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Short Communication: Trichomes plasticity in *Lallemantia royleana* under different stress

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Abstract. *Askary M, Talebi SE, Amini F, Sarabadani B. 2016. Trichomes plasticity in* Lallemantia royleana *under different stress. Nusantara Bioscience 8: 128-133. Lallemantia royleana* is an annual herb of Lamiaceae family, which naturally grows in various parts of Iran. This medicinal plant is used in traditional medicine. Resemble to many members of this family, the aerial parts of *L. royleana* were covered by different kinds of trichomes. In this study, effect of different concentrations of NaCl and nanoparticles of iron oxide were examined on the trichomes morphology as well as density. The finding results showed that two main groups of trichomes, glandular and non-glandular, were presented on outer side of epidermal cells. The ANOVA test showed significant variations in trichomes number among various treatments. The main types of glandular hairs were peltate and also capitate. The mentioned forms are mainly relevant to production and also secretion of essential oil. Nanoparticles of iron oxide had positive effects on creation of palate trichomes, while increment of salt concentrations, decreased formation of them as well as short-stalked capitate.

Keywords: Iron nanoparticles, *Lallemantia royleana*, trichomes morphology, salt

INTRODUCTION

Lallemantia Fisch & Mey. is a genus of family Labiatae which includes five species in Iran. All of these species distributed in different regions of the country(Rechinger 1982), while some members of it grow in other countries such as Afghanistan, China, India, Kazakhstan, Kyrgyzstan, Pakistan, Russia, Tajikistan, Turkmenistan, Uzbekistan, SW Asia, and Europe (Li and Hedge 1994). *Lallemantia royleana* (Benth. in Wall.) Benth. is one of these species that widely distributed in different regions of Iran (Jamzad 2012) and different vernacular names were proposed for it, such as Balangu or Balangu Shirazi (Naghibi et al. 2005). This aromatic herb is used in traditional medicine for a number of disorders. Studies confirmed that aerial parts of this plant have antibacterial properties and can be a suitable remedy for different skin and gastrointestinal diseases (Mahmood et al. 2013).

Plant hairs were defined as protuberances with a morphogenetic origin in epidermal cells, and that have relatively well-defined size parameters (Wagner et al. 2004). Two types of hairs, glandular and non-glandular, were seen in plants. Glandular trichomes can reserve, and sometimes synthesize secondary metabolic compound and also confiscate various components that are undesirable in the plants in solitary instruments. Previous investigations (Levin 1973; Wagner 1991) showed that 20-30% of all vascular plants lay significant resources in construction, retain as well as filling secretary hairs on the aerial parts of body.

Although iron is one of very important and necessary element for all plants (Rashno et al. 2013), its nutrition is confused by plants that grow in soils with high amount of calcium (Bakhtiari et al. 2015). In addition, Bakhtiari et al. (2015) suggested that nanoparticles of iron oxide are very useful in technological systems development that maximizing fertilizer as well as pesticide applications. For the mentioned reasons, in the present study, effects of different concentrations of NaCl and also nanoparticles of iron oxide were examined on the trichomes plasticity and density on the organs of *L. royleana*. We did not find any comprehensive examination on the effect of different salinity as well as iron nanoparticles treatments on trichomes density and morphology in the plant.

MATERIALS AND METHODS

Plant material was obtained from the seeds of the same native population of *L. royleana* from northeast of Iran during spring 2015. Plant samples were identified on the bases of descriptions have been provided in Flora Iranica (Rechinger 1982) as well as Flora of Iran (Jamzad 2012). These examinations were done in a growth chamber maintained at an air temperature of 20 and 25°C (in night/ day respectively), daily light and dark period throughout the duration of the experiment were14 and 10 hours, respectively. Treated plants were grown in 14 × 12 cm flowerpots that filled with soil and perlite (1:1). Then plants were treated using sodium chloride (NaCl, MW 58.44 g) and iron solutions (as iron oxide nanoparticles and also Fe-EDTA). In total fifteen solutions, combinations of four concentrations of NaCl (0, 50, 100, and 150 mM) associated with four concentrations of iron (0, 10 and 20 µm of Fe_2O_3 nanoparticles and normal iron-chelate), were used (Table 1). Irrigations were performed with 100ml of

complete Hoagland solution possessing Iron-chelate as Fe-EDTA or without iron-chelate and containing various concentrations of iron oxide nanoparticles and salt as Weekly. Control plants (under 0 µm/mM concentrations of iron nanoparticles and salt) didn't receive any treatment. The method of Prasad et al. (2012) was used to prepare various concentrations of nanoparticles of iron oxide.

