

MULTI-OMICS OF GENUS *SOLANUM* L. TRICHOMES REVEALS THE INTERSECTION BETWEEN STRESS MEMORY AND THE MOLECULAR PATHWAYS FOR RECOVERY

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Trichomes are cosmopolitan biological textures originating from the aerial epidermis, which render as a superior model to investigate plant differentiation at the cell level. The main function of these epidermal protuberances is protection from abiotic and biotic stresses. Trichomes are classified to glandular and non-glandular types. Glandular trichomes are biochemical factories and storage compartments for a diverse array of specialized metabolites. However, little knowledge is available about the plant machinery to coordinate trichome formation, metabolism regulation, and stress resistance. In the present study, trichomes are proved to be the key link between stress memory and the molecular mechanism of Solanaceous species for recovery. Scanning with electron microscope "SEM" confirmed the distribution of four glandular and six non-glandular trichome types between the studied *Solanum* species. Multi-omics analysis including genomic, transcriptomic, and metabolomic studies revealed that *Solanum* species, which contain approximately equal density and same types of trichomes are closer genetically to each other. Several stress-responsive *cis*-elements were identified through in-silico analysis of the promoters of *MIXTA*, *MIXTA-like1* and *MIXTA-like2* the key genes of trichome formation. The transcriptomic results demonstrated that all genes were differentially expressed in the studied *Solanum* species due to trichome types and density according to its tolerance to stresses.

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This report shed light into endemism of *Solanum* species in their defined zones.

Keywords: trichomes, environmental challenges, stress memory, molecular pathways, *MIXTA* transcription factors, endemism, *Solanum* species

Trichomes are cosmopolitan biological textures originating from the surfaces of the leaf and stem, which render as a superior model to investigate plant differentiation at the cell level (Yang et al., 2011). Generally, these epidermal structures can be classified into glandular or non-glandular trichomes (Kim et al., 2012). These epidermal outgrowths have generally been considered as agro-trait of little worth, due to its negative sides including the lower preference of farmers and the causing factor for allergy. However, the trichomes have proved to be directly or indirectly involved in the plant protection from drought (Ewas et al., 2016), high salinity (Skaltsa et al., 1994), ultraviolet radiation (Espigares and Peco, 1995), high temperature (Thomas et al., 1992), cold (Dhawan et al., 2016), herbivores, and pathogen attacks (Ewas et al., 2017) along with heavy metals (Ager et al., 2003). Epidermal outgrowths of plants have various complex forms and functions, which can be present individually or in combination on surface of the same plant (Glover and Martin, 2000). The microscopic characteristics of trichomes are important tools in comparative systematics to shed light into the evolutionary relationships between and within plant species (Theobald et al., 1979). Plants frequently are covered with different types of trichomes that vary among species. Taxonomic characters of specific trichome types have been scrutinized in various plant species, in particular blossoming plants such as snapdragon (Dudareva et al., 2005), eggplant (*Solanum melongena*) (Frary et al., 2003), potato (*Solanum tuberosum*) (Plaisted et al., 1992), tomato (*Solanum lycopersicum*) (Levin, 1973 and Ewas et al., 2017), and *Arabidopsis* (Valverde et al., 2004).

Sundry different types of trichomes are present on the surface of hypocotyls, leaves, stems, floral organs, and fruits of a single tomato plant (*Solanum lycopersicum*) (Kang et al., 2010). Taxonomic survey of *Solanum lycopersicum* identified four morphologically distinguished glandular trichomes: type I trichomes described by a multicellular base and a tall multicellular stalk (~2 mm) with a small glandular end; shorter IV type (~0.3 mm) that have a unicellular base, shorter multi-cellular stalk than type I trichomes with a tiny glandular tip; type VI consisting of four-celled glandular tip above short multi-cellular stalk (~0.1 mm); and type VII trichomes containing an irregularly shaped 4 to 8 cell gland on a short unicellular stalk (< 0.05 mm). Both of types II and III are identical in length approximately (0.2–1.0 mm), but vary in multicellular and unicellular base presence, respectively, while type V trichomes characterized by a unicellular base shorter stalk (0.1–0.3 mm)

(Luckwill, 1943). There is a vast diversity of trichome customs and chemical makeup inside *Solanum* species (Schillmiller et al., 2008).

The genus *Solanum* is one of the most important economic value genera among the Angiosperms, while a few studies were offered in crop genetics and molecular biology (Oh et al., 2008; Yoon et al., 2008; Kim et al., 2010 and Yeom et al., 2011). Due to the association of diverse stress resistances with trichome-forming phenotype, molecular investigations were carried out on trichome-formation in pepper and tomato (Kim et al., 2011; Kim et al., 2012; Ewas et al., 2016 and 2017). R2R3 genes that encoding the MIXTA-like MYB transcription factors are responsible for establishing the conical cells in the petals of Angiosperms (Noda et al., 1994) and are distinctly possible responsible for regulating trichome formation in different species (Gilding and Marks, 2010). These pursuits involve biochemical functions in organizing cuticle formation (Oshima et al., 2013), which impact responses to various environmental stresses (Gilding and Marks, 2010). Association of trichome formation and density with resistance to tomato mosaic virus (TMV) (Ewas et al., 2017) and pepper mottle virus (PepMoV) (Kim et al., 2011) was examined. However, the classification of trichome development due to environmental stresses was not reported in the genus *Solanum* L. (Kim et al., 2012). In the present study, electron microscope was used to observe trichome types on the epidermal surface of five *Solanum* species grown under normal and drought stress conditions. Also, the expression level of trichomes-key regulatory genes was examined to explore the relationship between plant stress memory and trichome formation.

