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METABOLOMIC ANALYSES TO EVALUATE THE EFFECT OF DROUGHT STRESS ON CHILI PEPPER (*CAPSICUM ANNUUM* L.) AT VEGETATIVE STAGE

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ABSTRACT

Water shortage due to competition from the various sector, global warming, and climate change leading to less-irrigated field occurred is serious now and future problems for all cultivated plants involving chili pepper. In the situation drought stress give high and significant effect on reducing plant production as a result of alteration in metabolite homeostasis in vegetative tissues. To reveal metabolomic alteration on chili pepper to the stress as main objective of the study, three capsicum genotypes with different tolerance levels to less irrigation condition compared to normal condition were evaluated. Leaves extract of three different chili genotypes grown in less irrigation and normal conditions were analyzed with Gas Chromatography-Mass Spectrophotometry (GC-MS) to reveal profiling of their volatile compounds. Based on principal component analysis (PCA), ten metabolites, i.e., neophytadiene, linolenic acid-ethyl ester, linolenic acid, gamma-sitosterol, phytol, alpha-tocopherol, squalene, i-propyl 7,10,13,16,19-docosapentaenoate, ergost-5-en-3-ol and bicyclo [10.1.0] tridec-1-ene were known to contribute to the existence of diversities in the three genotypes. Furthermore, under hierarchical cluster analyses (HCA) from the ten metabolite compounds, there were only three important metabolites of linolenic acid, gamma-sitosterol, and alpha-tocopherol revealing differences of them compared to the normal irrigation plants. In the less irrigated plants, lowering linolenic acid and increasing gamma-sitosterol and alpha-tocopherol were expressed to response the drought stress. Tolerance chili plants to the stress were affected by their capacity to reduce linolenic acid degradation and to increase alpha-tocopherol and gamma sitosterol accumulation.

KEY WORDS

Capsicum annum, drought stress, GC-MS, volatile compound, vegetative stage.

Chilli pepper (*Capsicum annum* L.) is one of important and strategic commodities in Indonesia giving a significant effect on the dynamic Indonesian economy and its inflation. In one hand, the high economic value is performed by total cultivation areas reaching 308,547 ha with Central, West and East Java provinces as main cultivation areas; 2.5 million ton total production 2018; 7.78 tonnes/ha productivity (BPS 2019) and high dynamic prices from Rp.4,000 to 120,000 per kg (Republika 2019). On the other hand, Kompas (2021) published that inflation index of consumer price in January 2021 was 0.21% caused by administered prices and volatile food. one of the biggest contributors to inflation is chili pepper. Though the chili pepper has high economic value and gives serious problem in Indonesian economic stability, there is difficult task, especially in balancing between its supply and demand. There is a big problem faced in maintaining and sustaining high plant productivity, especially in the dry season due to drought stress occurred during cultivation of the plants. For the chili pepper plants that have highly sensitive to the stress, drought condition generally decreased the plant production drastically.

In cultivation aspects, the chili plants are plants that require much water for their growth and are sensitive to irrigation practices (Abdul-Ganiyu et al. 2012). Lack of water in the plants can cause drought stress leading to a very significant yield loss (Gençoğlu et al., 2006; Demirtas and Ayas, 2009). Drought stress generally affected physiological, biochemical, and molecular processes such as photosynthesis, respiration, translocation, and metabolism (Pandey and Shukla, 2015). The stress interferes metabolite homeostasis and causes significant chemical composition changes in plants which require the temporal readjustments of the metabolic pathway (Azadeh et al. 2014), including carbohydrate, amino acid, and peptide metabolism (Huang and Gao 2000) and altering the metabolomic profiles of leaf tissues (source) that could reduce its yield (sink) potential. Furthermore, revealing chili metabolomic profile, especially in determination tolerance levels of the plants to drought stresses is important information for breeding programs, especially to establish selected tolerance candidates.