At ninety days after planting, mature leaves were selected for trichomes study using light microscopy (LM). For this, the embedded plant samples were used. The plant material was fixed in F.A.A. solution (formalin 5%: acetic acid 5% and 90% ethanol) for 48hours. Transverse hand sections of the lamina were made from the mid-part of fully-grown leaves. The sections were double-stained using methyl blue and carmine and mounted on the slides using Canada balsam. All observations were carried out on an Olympus light microscope (CH_2, Japan) at a magnification of 10 and 20x. Trichome numbers per leaf sides were determined. Trichomes densities were expressed as the ratio between mean number of trichome per leaf pair and the corresponding leaf area. The mean, as well as standard deviation of the trichomes number per each treatment, were determined. One-way Analysis of variance test (ANOVA) was used to assess the significance of variations in trichomes number difference among various treatments. SPSS ver. 9 software was used for statistical analyses.

RESULTS AND DISCUSSION

Results

In this study, effects of different concentrations of iron nanoparticles (Fe_2O_3) as well as salt (NaCl) were examined on the trichomes morphology and density of *L. royleana*. In total ten kinds of hairs were identified that were classified into two main groups; glandular and non-glandular. Glandular trichomes were seen in four shapes; peltate, capitate, digitate and sessile, while, non-glandular ones were recorded in one, two, three, four, five and six-celled forms (Table 2).

Table 1. Various treatments using iron oxide nanoparticles (µm) and NaCl (mM)

Treatment	NaCl	Iron	Treatment	NaCl	Iron
1 (control)	0	0	9	100	5
2	0	5	10	100	10
3	0	10	11	100	20
4	0	20	12	150	0
5	50	0	13	150	5
6	50	5	14	150	10
7	50	20	15	150	20
8	100	0			

ANOVA test showed significant variations ($p \leq 0.01$) for all observed trichomes types among treatments except those of sessile, digitate and six-celled hairs (Table 3). The most abundant glandular and non-glandular hairs among treated plants were long-stalked capitate and simple two-celled trichomes, respectively.

Peltate hairs had a short basal cell with a disc-shaped head consisting of six to eight cells (Figure 1.A). The numbers of peltate hairs varied between treatments, the largest number (30) was recorded in treatment no.4, while the lowest (0) were seen in treatments 2, 6,8,12 and 15. Capitate hairs were occurred in the shapes of long/ short-stalked. These kinds of hairs have an uni- or bi-celled head with2-3 basal cells. The main differences of them were related to length of basal cells and absence or presence of neck cell. In the studied samples, the long-stalked hairs were more abundant in comparison to other ones (Figure 1.B). The largest (46) and the lowest (0) values of long-stalked trichomes were recorded in treatments no 12 and 8, respectively. This issue differed for short-stalked trichomes and the largest number (12) was observed in treatment no.3, but its smallest number (0) was found in treatment no. 10. Sessile glandular hairs were observed as one and bi-celled. Number of sessile glandular trichomes varied among treatments, therefore its largest amounts were registered in treatment no. 9; however there was not any trichome of this form in treatments no. 3, 7, 8 and 11. Digitate hairs were seen rarely and were occurred in treatment no. 6 and 10.