MATERIALS AND METHODS

1. Plant Materials and Growth Conditions

The present study was carried out on five *Solanum* species including *S. lycopersicum*, *S. tuberosum*, *S. melongena*, *S. nigrum* and *S. incanum*. The taxonomic position and source of the studied species are shown in table (1). All plant species were grown in a greenhouse under a 12 h light/12 h dark regime ($180 \text{ mmol m}^{-2} \text{ s}^{-1}$ light intensity) with a regulated temperature of between 25–28°C (Qian et al., 2015). One-month-old seedlings were used as the non-treatment control, R0, after that all plants were exposed to air-drying at 28°C for 80 min (approximate water loss to 45%; used as the first drought stress treatment, S1) and then all plants were well re-watered for one day (as the first re-watering treatment, R1). Then, half of the R1 plants were grown under normal condition (as the no-stress memory treatment control, C). Two or three cycles of drought and re-watering treatments were applied to improve the stress memory.

Table (1). The taxonomic position of the studied species according to D'Arcy (1972, 1991) as well as its distribution in Egypt.

Species	Plant material	Source
<i>S. incanum</i> L. (= <i>Solanum bojeri</i> Dunal, <i>S. sanctum</i> L.)	Seeds and leaves	Gebel Elba and the surrounding mountainous regions, Egypt
<i>S. nigrum</i> L. (= <i>Solanum humile</i> Lam.)	Seeds and leaves	The Nile region including the delta, Egypt
<i>S. melongena</i> L. var. <i>esculenta</i>	Seeds	Vegetable Crops Department, Faculty of Agriculture, Alexandria University, Egypt
<i>S. lycopersicum</i> L. var. Ailsa Craig (AC)	Seeds	Vegetable Department, Faculty of Horticulture, Huazhong Agriculture University, China
<i>S. tuberosum</i> L. cv. E-potato 3, (E3)	Tubers	Vegetable Department, Faculty of Horticulture, Huazhong Agriculture University, China

2. FSEM Observation

The third leaf from the top was collected from the wild species (*S. nigrum* and *S. incanum*) and the two-month-old cultivated species (*S. lycopersicum*, *S. tuberosum* and *S. melongena*). Leaves were cut into ~0.1 cm² pieces then fixed with 2% glutaraldehyde for roughly 24 h. All samples then were washed in cacodylate buffer 0.1 mol/L, followed by dehydration in a graded ethanol series, and then dried in a desiccator (HCP-2; Hitachi). The dried samples were coated with a film of gold. Electron microscope model JSM-6390/LV was used for scanning (Yang et al., 2011).

3. Measurement of Drought-Stress-Related Biochemical Parameters

Drought-stress-related biochemical markers were analyzed in the studied species after exposing to different drought stress memories and well-watered treatments. The relative water content was estimated at the four-leaf stage (as drought stress treatment was initiated). Proline and soluble sugars and malondialdehyde (MDA) contents were measured with three replicates according to the previous methods described by Bates et al. (1973) and Orozco and Ryan (1999).

4. Expression Analyses

Total RNA was extracted using Trizol reagent (Invitrogen), while the first-strand cDNA was synthesized using 200 U of M-MLV reverse transcriptase (Invitrogen) and 3 µg of RNA according to the producer's protocol. Real-time PCR was carried out on an optical 96-well plate using an AB StepOnePlus PCR system (Applied Biosystems) by using SYBR Premix Reagent F-415 (Thermo Scientific). Also, actin gene was used as an internal control, which was amplified with 24 cycles. Gene expression level was

calculated using a relative quantification method described by Schmittgen and Livak (2008). All primers used in this study are shown in supplemental table (S1).

RESULTS

1. Taxonomic Survey of Trichome Types and Density in Five Different *Solanum* Species

Field Emission Scanning Electron Microscopy (FESEM) was used for trichomes morphology examination of detached leaves from five well-watered *Solanum* species: *S. lycopersicum*, *S. tuberosum*, *S. melongena*, *S. nigrum* and *S. incanum*. FESEM observation recognized 10 distinct types of trichome on the epidermis of leaves and illustrated in detail in table (2) and Fig. (1). Furthermore, SEM revealed that density of glandular and non-glandular trichomes was much higher in the leaves of *S. nigrum*, *S. incanum* and *S. melongena*, respectively, than those in leaves of *S. lycopersicum* and *S. tuberosum*, particularly the trichome type I, that previously described had a small glandular end (Table 2 and Fig. 1). Moreover, all trichome types in *S. melongena* (Fig. 1q, r, s, t, u) and *S. incanum* leaves were shorter than those of *S. nigrum*, *S. lycopersicum* and *S. tuberosum* (Fig. 1v, w, x, y, z).

Table 2. Distribution of trichome types in the leaves of five studied *Solanum* species.

Trichome type	I	II	III	IV	V	VI	VII	VIII	III-like-A	III-like-B
<i>Solanum species</i>										
<i>S. lycopersicum</i>	+		+	+	+	+			+	+
<i>S. tuberosum</i>	+		+	+	+		+	+		
<i>S. melongena</i>	+	+	+	+	+	+	+			
<i>S. nigrum</i>	+	+	+	+	+	+	+	+		
<i>S. incanum</i>	+	+	+	+	+	+	+	+		