Several plant metabolomic profile studies were successfully reported previously. Wahyuni et al. (2013) found that a large group of acyclic diterpenoid glycosides, called capsianosides, was in highly abundant in all *C. annuum* genotypes studied. Metabolomic profiling demonstrated that in soybeans, keeping up with sugar and nitrogen metabolism is of prime significance, along with phytochemical metabolism under drought and heat stress conditions (Das et al., 2017). In African eggplant, proline, glutamate, sucrose, fructose and tricarboxylic acid cycle metabolites were shown to be positively correlated with stress (Mibei et al., 2017). In sugar beets, drought triggered changes in primary metabolism, especially increases in amino acids in both organs, but leaves and roots responded with different dynamics to re-watering. After a transient normalization of most metabolites within eight days, a second accumulation of amino acids in leaves might indicate a stress imprint beneficial in upcoming drought events (Wedeking et al., 2018). While metabolomic profiling on *Capsicum annuum* L dealing with the establishment tolerance level of chili pepper is not reported yet.

In the study, important information was successfully revealed based on comparing three selected chili genotypes in different tolerance levels to drought stress with the normal genotype as the main objective of the research. Results of the study gave evidence that different level of tolerances on chili pepper was affected by the existence of linolenic acid, gamma-sterol, and alpha-tocopherol. The metabolomic profiling of chili pepper according to its response to drought, was discussed in detail in this paper.

MATERIALS AND METHODS OF RESEARCH

The experiment used Gada MK, SSP, and C10 genotypes which were collections of the Laboratory of Genetic-Education and Plant-Breeding, IPB. The selection of genotypes was based on tolerance level grouping by Fischer and Maurer (1978) on vegetative and yield characters. Gada MK and SSP represent tolerant genotypes, whereas C10 represents sensitive genotypes.

The seeds of 3 chili genotypes were germinated in trays containing a mixture of soil and compost as sowing media. After four weeks of sowing or have at least four adult leaves, the seedlings were transplanted to polybags (30 cm in diameter) containing a mixture of soil, sand, and manure (2: 2: 1, v/v/v). Before mixing, each component of the media is filtered with a 0.5 cm diameter hole sieve to obtain fine granules, uniform and free of impurities, then dried. The polybags filled with soil are then saturated with water. Planting was done in the afternoon (one plant per polybag). The chili plants were then placed in the greenhouse and arranged in rows with a distance of 50 cm x 50 cm and a distance between treatments 70 cm. Normal irrigation was maintained before and three weeks after transplanting of the seedlings to keep the soil moisture at over 90% field capacity.

The experiment consisted of two treatments, i.e., full irrigation (as control) and less irrigation (drought), arranged in randomized complete block design with three replications. Drought treatment was started at three weeks after transplant applied by stopping irrigation in 7 days. Soil moisture measurement was carried out every day using a soil moisture sensor

based on electrical impedance, and the humidity of media was maintained at the range of 50-60% field capacity.

Metabolomics analysis was conducted using Agilent Technologies 7890 GC system with Auto Sampler, 5975 Mass Selective Detector and Chemstation data, which was equipped with a capillary column HP Ultra 2 (30 m x 0.20 mm ID x 0.11 μ m Film Thickness). Samples were taken from each treatment one week after the application of the treatments (four weeks after transplant or at the end of the vegetative period). Fresh leaves (10 g) from each treatment were sliced into small pieces and extracted with pro analysis ethanol (50 mL) then macerated for approximately five days in a closed container at room temperature. After maceration, 10 ml of the extract was poured into the tube and then dried at 60°C for 1 hour. After drying, the extract was dissolved again with the remaining 200 μ L extracts and use as a sample in GC-MS analysis. Each component of metabolites was identified by matching the recorded mass spectrum from the GC-MS data system. Metabolite components were identified by the name of the compound, molecular formula, and molecular weight using a peak spectrum comparison in Wiley and PubChem databases.

Data were analyzed statistically by using the R package (Xia and Wishart, 2016). Principal Component Analysis (PCA) was applied to determine the metabolites that contribute significantly to the diversity of chili genotypes. Hierarchical Cluster Analysis (HCA) was conducted to assess the relationship between chili genotypes in response to normal and drought conditions. The t-Students test was conducted to show metabolite differences between treatments.