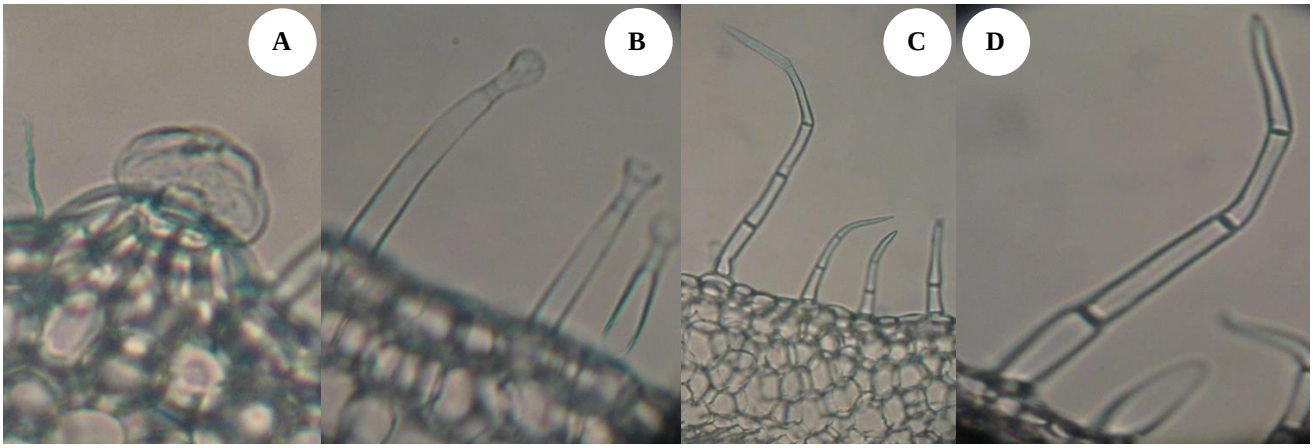

Figure 1. Light image of some trichomes kinds in the treated plants. A. Peltate, B. Long-stalked capitate, C. Left to right simple five, three and bi-celled hairs, D. Four-celled hair

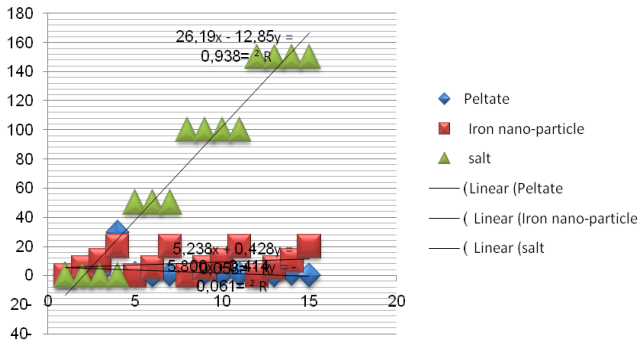

Figure 2. Variations in peltate trichomes numbers in the treated samples

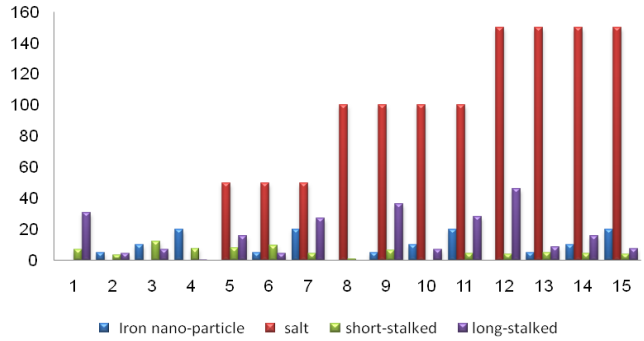

Figure 3. Comparison among treatments effects stalked- hairs numbers

Table 2. Average numbers of trichomes in treated samples

		One-celled	Two-celled	Three-celled	Four-celled	Five-celled	Short-stalked	Long-stalked	Sessile	Peltate
Control	Mean	2.00	33.33	48.33	12.33	4.33	7.00	30.66	1.66	.66
	N	3	3	3	3	3	3	3	3	3
	SD	1.73	8.50	1.26	5.13	4.50	0.00	9.29	0.57	0.57
2	Mean	2.33	20.33	17.00	2.33	1.00	8.00	15.66	1.33	1.00
	N	3	3	3	3	3	3	3	3	3
	SD	1.15	1.45	2.64	2.51	1.00	1.73	4.72	1.52	1.73
3	Mean	3.66	50.33	.00	.00	30.00	12.33	7.00	.00	1.00
	N	3	3	3	3	3	3	3	3	3
	SD	2.51	2.49	0.00	0.00	1.08	7.50	2.64	0.00	0.00
4	Mean	.66	6.33	14.00	.66	.00	7.33	.33	1.00	29.33
	N	3	3	3	3	3	3	3	3	3
	SD	.57	2.516	3.60	1.15	0.00	1.52	.57	1.00	3.05
5	Mean	1.33	1.00	6.33	9.33	1.66	3.33	4.66	1.00	0.00
	N	3	3	3	3	3	3	3	3	3
	SD	0.57	1.00	3.05	7.37	2.88	1.52	0.57	1.00	0.00
6	Mean	.33	8.66	2.00	1.33	.50	9.66	4.33	.66	.00
	N	3	3	3	3	2	3	3	3	3
	SD	.57	2.51	1.00	0.57	0.70	2.08	0.57	1.15	.00
7	Mean	8.00	32.33	3.66	.00	.00	4.33	27.00	.00	.66
	N	3	3	3	3	3	3	3	3	3
	SD	2.64	6.65	1.52	0.00	0.00	2.51	3.00	0.00	1.15
8	Mean	0.66	15.00	0.33	1.00	16.33	1.00	0.00	0.00	0.00
	N	3	3	3	3	3	3	3	3	3
	SD	1.15	3.46	0.57	1.00	2.08	1.00	0.00	0.000	0.00
9	Mean	2.66	9.33	16.33	7.33	3.00	6.66	36.33	2.66	1.66
	N	3	3	3	3	3	3	3	3	3
	SD	1.15	4.04	3.21	7.02	2.64	2.08	8.38	2.08	0.57
10	Mean	2.66	2.00	3.33	2.33	7.00	0.00	7.00	1.33	.66
	N	3	3	3	3	3	3	3	3	3
	SD	2.51	1.73	0.57	1.52	3.46	0.00	5.56	.57	.57
11	Mean	4.33	25.33	2.33	0.00	0.00	4.33	28.33	0.00	.66
	N	3	3	3	3	3	3	3	3	3
	SD	2.08	6.11	1.52	0.00	0.00	1.15	9.29	0.00	1.15
12	Mean	1.33	76.66	4.66	0.00	0.00	4.00	46.00	1.66	0.00
	N	3	3	3	3	3	3	3	3	3
	SD	2.30	1.64	3.78	0.00	0.00	2.00	9.64	1.15	0.00
13	Mean	2.33	2.00	3.66	4.66	.00	5.00	8.66	1.66	0.33
	N	3	3	3	3	3	3	3	3	3
	SD	0.57	2.64	2.08	1.52	0.00	1.73	2.88	1.52	0.57
14	Mean	1.66	3.33	10.66	3.66	1.00	4.33	15.66	.33	1.33
	N	3	3	3	3	3	3	3	3	3
	SD	0.57	1.52	2.51	2.08	1.00	0.57	5.68	0.57	0.57
15	Mean	0.33	5.00	22.66	0.00	4.66	4.00	7.33	.33	.33
	N	3	3	3	3	3	3	3	3	3
	SD	0.57	1.73	6.42	0.00	5.68	3.60	0.57	0.57	0.57

