



Effects of Potassium and Calcium Salts on Chlorophylls and Carotenoids Contents, and some Stomata Properties of *Vicia faba* L

Ikbal Muhammed Gharib AL-Barzinji¹ & Aroul Muhson Anwar¹

¹ Faculty of Science and Health- Koya University, Daniel Mitterrand Boulevard, Koya KOY45 AB64, Kurdistan Region - Iraq

E-mail: ikbal.tahir@koyauniversity.org

Article info	Abstract
Original: 30/10/2017 Revised: 29/12/2017 Accepted: 06/02/2018 Published online:	This study was carried out by using foliar spraying with two cations and two anions that where potassium chloride (KCl), potassium sulphate (K ₂ SO ₄), calcium chloride (CaCl ₂ .2H ₂ O), and calcium sulphate (CaSO ₄), in addition to the control plants that sprayed with tap water, two concentration of each treatment (5 and 7.5 g/l) were used to investigate their effects on chlorophylls, carotenoids contents and some stomata characteristics of <i>Vicia faba</i> L. var. Barcelona. The results show that KCl increased chlorophyll a, chlorophyll b and total carotenoids significantly compared to other treatments, except the CaSO ₄ treatment for chlorophyll b, whereas there were non-significant differences between salts concentrations on these pigments. Salts type had non-significant effects on stomata number on both of adaxial and abaxial leaves surfaces. Regarding stomata guard cell length in abaxial surfaces the differences between the treatments were non-significant, whereas in adaxial surfaces each of control and CaCl ₂ .2H ₂ O had the highest length compared to K ₂ SO ₄ treatment. Stomata guard cell width on abaxial surfaces increased significantly when CaSO ₄ salt was sprayed compared to the control and CaCl ₂ .2H ₂ O treatments, whereas the differences were non-significant in respect to adaxial leaves surfaces. There were non-significant differences between salt concentrations on the characteristics of stomata on adaxial and abaxial leaves surfaces.
Key Words: <i>Vicia faba</i> L. Potassium, Calcium, Chlorophyll, Carotenoids, Stomata.	

Introduction

Faba bean or broad bean (*Vicia faba* L.) is one of the most important Fabaceae crops in Iraq and other countries; its importance belongs to their rich protein seeds, in addition to its ability to fix atmosphere nitrogen by their root nodules and increasing soil fertility [1].

The problem of soil pollution as a result of the increase in using chemical fertilizers encouraged using foliar spraying. The latest is a practice commonly used by researchers and farmers to supplement fertilizer in the soil because foliar spraying are easy to implement and are relatively inexpensive, especially if foliar fertilizer applications are combined with pesticide applications [2]. Legumes, in general, require K and Ca elements through their growth in the field [3] and [4]. Potassium is required in large quantities by the plant, and it is present in the soil in the form of its various salts. Its adequate supply during growth period improves the water relations of plant and photosynthesis [5], maintains turgor pressure of cell which is necessary for cell expansion, helps in osmotic-regulation of plant cell, assists in opening and closing of stomata and activates many enzymes involved in respiration and photosynthesis [6]. Calcium is abundantly present in most of the soils both in the forms of its soluble as well as insoluble salts. It is required for the normal functioning of plant membranes and has been implicated as a second messenger for various plant responses to both environmental and hormonal signals [7]. Its ions (Ca²⁺) are used in the

