al.* 2016). The successive compounds, Bicyclo [3.1.1] heptane (0.58%) and epianastrephin (0.12%) are biologically undefined substances. Eucalyptol is reported to kill infectious organisms and preventing their spread (Wydro and Szedfo 2016). Allyldiethylamine inhibits the activity of harmful organisms (Shagun *et al.* 2016). 2-propenoic acid is a powerful biocide (Danish *et al.* 1995). Pinocarvone functions as antioxidant, antibacterial and possesses other biological activities (Lima *et al.* 2012). Naphthalene induces oxidative stress in *Trifolium* plants (Huang *et al.* 2017). Myrtenol, is anti-inflammatory flavoring compound (Gomes *et al.* 2017). Neophytadiene is a bioactive lipid component (Ahn *et al.* 2016). The tricosane is an antibacterial substance (Samadi *et al.* 2012), whereas hexacosane, is the potent antibacterial agent (Kotan *et al.* 2010). Squalane is unsaturated hydrocarbon and acts as antioxidant, skin hydrator, detoxifier and drug carrier (Kim and Karanediz 2012). Safrole acts as antifungal and considered carcinogenic compound (Yang *et al.* 2017). D-limonene, the insecticidal natural product (0.7564%, (Nyasembe *et al.* 2012). D-carvone is a compound of volatile oil characterized by medicinal properties as antimicrobial (Jirovetz *et al.* 2003).

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