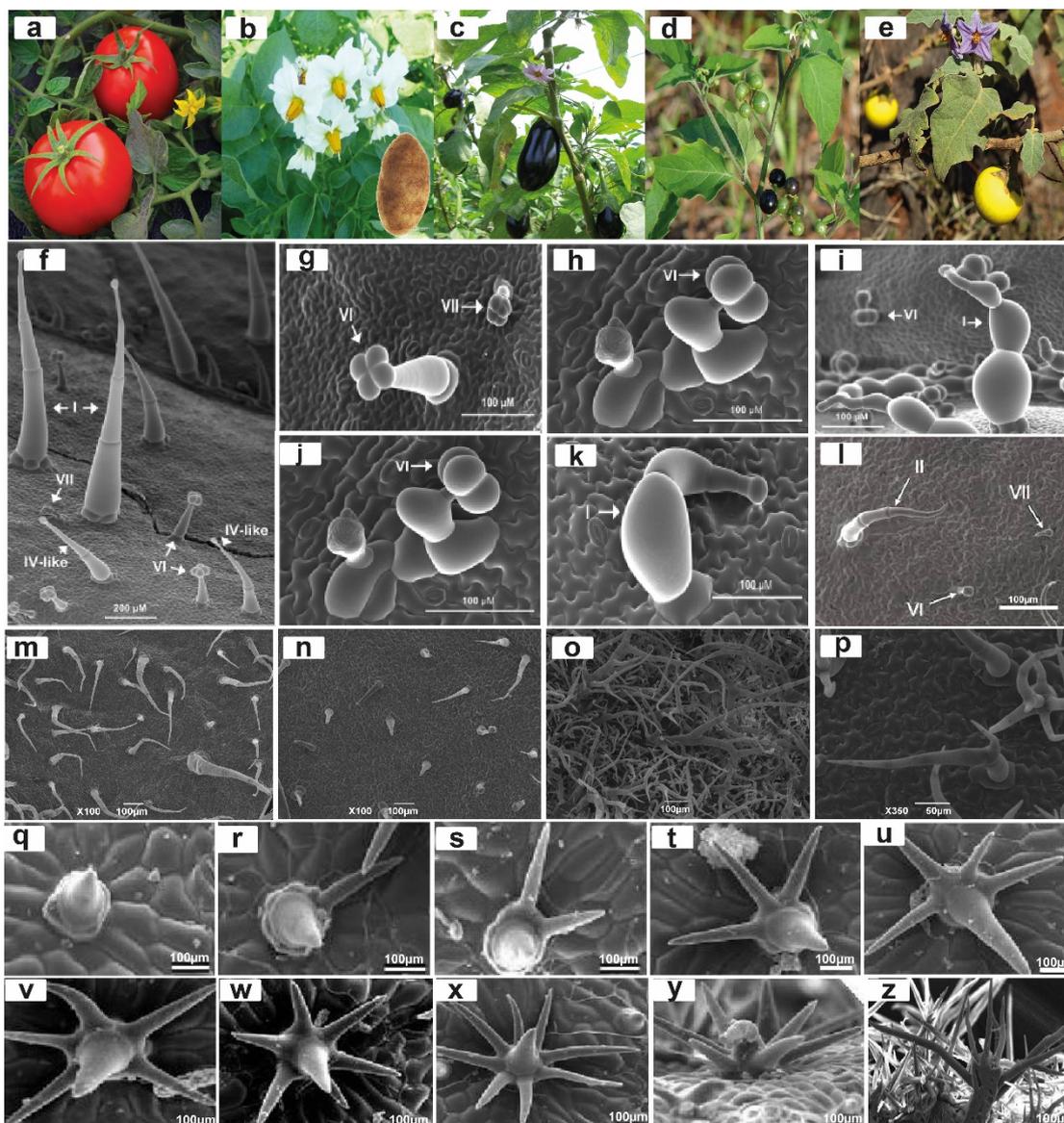


Fig. (1). Initial morphological characterization of investigated *Solanum* species. Phenotype of (a) *Solanum lycopersicum*; (b) *Solanum tuberosum*; (c) *Solanum melongena*; (d) *Solanum nigrum* and (e) *Solanum incanum*. (f-z) trichome types of studied *Solanum* species including glandular and non-glandular.

2. Seedlings of *Solanum* Species Display Drought Stress Memory Under Appropriate Water Deficient Stress

To explore whether *Solanum* genus exhibit a drought memory impact, the RWC of the five *Solanum* species subjected to multiple “drought training treatments” were estimated. The relative water content was reduced as air-drying time prolonged (0-80 min) for all treatment combinations. No significant differences were recorded in RWC between all stress memories treatments of all *Solanum* species at 0 min (Fig. 2a, b, c, d, e). The RWC of S1 plants distinctly dropped to 51.54, 48.11, 60.21, 59.71 and 61.42% in leaves of *S. lycopersicum*, *S. tuberosum*, *S. melongena*, *S. nigrum* and *S. incanum*, respectively, after 80 min of air drying (Fig. 2a, b, c, d, e). However, the RWC rates of S2-S4 plants were significantly higher than those of S1 plants, and the water content loss was markedly slower after more than two treatments. The significant changes in water loss during cycles of drought treatment indicated that all examined *Solanum* species showed drought memory after cycles of drought training.

3 Primary Physiological and Metabolic Parameters are Affected During Drought Memory

Proline, soluble sugars and MDA have been considered to be critical indicators of drought stress. To confirm the relation between drought stress memory and trichomes formation in *Solanum* species, proline, MDA and soluble sugars contents were measured after drought stress and re-watered treatments in the leaves of the studied *Solanum* species. Significant increases in proline values were recorded after drought stress memories S1 up to 2700, 2500, 3200, 3100 and 3500 in leaves of *S. lycopersicum*, *S. tuberosum*, *S. melongena*, *S. nigrum* and *S. incanum*, respectively. Notably, the increase in proline values of first stress memory S1 was much higher than those of S2 and S3 in leaves of all investigated *Solanum* species, except proline value of *S. incanum* and *S. nigrum* after stress memory S3, which was slightly higher and lower, respectively, than those values after stress memory S1 (Fig. 3a).