RESULTS AND DISCUSSION

A total of 83 volatile compounds identified from three chili genotypes grew under normal and drought conditions based on PCA (Table 1). From the 83 volatile compounds, 20 metabolites gave most contribution in forming diversity (Figure 1), however, only 10 metabolites, i.e: neophytadiene, linolenic acid-ethyl ester, linolenic acid, gamma-sitosterol, phytol, Alpha-tocopherol, squalene, i-Propyl 7,10,13,16,19-docosapentaenoate, Ergost-5-en-3-ol and Bicyclo [10.1.0] tridec-1-ene can differentiate genotypes based on the treatments applied (Figure 2 and 3). Furthermore, under HCA from the ten metabolites (Figure 3), there were three important volatile compounds detected in leaf samples derived from normal and drought stress condition, namely linolenic acid (M5), gamma-sitosterol (M9) and alpha-tocopherol (M15). This result was then confirmed by the T-Student test at the level of 5%. All chili samples grew in drought conditions showed a decrease in linolenic acid levels, and an increase in gamma-sitosterol and alpha-tocopherol (Figure 4).

At normal growth condition, the highest linolenic acid content was recorded at the C10 as sensitive genotype and indicated significant difference compared to the Gada and SSP as tolerant genotypes (Figure 5a). The highest content of alpha-tocopherol during normal condition was also expressed by C10 genotype, but there was no significant difference compared to Gada and SSP genotypes (Figure 5b) while the lowest gamma-sitosterol content in the normal condition was noted in the C10 genotype and indicated significant difference compared to the Gada and SSP as tolerant genotypes (Figure 3c).

Under drought stress conditions, in sensitive genotypes (C10) there was a significant decrease in linolenic acid content with a slight increase in alpha-tocopherol and gamma-sitosterol content, whereas in tolerant genotypes (SSP and Gada MK) there was no significant decrease in linolenic acid content, but a significant increase in alpha-tocopherol and gamma-sitosterol content (Table 2). Table 2 was strengthened by the change concentration of the three important metabolites (Figure 6).

Entirely the study successfully revealed evident that different growth of chili pepper in normal and drought stress condition leading to different metabolite compound profiling. All chili samples grew in drought conditions showing a decrease in linolenic acid and an increase in alpha-tocopherol and gamma-sitosterol levels.