Table 3. ANOVA test results among various trichome types

Trichomes type		Sum of Squares	df	Mean Square	F	Sig.
Peltate	Between Groups	2323.244	14	165.946	146.423	.000
	Within Groups	34.000	30	1.133		
	Total	2357.244	44			
Short-stalked capitate	Between Groups	423.644	14	30.260	4.465	.000
	Within Groups	203.333	30	6.778		
	Total	626.978	44			
Long-stalked capitate	Between Groups	8576.133	14	612.581	20.541	.000
	Within Groups	894.667	30	29.822		
	Total	9470.800	44			
Sessile	Between Groups	27.644	14	1.975	1.975	.058
	Within Groups	30.000	30	1.000		
	Total	57.644	44			
Digitate	Between Groups	.000	1	.000	.000	1.000
	Within Groups	1.333	4	.333		
	Total	1.333	5			
Simple six-celled	Between Groups	.000	1	.000	.000	1.000
	Within Groups	1.333	4	.333		
	Total	1.333	5			
Simple Five-celled	Between Groups	2827.561	14	201.969	14.385	.000
	Within Groups	407.167	29	14.040		
	Total	3234.727	43			
Simple four-celled	Between Groups	622.000	14	44.429	4.503	.000
	Within Groups	296.000	30	9.867		
	Total	918.000	44			
Simple three-celled	Between Groups	6648.978	14	474.927	26.127	.000
	Within Groups	545.333	30	18.178		
	Total	7194.311	44			
Simple two-celled	Between Groups	19350.133	14	1382.152	15.655	.000
	Within Groups	2648.667	30	88.289		
	Total	21998.800	44			

All observed types of non-glandular trichomes were unbranched, while the cell number(s) of each kind varied between treatments. For example, highest as well as lowest amounts of three (Figure 1.C) and four-celled (Figure 1.D) simple hairs were found in treatments no. 1 and 3, respectively. In addition, six-celled simple hair was rarely recorded and was observed in no. 2 and 15. Paired T-test analyses showed significant differences between salt concentrations with all observed non-glandular trichomes number.

Significant negative/positive correlations were found between trichomes densities and salt and nanoparticles of iron oxide concentrations. For example, significant negative correlations were existed between iron nano-particles concentrations with simple four-celled ($p \leq 0.01$, $r = -0.39$) and sessile ($p \leq 0.05$, $r = -0.34$) trichomes, but this component had significant positive correlations with peltate ($p \leq 0.01$, $r = 0.40$) and also simple one-celled ($p \leq 0.05$, $r = 0.31$) ones (Figure 2). Furthermore, significant negative correlations were found between salt concentrations with peltate and short-stalked capitate hairs (Figure 3).