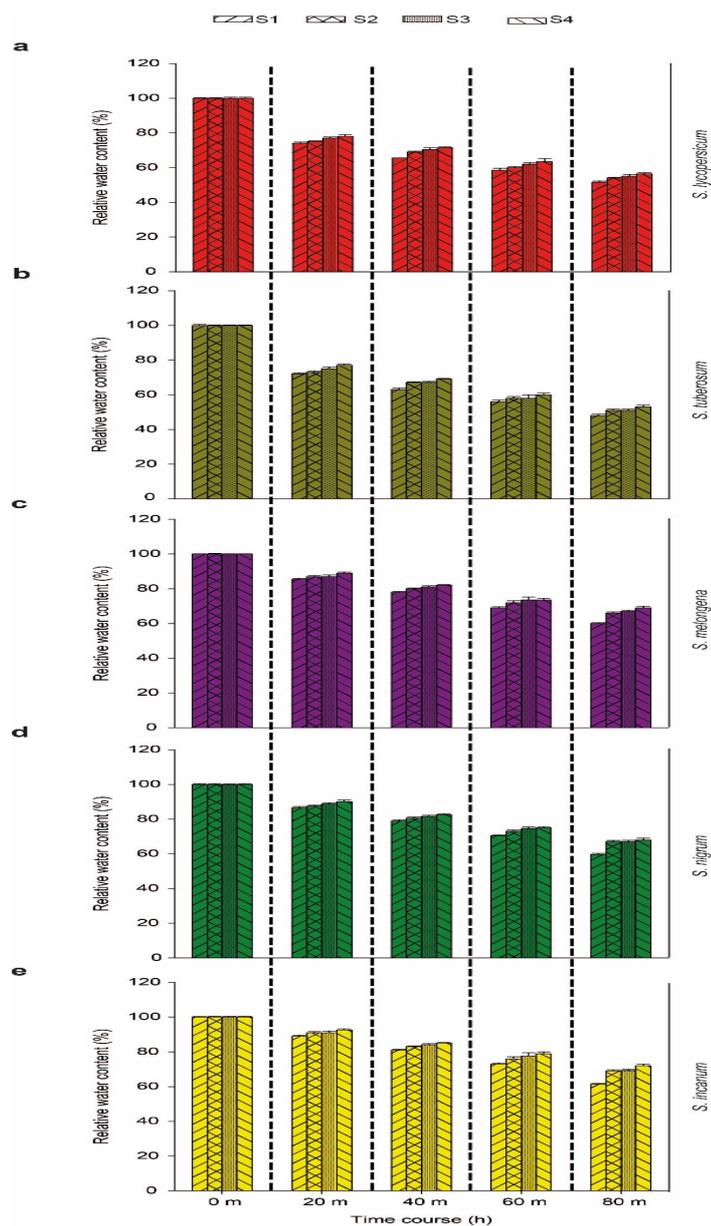


Fig. (2). Plant response parameters during drought training treatment. Relative water content (RWC) of the sampled leaves of *Solanum lycopersicum* (a); *Solanum tuberosum* (b); *Solanum melongena* (c); *Solanum nigrum* (d) *Solanum incanum* (e), after air drying for the indicated times. Values are the mean \pm SD (n = 8).

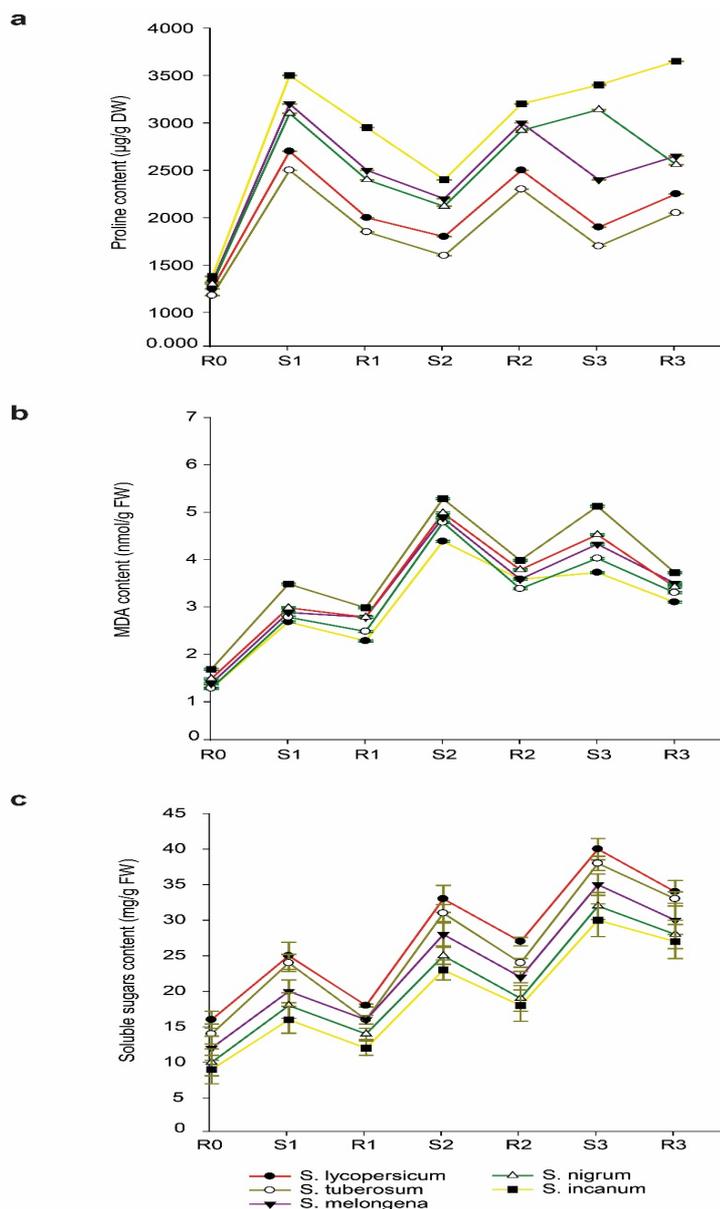


Fig. (3). Plant response parameters during drought training treatment the leaves of investigated *Solanum* species. **(a)** Proline content; **(b)** MDA content and **(c)** Soluble sugars content. Data are means of three biological and error bars are \pm SE from three independent experiments, each performed with 6–8 leaves from five separate plants.

Interestingly, MDA content was associated with trichome density in the investigated *Solanum* species, this idea was confirmed by the MDA content results, which showed that MDA values were significantly higher in the leaves of *S. lycopersicum*, *S. tuberosum* and *S. melongena* than those of *S. incanum* and *S. nigrum* after all stress memories.