Table 1 – The concentration of volatile compounds derived from the leaf samples of three chili genotypes at normal and less irrigation condition

No	Label	Compounds	Molecular Formula	Molecular Weight (g/mol)	Retention Time	Peak area (%) of Chilli genotypes					
						Normal Condition			Drought Condition		
						SSP	Gad a	C10	SSP	Gada	C10
1	M1	Neophytadiene	C20H38	278.52	27.37	8.35	9.05	4.28	16.79	10.71	8.27
2	M65	6,10-Dimethyl-9-Undecen-2-One	C13H24O	196.33	27.42	0.00	0.36	0.00	0.00	0.00	0
3	M77	1-Hexadecyne	C16H30	222.42	27.46	0.00	0.00	0.70	0.00	0.00	0
4	M16	6-Tetradecyne	C14H26	194.36	27.62	0.00	0.00	0.00	0.00	0.00	0
5	M66	1,E-8,Z-10-Tridecatiene	C13H22	178.32	27.62	0.00	0.30	0.00	0.00	0.00	0
6	M17	1- Bromo-8-Heptadecyne	C17H31Br	315.34	27.79	0.00	0.50	0.00	0.00	0.00	0
7	M42	Scleral (Sclareolide Lactol)	C16H28O2	252.40	28.08	6.08	0.00	1.67	0.00	0.00	0
8	M62	2,2,7-Trimethyl-3-octyne	C11H20	152.28	28.09	0.00	3.93	0.00	0.00	0.00	0
9	M18	.alpha.-Selinene	C15H24	204.36	28.50	0.00	0.00	0.00	0.00	0.00	0
10	M43	i-Propyl 7,10,13,16,19-docosapentaenoate			28.51	0.54	0.00	0.00	0.93	0.00	0
11	M74	1,5-Cyclodecadiene, (E,Z)-	C10H16	136.24	28.60	0.00	0.00	0.00	0.00	2.85	0
12	M80	1-Cyclododecyne	C12H20	164.29	28.61	0.00	0.00	1.61	0.00	0.00	0
13	M70	Acetamide, N-(IMIDAZOL-1-ylpropyl)-2-Methoxy			28.61	0.00	0.00	0.00	0.00	1.94	0
14	M26	Stearate	CH3(CH2)16COOH or C18H36O2	284.48	28.72	0.00	0.00	0.00	0.00	0.00	0
15	M3	Palmitic Acid	C16H32O2	256.43	28.78	8.24	9.83	11.58	6.44	8.33	7.24
16	M48	Palmityl Chloride	C16H33Cl	260.89	29.48	0.00	0.00	0.00	0.34	0.54	0
17	M52	3-Tetradecene	C14H28	196.38	29.48	0.00	0.00	0.00	0.60	0.00	0
18	M57	Cyclododecane	C12H24	168.32	29.48	0.00	0.00	0.00	0.58	0.00	0
19	M83	Cyclohexadecane, 1,2-diethyl-	C20H40	280.54	29.48	0.00	0.00	0.00	0.00	0.00	0.36
20	M10	Phytol	C20H40O	296.54	29.50	10.03	6.66	7.31	35.44	7.16	6.35
21	M75	Phytol Isomer	C ₂₀ H ₄₀ O	296.53	29.54	0.00	0.00	0.00	0.00	6.78	0
22	M4	benzyl (dideuterated) methyl EtHe			29.56	0.00	0.00	15.92	0.00	14.51	0
23	M39	Cyclodecacyclotetradecene, 1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20-eicosahydro-	C22H40	304.56	29.62	0.30	0.00	0.00	0.00	0.00	0
24	M44	1,4,9-Decatriene, (Z)-	C ₁₀ H ₁₆	136.238	29.62	0.29	0.00	0.00	0.00	0.00	0
25	M60	6-[1-(Hydroxymethyl)Vinyl]-4,8a-Dimethyl-4a,5,6,7,8,8a-Hexahydro-2(1h)-Naphthalenone	C ₁₅ H ₂₂	234.334	29.63	0.00	0.34	1.67	0.00	0.00	0
26	M63	1,2-15,16-Diepoxyhexadecane	C16H30O2	254.41	29.63	0.00	0.33	0.00	0.00	0.00	0