Discussion

L. royleana is an aromatic medicinal plant which widely distributed in different regions of central as well as northeast parts of the country. Looking like other members of family Labiatae for example, *Lagochilus*, *Ziziphora* (Talebi et al. 2012a) and *Stachys* (Rezakhanlou and Talebi 2010), *Acinos* (Talebi and Shayestehfar 2014), the aerial parts of this plant were covered by different types of trichome. These structures have various roles in plant. Different studies (e.g. Johnson 1975; Wagner et al. 2004; Simmons and Gurr 2005; Serna and Martin 2006) have confirmed that the functions of glandular and non-glandular hairs in plants are decrement of heat load, increment of plant tolerance to freezing, aid to dispersal of seeds and also propagules, enhance absorption of water, protect plant bodies from the harmful effects of UV-B, serve as insect repellent, and provide tools for protection plant aerial parts against different herbivores as well as pathogens.

It seems that physiological conditions of plant can affect on trichomes morphology as well as densities. Plants produce different types of secondary metabolite compounds under various environmental conditions and each of these components was stored in the specific structures. Therefore, on the basis of their amounts, different numbers of specialized structures were produced. This condition holds true for glandular trichomes. Glandular trichomes linked to creation, storage or secretion of essential oil and also other secondary metabolites. Based on the type of manufactured metabolite, the kind of created

trichomes varied. As Bini Maleci et al. (1983) suggested morphology of glandular trichomes is often connected to the secreted secondary product type.

Our findings confirmed that increment of salt concentrations decreased number of peltate trichomes, thus salt treatment had preventive effects on the formation of this type of trichomes. In contrast, the largest number of this kind of glandular trichomes occurred in treatment that received the highest value of iron oxide nanoparticles with no salt concentration. These conditions hold true for other plant species, for example, in *A. annua* and other glandular trichomes bearing plants relatively modest stress treatments significantly increased densities of glandular hairs (Gonzales et al. 2008; Maes et al. 2008; Liu et al. 2009). For this reason, presence of this component stimulated creation of peltate trichome. Previous investigations (for example, Bini Maleci et al. 1983; Werker et al. 1985b; Siebert 2004) confirmed that the peltate hairs are considered as the production and storage sites for essential oil and peltate trichomes accumulate essential oil in their structure. A recent study showed that the amount of produced essential oil varied under various treatments (Askary et al. 2016) therefore the frequencies of peltate hairs are depended on the amount of reserved essential oil.

Not only capitate trichomes differed from peltate ones in their morphology and structures, but also each of them contains different metabolic components. These types of glandular hairs contain mostly polysaccharides and only small essential oil amounts (Bini Maleci et al. 1983; Werker et al. 1985b). Capitate trichomes were seen in two shapes namely short and long-stalked and these forms had different manners. These conditions were seen in different plant species. The study of Werker et al. (1985b) showed that in capitate trichomes some features such as stalk length, head shape, and secretion process varied, therefore on the bases of the mentioned traits, capitate hairs can be subdivided into different kinds. Highest frequency of short-stalked ones was recorded in the treated plants with ten micrograms of iron oxide nanoparticles without any salt, while long-stalked trichomes were found in the treated samples, which contain highest amount of salt with no iron nanoparticles. Variations in the numbers of these kinds of trichomes are very important for plant, because each of mentioned trichomes corresponds to specific compounds that were made or accumulated in these hairs. The investigations of Werker et al. (1985a) confirmed our hypothesis. They stated that the different morphological structure of some capitate hair might link to the formation of the various compound. Investigations (for example, Askary et al. 2016) showed that amount, as well as type of essential oil compounds, differed in treated plants and for this reasons the kind and also numbers of essential oil storage tissue varied under plant treatment. Although the amounts and also composition of essential oils were not checked out in this study, trichomes investigations proved amounts of created essential oil, as well as their composition, will change between studied samples.

Non-glandular trichomes were seen in different shapes and found on the aerial parts of all studied treated plants, while some kinds of them were rarely observed. It seems that these kinds of trichomes have prominent roles in physical defense of plant against biotic as well as abiotic agents. Non-glandular trichomes do not have any storage valence, while, no popular trait can determine the structural diversity. These trichomes can emerge in different shapes as hairs, hooks, needles, plates, "umbrellas", etc. and have many different roles, such as desiccation or drench forbidding, help for scattering of seeds, insulation of cold and heat, herbivore inhibition. Perhaps the most prominent non-glandular trichomes to man are the hair-like trichomes on the cotton seeds (Levin 1973; Wagner et al. 2004).

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