Surprisingly, the accumulation of MDA after the third drought stress cycle was lower than those of the first and second cycles. On the other hand, a gradual increase in MDA content was correlated with the number of stress memories, especially stress memory S3 of all studied species (Fig. 3b).

Consistent with proline results, a marked increase of soluble sugars content was recorded in leaves of all investigated species after all drought stress memories. The increase in soluble sugars content ranged between 25-40, 24-38, 20-35, 18-32 and 16-30 mg/g FW in the leaves of *S. lycopersicum*, *S. tuberosum* and *S. melongena*, *S. nigrum* and *S. incanum*, respectively, (Fig. 3c).

4. Genomic Detection of Regulatory Transcription Factors of Trichome Formation in *Solanum* Species

Genomic sequences of *MIXTA*, *MIXTA-like1* and *MIXTA-like2*, the key genes in trichome formation and development were used to design specific primers (forward and reverse) of these genes for detection. The transient analysis indicated the presence of trichome regulatory genes in the genomic DNA of all investigated species including *S. lycopersicum*, *S. tuberosum* and *S. melongena*, *S. nigrum* and *S. incanum* (Fig. 4a, b, c). Notably, the bands of the regulatory genes of trichome formation were obtained at the specific molecular weight that previously reported in different studies.

5. Transcripts Related to Trichome Formation Could Play Important Functions in *Solanum* Species Drought Memory

Many metabolic pathways, in addition to trichomes on the aerial epidermis and hairy roots, play a vital role in plant stress resistance (Ewas et al., 2017 and Li et al., 2019). To explore the cross-link between trichomes and drought stress memory formation in *Solanum* species, expression level was measured for three transcription factors related to trichome formation after three cycles of drought stress.

SiMIXTA, *StMIXTA*, *SmMIXTA*, *SnMIXTA* and *SiMIXTA* were assessed in the leaves of *S. lycopersicum*, *S. tuberosum*, *S. melongena*, *S. nigrum* and *S. incanum*, respectively, after three drought stress memories. Water deprivation strongly induced expression level of these genes after first stress memory S1 compared to their transcripts after re-watered R1, while it was slightly expressed after both of stress memories S2 and S3 in comparison with R2 and R3 (Fig. 5a, b, c, d, e). On the other hand, oscillated expression was recorded in the transcription level of *SiMIXTA-like1*, *StMIXTA-like1*, *SmMIXTA-like1*, *SnMIXTA-like1* and *SiMIXTA-like1*, while retaining the

highest gene expression after the first drought stress memory S1 compared to S2 and S3 (Fig. 5f, g, h, i, j).

Interestingly, the expression level of *SMIXTA-like2*, *StMIXTA-like2*, *SmMIXTA-like2*, *SnMIXTA-like2* and *SiMIXTA-like2* was gradually increased, especially after dehydration memories S1 and S2 (Fig 5k, l, m, n, o). Furthermore, the bioinformatics analysis of promoter regions for *MIXTA*, *MIXTA-like1* and *MIXTA-like2* genes also revealed presence of some drought stress-responsive elements such as DRE2-motif, DBF2-motif, MYC-motif, ABRE, MYB-motif, MYC-motif, NAC-motif, bZIP-motif and TC-rich elements (Table 3).

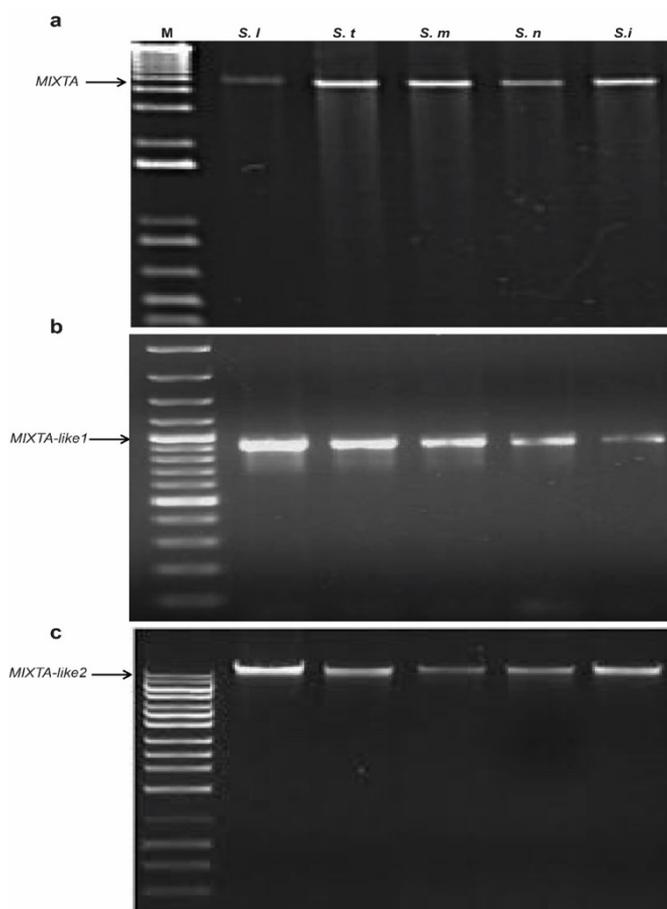


Fig. (4). Genome detection of the key genes involved in trichome formation. *MIXTA* (a); *MIXTA-like1* (b); and *MIXTA-like2* (c). DNA was extracted from three biological replicates of each species and detection was carried out using PCR.