Table 1 Continue

No	Label	Compounds	Molecular Formula	Molecular Weight (g/mol)	Retention Time	Peak area (%) of Chilli genotypes					
						Normal Condition			Drought Condition		
						SSP	Gada MK	C10	SSP	Gada MK	C10
27	M81	2,5,8-Heptadecatrien-1-ol			29.64	0.00	0.00	0.50	0.00	0.00	0
28	M27	Linoleic Acid	C18H32O2	280.45	29.75	0.00	0.00	1.52	0.93	0.30	0
29	M2	Linolenic acid, ethyl ester	C20H34O2	306.49	29.79	0.00	0.00	0.00	3.95	0.00	0
30	M5	Linolenic acid	C18H30O2	278.44	29.97	24.05	26.30	34.94	19.47	19.90	20.92
31	M53	Bicyclo [10.1.0] tridec-1-ene	C13H22	178.32	30.01	0.00	4.72	0.00	1.21	1.51	0
32	M19	Methyl 8, 11, 14- heptadecatrienoate	C18H30O2	278.44	30.01	4.08	2.60	3.35	1.63	0.00	0
33	M78	6-Dodecyne	C12H22	166.31	30.10	0.00	0.00	3.31	0.00	0.00	0
34	M61	i-propyl 6,9,12-hexadecatrienoate	C19H32O2	292.46	30.20	0.00	1.96	0.00	0.00	0.00	0
35	M82	(4as,5r,8s,8as)-8-Isopropyl-5-Methyl-3,4,4a,5,6,7,8,8a-Octahydro-2-Naphthalenemethanol			30.34	0.00	0.00	1.31	0.00	0.00	0
36	M33	S-[2-[N,N- Dimethylaminol]ethyl]n,n-dimethylaminol thiocarbohydroximate	C8H17N3O2S	219.30	30.39	1.46	0.00	0.00	0.00	0.00	0
37	M68	14-Methyl-8-Hexadecyn-1-ol	C17H32O	252.44	30.39	0.00	0.99	0.00	0.00	0.00	0
38	M76	Labda-8(17), 13e-dien-15-al			30.40	0.00	0.00	0.00	0.00	1.65	0
39	M20	Santolina epoxide	C10H16O	152.24	30.41	0.00	0.00	0.00	0.00	0.00	0
40	M22	S-[2-[N,N- Dimethylamino]ethyl]N,N-dimethylcarbamoyl thiocarbohydroximate	C8H17N3O2S	219.30	30.43	0.00	0.00	0.00	0.00	0.00	0
41	M79	Iso-Dihydrocarveol	C10H18O	154.25	30.45	0.00	0.00	1.63	0.00	0.00	0
42	M40	Cyclopentanemethanol, 2-(1-Butenyl)-,[1r-[1.alpha.,2.alpha.(Z)]]			30.45	1.11	1.05	0.00	0.00	0.00	0
43	M11	2-Oxatricyclo(4.4.1.0)Undecan-4-One			30.47	0.00	0.00	0.00	0.00	0.00	0
44	M34	Vulgarol A	C20H36O2	308.51	30.55	1.29	0.91	0.00	0.00	0.00	0
45	M23	1-Ethoxycarbonyl-.Beta.-Carboline	C14H12N2O2	240.26	30.57	0.00	0.00	0.00	0.00	0.00	0
46	M54	Patchoulol	C15H26O	222.37	30.57	0.00	0.00	0.00	0.96	0.00	0
47	M49	12-Methyl-E,E-2,13-octadecadien-1-ol	C19H36O	280.50	30.57	0.00	0.00	0.00	1.77	0.00	0
48	M85	EICOSYNE	C20H38	278.52	30.57	0.00	0.00	0.00	1.19	0.00	0
49	M71	(9e, 12e)-9,12-Octadecadienoyl Chloride	C18H31ClO	298.90	30.57	0.00	0.00	0.00	0.00	1.57	0
50	M84	Pregnan-20-one, 3(acetyloxy)-14-hydroxy-, (3.beta., 5.beta., 14.beta.)-	C ₂₃ H ₃₆ O ₄	376.530	30.57	0.00	0.00	0.00	0.00	0.00	2.19
51	M45	Agatholol	C20H32O2	304.47	30.66	0.67	0.00	0.00	0.00	0.00	0
52	M55	Caryophyllene oxide	C15H24O	220.36	30.70	0.00	0.00	0.00	1.80	0.00	0
53	M12	.gamma.-Gurjunene	C15H24	204.36	30.91	0.00	0.00	0.91	0.00	0.00	0.92
54	M50	[2,6,6-Trimethyl-4-(3-Methyl-2-Butenyl)-1-Cyclohexen-1-Yl]Methanol	C15H26O	222.37	30.92	0.00	0.00	0.00	0.92	0.00	0