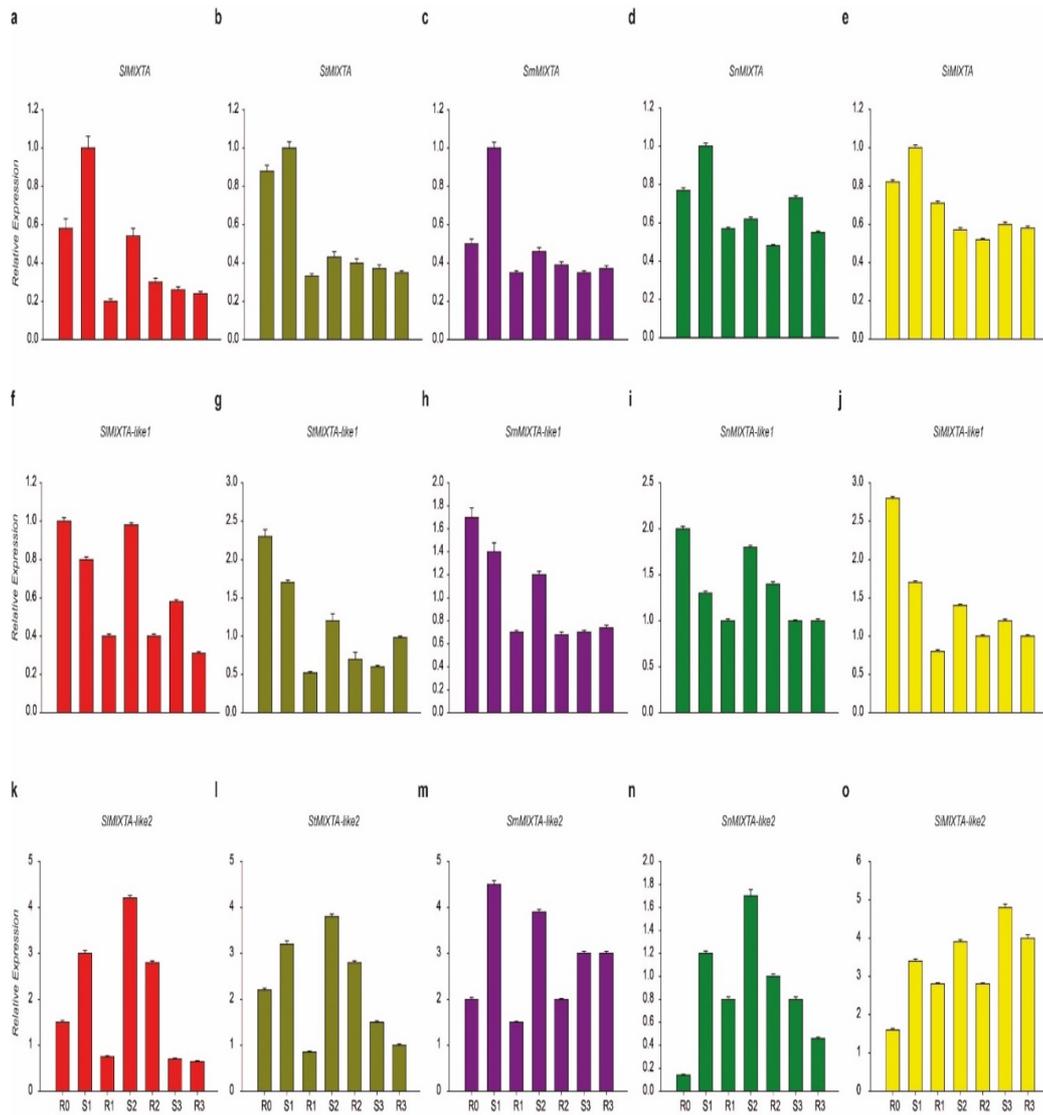


Fig. (5). Real-time quantitative PCR analysis for the transcripts of trichome formation genes involved in stress memory during the drought stress cycles. Actin was used as an internal control. Data are means of three biological replicates and error bars are \pm SE from three independent experiments, each performed with 6–8 leaves from five separate plants.

Table (3). *Cis*-regulatory elements responsive to drought stress, salinity stress, osmotic stress and ABA in the promoter regions of *MIXTA*, *MIXTA-like1* and *MIXTA-like2*.

Pattern	Description	<i>MIXTA</i>	<i>MIXTA-like1</i>	<i>MIXTA-like2</i>
ACGT	This sequence required for etiolation-induced expression of <i>erd1</i> .	+	+	+
ACCGAC	DBF2" bound to "DRE2"; <i>rab17</i> is expressed during late embryogenesis, and is induced by ABA.	+	-	+
WAACCA	MYB recognition site found in the promoters of the dehydration-responsive gene <i>rd22</i> .	-	+	-
CANNTG	MYC recognition site found in the promoters of the dehydration-responsive gene <i>rd22</i> and many other genes in <i>Arabidopsis</i> . Binding site of ATMYC2.	+	+	-
ACGTGKC	DRE and ABRE are interdependent in the ABA-responsive expression of the <i>rd29A</i> .	-	+	+
YAACKG	MYB recognition site found in the promoter of the dehydration-responsive gene <i>rd22</i> .	-	+	-
RYACGTGGYR	ABRE in <i>Arabidopsis</i> dehydration-responsive gene <i>rd22</i> .		+	
CCACGTGG	ABRE; ABA and water-stress responses.	+	+	+
YACGTGGC	ABA responsive element found in the promoter of stress regulated.	+	+	+
CACATG	MYC binding site in <i>rd22</i> gene of <i>Arabidopsis thaliana</i> ; ABA-induction.		+	+
CATGTG	MYC recognition sequence necessary for expression of <i>erd1</i> in dehydrated <i>arabidopsis</i> .	-	-	+
CNGTTR	ATMYB2 is involved in regulation of genes that are responsive to water stress in <i>Arabidopsis</i> .	+	+	-
CCGAC	Core of low temperature responsive element (LTRE) of <i>cor15a</i> gene in <i>Arabidopsis</i> .	+	+	-
TACCGACAT	Related to responsiveness to drought, low temperature or high-salt stresses.	+		
TAACTG	AtMYB2 is involved in regulation of genes that are responsive to water stress in <i>Arabidopsis</i> .	+	+	+
CTAACCA	Binding site for MYB in dehydration-responsive gene, <i>rd22</i> .	+	+	+
WK[TAGC]CGTR	NAC binding site (NACBS).	+		+
NT CYC1	Trichome specific expression of the tobacco (<i>Nicotiana sylvestris</i>) cembratrien-ol synthase genes is controlled by both activating and repressing cis-regions.	+	+	

Table (3). Cont.

Ns CBTS-2a	Trichome specific expression of the snapdragon (<i>Anthurium majouras</i>).	+	-	-
Ns CBTS-2b	Trichome specific expression of the <i>Arabidopsis</i> (<i>Arabidopsis thaliana</i>).		+	
Ns CBTS-3	Trichome specific expression of the rice (<i>Oryza sativa</i>).	-	+	+
ACACNNG	Binding core bZIP, ABA response; related to trichome formation.	+	+	+
ACACTTG	Binding core bZIP, ABA response; related to trichome formation.	+	+	+

Note: The symbol W was used in addition to A or T; the symbol R was used in addition to A or G; the symbol Y was used in addition to C or T; the symbol K was used in addition to G or T; the symbol W was used in addition to T or A; and the symbol N was used in additional to A, C, G or T.

DISCUSSION

1. *Solanum* Species Show Distinctive Drought Memory Patterns

Plants that formerly exposed to abiotic stresses may alter their responses to posterior stresses (Barrett and Campbell, 2006; Harb et al., 2010; Ding et al., 2012, 2013, 2014). A recent study of a dryland genotype of *Solanum lycopersicum* (Summer-set) revealed a memory effect stimulated by drought pre-treatment (Li et al., 2019). Water deprivation can strongly induce the water loss in plant leaves (Osakabe et al., 2014). In order to reveal the ability of *Solanum* species memory, the relative water content was measured as air-drying time extended (0–80 min). The more delayed loss of water, during the equal periods of water-deficiency treatment cycles, might elucidate the increase of the water-retaining ability and it divulged a superior adaptive capability for drought. Stress memory often arises after a plant is exposed to several cycles of that stress. This idea was confirmed by the results of the current study on the relative water content in the leaves of the studied *Solanum* species after exposure to similar cycles of drought. These results are consistent with several previous studies on *Arabidopsis* (Ding et al., 2012), aptenia (Fleta-Soriano and Munne-Bosch, 2016) and rice (Li et al., 2019).

2. Proline and Soluble Sugars Could Contribute to Drought Memory Formation

It is well known that both of proline and soluble sugars are vital molecules for many biological processes within the plant, including protection from various environmental stresses (Valverde et al., 2004 and Ewas et al., 2017). One of these severe stresses is drought, which causes cell damage. Plants respond to drought stress by increasing the synthesis level of proline and soluble sugars, which in turn can scavenge reactive oxygen species as well

as serving as a molecular chaperone to sustain protein structure (Szabados and Savoure, 2010; Spoljarevic et al., 2011 and Auler et al., 2017). However, biochemical and molecular evidence was lacking in *Solanum* species concerning whether proline and soluble sugars play crucial functions in short-term drought memory formation. The impact of exposure to dehydration resulted in the accumulation of proline and soluble sugars in rice and *Arabidopsis* leaves (Hare and Cress, 1997 and Li et al., 2019). In the present study, levels of proline and soluble sugars were gradually accumulated as drought stress memory cycles increased to prevent the increasing effect of oxidative lipid (Malondialdehyde) in *Solanum* species. This accumulation was higher in the *Solanum* wild species leaves including *S. nigrum* and *S. incanum* than those of the cultivated species such as *S. melongena*, *S. lycopersicum* and *S. tuberosum*. This conclusion, in turn, explains the close relationship between the accumulation of these compounds in *Solanum* species and drought stress memory formation. This evidence is consistent with the results of previous studies by Hanna (2004) and Wu et al. (2014).

3. Trichome Development Signaling is Involved in Drought Memory Formation

The results of SEM in the present study confirmed the presence of ten types of trichomes, including glandular and non-glandular trichomes in leaves of the five studied *Solanum* species. Also, the survey indicated that trichomes density in the leaves of *Solanum* wild species, including *S. nigrum* and *S. incanum* is higher than those in cultivated species such as *S. lycopersicum* and *S. tuberosum*. Previous studies have confirmed a significant increase in trichome density in the leaves of dry and desert plants more than those of the relative cultivated species such as pepper (Kim et al., 2012), tomato (Galdon-Armero et al., 2018) and rice (Li et al., 2019). These results are consistent with the results of proline and soluble sugars accumulation after exposure to drought stress in the same *Solanum* species. The results of the bioinformatics analysis also confirmed the abundance of *cis*-regulatory elements responsive to drought stress in the promoter regions of *MIXTA*, *MIXTA-like1* and *MIXTA-like2*, referring to the close relationship between these genes and drought stress tolerance. Furthermore, induction in the expression level of *MIXTA*, *MIXTA-like1* and *MIXTA-like2* "the key genes of trichomes formation" after different drought stress treatments suggest the cross-talk between trichomes and stress memory formation in *Solanum* species. Interestingly, the transcription level of these genes was significantly higher in the leaves of wild species including *S. nigrum* and *S. incanum*, followed by *S. melongena* than those of cultivated species such as *S. lycopersicum* and *S. tuberosum*. The induction in transcription level of *MIXTA* genes under drought stress condition was also reported in other plant species, including *AmMIXTA1* from *Antirrhinum majus*, *PhMIXTA1* of *Petunia hybrida*, *AtMIXTA16*, *AtMIXTA17*, *AtMIXTA106* in *Arabidopsis thaliana*, *GhMIXTA25*, *GhMIXTA25-like* in

Gossypium hirsuta, DcMIXTA1 from *Dendrobium crumenatum*, MtMIXTA3 of *Medicago truncatula* and SIMIX1 from *Solanum lycopersicum* (Perez-Rodriguez et al., 2005; Baumann et al. 2007; Gilding and Marks, 2010; Pastore et al., 2011; Machado et al., 2009; Walford et al., 2011 and Ewas et al., 2016), respectively. These findings indicate the role of trichomes in drought stress tolerance in *Solanum* species. The idea of stress memory is associated with trichome density was confirmed previously in several desert species, including *Achillea fragrantissima*, *Seriphidiom herba-alba*, *Artemisia judaica*, *Launaea spinosa*, *Heliotropium arbainense*, *Moltkiopsis ciliata*, *Zilla spinosa*, *Cleome droserifolia*, *Convolvulus lanatus*, *Chrozophora obliqua*, *Acacia tortilis* subsp. *raddiana*, *Astragalus spinosus*, *Crotalaria aegyptiaca*, *Salvia deserti*, *Teucrium pilosum* and *Ochradenus baccatus*, *Lycium shawii* Roem and *Fagonia arabica* (Gamal, 1997).