Table 1 Continue

No	Label	Compounds	Molecular Formula	Molecular Weight (g/mol)	Retention Time	Peak area (%) of Chilli genotypes					
						Normal Condition			Drought Condition		
						SSP	Gada MK	C10	SSP	Gada MK	C10
55	M28	(2 .Alpha., 6 .Alpha.)-Trans-9,10-Dimethyl-4-Oxatetracyclo [6.3.0.0(2,6).0(7,11)Undecane			30.93	0.00	0.00	0.00	0.00	0.00	0
56	M21	(-)-.Beta.-Elemene	C15H24	204.36	30.94	0.00	0.00	0.00	0.00	0.00	0
57	M6	(z) Nerolidol	C15H26O	222.37	31.03	0.00	0.00	0.00	0.00	0.00	0
58	M56	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	C15H26O	222.37	31.12	0.00	0.00	0.00	0.28	0.00	0
59	M72	Chenodiol	C24H40O4	392.58	31.14	0.00	0.00	0.00	0.00	0.39	0
60	M73	Diltiazem	C22H26N2O4S	414.52	31.16	0.00	0.00	0.86	0.00	1.15	0
61	M29	1,3,3-trimethyl-2-hidroxymethyl-3,3-dimethyl-4-(3-Methylbut-2-enyl)-cyclohexene			31.25	0.00	0.00	0.00	0.00	0.00	0
62	M58	Methanol, [6,8,9-trimethyl-4-(1-propenyl)-3-oxabicyclo[3.3.1]non-6-en-1-yl]-	C ₁₅ H ₂₄	236.350	31.32	0.00	0.00	0.00	0.63	0.00	0
63	M35	OXACYCLOTRIDECAN-2-ONE	C12H22O2	198.31	31.47	0.58	0.00	0.00	0.00	0.00	0
64	M46	5-Chloro-6-nitrocholestan-3-ol	C27H46ClNO3	468.12	31.47	0.55	0.00	0.00	0.00	0.00	0
65	M51	Limonene Dioxide	C10H16O2	168.24	31.48	0.00	0.00	0.00	0.68	0.00	0
66	M59	7-Isopropyl-4a-Methyloctahydro-2(1h)-Naphthalenone	C14H24O	208.35	32.21	0.00	0.00	0.00	0.67	0.00	0
67	M30	13-Tetradecene-11-yn-1-ol	C14H24O	208.35	32.21	0.00	0.00	0.00	2.66	0.00	0
68	M36	Dodecahydro-as-indaceno[4,5-b]oxirene	C12H18O	178.28	32.27	0.30	0.00	0.00	1.16	2.38	0
69	M64	Eicosane	C20H42	282.56	32.27	0.00	0.29	0.00	0.00	0.00	0.43
70	M31	Androstan-3-ol	C19H32O	276.46	32.28	0.00	0.00	0.00	0.00	0.00	0
71	M37	6-Cyclohexylidene-1-Hexyn-3-Ol			32.40	0.93	0.54	0.00	0.00	0.00	0
72	M7	1,5,9,13-Tetradecatetraene	C14H22	190.33	32.41	1.18	0.00	0.00	0.00	0.00	0
73	M38	9,12-Tetradecadien-1-ol, acetate			32.45	1.84	0.00	0.00	0.00	0.00	0
74	M13	beta-monolinolein	C21H38O4	354.53	32.45	0.00	0.00	0.00	0.00	0.00	0
75	M14	cis,cis,cis-7,10,13-hexadecatrienal	C16H26O	234.38	32.50	0.00	1.28	0.00	0.00	0.00	0
76	M24	1,3,12-Nonadecatriene	C19H34	262.48	32.50	0.00	0.00	0.00	0.00	0.00	0
77	M32	Tridecanedial	C13H24O2	212.33	32.50	0.00	0.00	0.00	0.00	0.00	0
78	M8	3-vinyl-1-cyclooctene	C10H16	136.24	32.53	0.00	0.00	0.00	0.00	0.00	0
79	M25	Squalene	C30H50	410.73	33.12	0.33	0.00	0.83	0.96	0.92	0.71
80	M15	.Alpha. Tocopherol	C29H50O2	430.72	36.14	1.25	0.56	1.95	5.12	4.26	3.57
81	M47	ERGOST-5-EN-3-OL	C28H48O	400.69	37.39	1.37	0.00	0.00	1.74	0.00	0
82	M9	.gamma. -sitosterol	C29H50O	414.72	38.96	1.95	1.40	1.23	3.54	3.23	2.42
83	M41	3-Heptadecen-5-Yne	C17H30	234.43		2.02	0.00	0.00	0.00	0.00	0

Figure 1 – Biplot of 83 volatile compounds from the leaf samples of three chili genotypes at normal and less Irrigation Condition

Figure 2 – Graphic of the contribution of metabolite at PC1, PC2, and PC3

Figure 3 – Heatmap of 10 metabolite compounds from leaf samples of three chili genotypes at normal and drought stress conditions.

Figure 4 – The average concentration of metabolite compounds that show differences in chili leaf samples grown under normal and drought conditions.

Figure 5 – The linolenic acid content (a), Alpha Tocopherol (b) and Gamma sitosterol, (c) in the Gada MK, SSP and C10 genotypes under normal condition

Table 2 – The concentration of metabolites in Gada MK (tolerant), SSP (tolerant) and C10 (sensitive) genotypes under normal and drought conditions

Genotype	Metabolites	Average concentration (%)		Pr > t
		Normal (Sufficient Irrigation)	Drought (Less Irrigation)	
Gada MK	Linolenic acid	26.3	19.9	0.192
	Alpha-Tocopherol	0.6	4.3	0.001 **
	Gamma-sitosterol	1.4	3.2	0.009 **
SSP	Linolenic acid	24.1	19.5	0.233
	Alpha-Tocopherol	1.2	5.1	0.001 **
	Gamma-sitosterol	1.9	3.5	0.035 *
C10	Linolenic acid	34.9	20.9	0.004 **
	Alpha-Tocopherol	2.0	3.6	0.490
	Gamma-sitosterol	1.2	2.4	0.018 *

Note: * = significant at $\alpha = 0.05$; ** = very significant at $\alpha = 0.05$.

Figure 6 – Response in concentration changes (Δ) of linolenic acid, alpha-tocopherol, and gamma-sitosterol in each chili genotype due to drought condition

The results of our study concur with the findings of other studies that have reported a decrease in linolenic acid content (Pham Thi et al., 1987; Pham Thi et al., 1990; Repellin et al. 1997), an increase in phytosterol content (Kumar et al. 2015), and an increased in alpha-tocopherol content (Tanaka et al., 1990; Moran et al., 1994; Price et al., 1989; Bartoli et al., 1999; Munn'e-Bosch et al., 1999; Munn'e-Bosch and Alegre, 2001) in plants response to drought stress.

Pham Thi et al. (1990) and Repellin et al. (1997) stated that tolerant plants tend to contain less linolenic acid than sensitive plants. Cowpea (*Vigna unguiculata*) and cotton (*Gossypium hirsutum*) which are tolerant to drought contain smaller amounts of polar lipids and MGDG and higher saturation levels of MGDG because the content of linolenic acid is less than sensitive plants at normal conditions (Pham Thi et al. 1990). This previous study similar to our study, where linolenic acid content in sensitive genotype (C10) is higher than in tolerant genotypes (Gada and SSP). C10 genotype contains the highest, and significantly different linolenic acid content compared to Gada and SSP genotypes at normal condition.

The previous study shows that the percentage of linolenic acid in the drought-susceptible variety of cowpea and cotton significantly decreases when exposed to drought, while in the drought-tolerant variety it is only slightly decreasing or does not vary (Pham Thi et al., 1990). In accordance with the previous results, in this study, there was a significant

decrease in linolenic acid content in sensitive genotype (C10) when exposed to drought, whereas in resistant genotypes (Gada MK and SSP) there was no significant decrease in linolenic acid content (Table 2.)

The increase in gamma-sitosterol as one of the phytosterols in chili plants exposed to drought in this study is similar to some previous studies in other plants. Kumar et al. (2015) reported that in rice plants exposed to drought stress there was an increase in phytosterol, and the tolerant cultivars ability to survive in drought condition are due to its better ability to accumulate phytosterols rather than susceptible cultivars. In this study gamma-sitosterol content were significantly increased in the three genotypes when exposed to drought, but it seems that tolerant genotypes (Gada MK and SSP) accumulate gamma-sitosterol better than sensitive genotype (C10) (Figure 6.)

Studies in different plants have shown a positive relationship between biosynthesis/tocopherol accumulation and water stress (Munn'e-Bosch et al., 1999; Munn'e-Bosch and Alegre, 2003). Some Reports have shown a remarkable increase in alpha-tocopherol in conditions of water shortages in pea (Tanaka et al., 1990; Moran et al., 1994), wheat and cereals (Price et al., 1989; Bartoli et al., 1999), rosemary (Munn'e-Bosch et al., 1999), and lavender (Munn'e-Bosch and Alegre, 2001). In accordance with the results of these studies, our study also showed an increase in alpha-tocopherol content in chili plants at conditions of water shortages.

In a recent report, Kumar et al. (2013) observed that alpha-tocopherol-enriched transgenic *Brassica juncea* plants constitutively overexpressing the γ -TMT gene showed enhanced tolerance to drought stress compared to wild-type plants. Hasanuzzaman et al. (2014) stated that the elevated level of alpha-tocopherol might enhance the photosynthetic efficiency and lower lipid peroxidation leading to better oxidative protection. According to this statement, in this study, there was a very significant increased of alpha-tocopherol content in tolerant genotypes (Gada MK and SSP) (Table 2. and figure 6.) and only slightly increased of this metabolite in the sensitive genotype (C10).

From this study, it was revealed that maintaining linolenic acid contents and increasing alpha-tocopherol and gamma-sitosterol recorded in Gada MK and SSP genotypes were a mechanism for both genotypes to respond and adapt to the drought stress condition.

CONCLUSION

Our study showed evidence of drought stress responses in chili pepper, as indicated by a decreased level of linolenic acid, and increased levels of alpha-tocopherol and gamma-sitosterol. The study displayed differences in the levels of linolenic acid, alpha-tocopherol, and gamma-sitosterol in response to drought stress, and this was dependent on genotype.

Genotypes that are tolerant to drought stress (Gada and SSP) produce a low level of linolenic acid in normal condition and have not significantly decreased in content when exposed to drought. The tolerant genotypes produce high gamma sitosterol and alpha-tocopherol in normal condition and also had a significant increase in content when exposed to drought.

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