CONCLUSION

This study provides a deep insight into drought stress memory formation in the studied *Solanum* species, which can be summarized by the presence of three distinctive mechanisms related to trichomes density in these species. The first is a physiological mechanism responsible for increasing trichomes density and this, in turn, reflects the sunlight and reduces the relative water loss by controlling stomatal closure. The second is a biochemical mechanism within the trichomes of these species. The current study reveals that trichomes of *Solanum* species are a prolific source of a vast number of metabolites. These metabolites are generated within the glandular and non-glandular trichomes by essential enzymes required in the biosynthesis of proline, soluble sugars, flavonoids and terpenoids. Regulation of the biosynthetic genes of these metabolites and key genes of trichome formation are associated with drought stress memory and this is the third mechanism (Johansen, 1940; Kaur and Goel, 2011; Pattanaik et al., 2014 and Ewas et al., 2016). This report shed light into endemism of *Solanum* species in their defined zones.

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Table (S1). Primer pairs used in this study for gene detection and expression analysis.

Experiment	Primer name	Primer Sequence
Gene detection	<i>MIXTA</i> -FW	TTCAGTCGACATGGGTCGATCTCCGTGT
	<i>MIXTA</i> -RV	TGGTCTAGAGGTCAGGCTTTCAGCACG
	<i>MIXTA-like1</i> -FW	GGTAGTATTTGAGAACTGGGCTCTCT
	<i>MIXTA-like1</i> -RV	ATATCTCGAGGCCGACCACCTATTCCC
	<i>MIXTA-like2</i> -FW	ATGGATTCTTTCTCTACTTCTTCACTTT
	<i>MIXTA-like2</i> -RV	TTACATTCTTGGTTTAATTTTAGCTCTT
Expression analysis	<i>MIXTA</i> -FW	GCGAGCGCTAGTGCTGGTAT
	<i>MIXTA</i> -RV	TAATATGTTGCGCATTTTCGAAA
	<i>MIXTA-like1</i> -FW	TTTCATTTTTCACAATTTCCGCC
	<i>MIXTA-like1</i> -RV	CTGGAGGTCCGGGAGGTAG
	<i>MIXTA-like2</i> -FW	TTAAAGGGTCCACGTGTTTGG
	<i>MIXTA-like2</i> -RV	CACGCGCGGAGATTATCTACT

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التحليل الجزيئي المتعدد المستويات للزغب في جنس السولانم يكشف التقاطع بين المعلوماتية الحيوية للضغوط المختلفة والمسارات الجزيئية للمقاومة

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يعد الزغب في النبات تراكيب بيولوجية فريدة تنشأ من السطح العلوي للبشرة كما أنها تمثل نموذج مثالي لدراسة التشفير على المستوى الخلوي، حيث تؤدي وظائف هامة لحماية النبات من الضغوط البيئية سواء الإحيائية أو اللاإحيائية. ويصنف الزغب في النباتات إلى أنواع غدية وأنواع غير غدية، حيث تمثل أنواع الزغب الغدية المصانع البيوكيميائية في النبات إضافة لكونها أماكن تخزين للعديد من مركبات الأيض الثانوية الهامة. ومع ذلك فإن الدراسات السابقة قليلة جداً عن الآلية الوراثية في النبات نحو تنسيق تشكيل ونشأة الزغب وكذلك علاقتها بالتنظيم الأيضي ومقاومة الظروف البيئية المختلفة. في حين تؤكد الدراسة الحالية على أن الزغب هو الرابط الرئيسي بين ذاكرة الإجهاد والآلية الجزيئية في تحمل أنواع السولانم للظروف البيئية المعاكسة. كما أكدت نتائج الفحص بالميكروسكوب الإلكتروني وجود أربعة أنواع من الزغب الغدية وستة أنواع غير غدية في أوراق أنواع السولانم محل الدراسة، سواء الأنواع البرية مثل *S. nigrum* and *S. incanum* أو الأنواع المنزرعة مثل *S. lycopersicum*, *S. tuberosum* and *S. melongena*. كما أكدت نتائج التحاليل المتعددة على المستوى الجزيئي والأيضي والتعبير الجيني إلى أن أنواع السولانم المحتوية على نسب متساوية من نفس أنواع الزغب كانت أكثر تقارباً وراثياً من بعضها. كذلك كشف تحليل المعلوماتية الحيوية عن وفرة عناصر النسخ الخاصة بالضغوط البيئية *cis-elements* في مناطق البادئات الخاصة بكلاً من جينات *MIXTA*, *MIXTA-like1* and *MIXTA-like2* وهي الجينات المسؤولة عن تشكيل ونشأة الزغب. كما أكدت النتائج على اختلاف التعبير الجيني لتلك الجينات في الأنواع محل الدراسة طبقاً لأنواع الزغب ووفرته في الأنواع المدروسة فيما يفسر ارتباط التعبير الجيني للجينات المسؤولة عن تكوين الزغب مع ذاكرة تحمل الإجهاد المائي. هذا ويسلط البحث الضوء على آلية توطن تلك الأنواع محل الدراسة في مناطقها البيئية المحددة.