synthesis of new cell walls, particularly the middle lamellae [6]. The low mobility of Ca in the plant [8] encouraged foliar spraying because it may be beneficial for correcting local deficiencies. Some studies have demonstrated that foliar spraying of calcium can be beneficial, even in soil with adequate levels of this nutrient [9]. Bai et al. (2008) [10] showed that foliar treatment with Ca-chloride and Ca-hydroxide in combination led to transient changes in the rate of photosynthesis and stomatal conductance of apple and bean leaves. Recently, Kraemer et al. (2009) [11] found that the rate of penetration of CaCl₂ was always higher than that of Ca-acetate. Another plant nutrient is sulfur which is present in the soil mostly as sulfates, it absorbed primarily as SO₄⁻² ions, and its translocation is both active and passive. Leaves can absorb appreciable amounts of SO₂ gas. Sulfur is constituent of various plant structures, mainly as a component of protein, activated sulfate occurs in compounds analogous to ATP. The element chlorine is present in plants either as free anions or as chloride. The deficiency of chloride is rare. Chlorine is essential for photosynthesis for Hill reaction, it is believed that chloride increases the cell acidity and accelerates enzyme action. Chloride foliar spraying has also been found to increase water and chlorophyll contents of plant tissues [12].

The aim of this study

To determine the effects of foliar spraying of some potassium and calcium salts and their companion ions on some photosynthesis pigments and stomata characteristics of broad bean grown in Koya city, Erbil, Iraq.

Materials and Methods

Plant material and treatments

The experiment was carried out at the agricultural research center in Koya city, Erbil, using *Vicia faba* L. var. Barcelona at the winter season 2013-2014. Seeds were planted on 15, October 2013. Table 1 shows some of the chemical and physical properties of the study soil. Foliar spraying with four different salts was applied by using potassium chloride (KCl), potassium sulphate (K₂SO₄), calcium chloride (CaCl₂.2H₂O) and calcium sulphate (CaSO₄), in addition to the control plants (sprayed with tap water). Two concentration (5 and 7.5 g/l) of each salt were used. The salts were sprayed until run-off point in two different times; the first was 50 days after planting, where the plants were small and still at the vegetative stage because of low temperature as it shown in table 2. The second does spraying was two weeks after the first. Each experimental unit was consisting of 20 plants. Some meteorological data in the field condition during the study were measured in Agro-Meteorological Station in Koya city as it shown in table 2.

Table-1: Some chemical and physical properties of the study soil.

Clay	Silt	Sand	Texture	HCO ₃	Na	Ca	Mg	K	P	N	O.M. (%)	EC (ds.m ⁻¹)	pH
%													
25.7	35.4	38.9	Loamy soil	11	74	129	1.46	55	4.3	0.91	0.93	0.68	7.43

Table-2: Maximum and minimum temperature, the relative humidity and the amount of rain fall during the study period.

Month	Air Temp. C ^o		Relative Humidity%	Rain fall (mm)
	Maximum	Minimum		
October 2013	28.32	18.77	30	1.5
November 2013	20.47	17.65	60	69.5
December 2013	10.26	5.81	61	117.7
January 2014	11.97	6.23	65	330.5

At the flowering stage the fifth leaf of five plants selected randomly from each experimental unit in order to measure the following:

- *Chlorophyll a, b and total carotenoids*: determined by weight fresh samples and extracted with acetone (80%). The extraction ratio was 1:50. The samples were grained using mortar and pestle and then the samples were filtered using filter paper. The supernatant was speared and concentration of the pigments was measured spectrophotometrically (721-2000 SPECTROPHOTOMETER, China). Three replicates were used for each treatment, and the amount of pigment present in each sample was calculated according to the following equations [13]:

- Chlorophyll a (Chla) [mg/l] = $11.75A_{662} - 2.35 A_{645}$
- Chlorophyll b (Chlb) [mg/l] = $18.61A_{645} - 3.96 A_{662}$
- Total Carotenoids (Car) [mg/l] = $1000 A_{470} - 2.27 Chla - 81.4 Chb/227$

Where:

A₆₆₂ absorbance at wavelength 662 nm

A₆₄₅ absorbance at wavelength 645 nm

A₄₇₀ absorbance at wavelength 470 nm

A₆₆₆ absorbance at wavelength 666 nm

A₆₅₃ absorbance at wavelength 653 nm

A₆₄₄ absorbance at wavelength 644 nm

For converting the concentrations from mg/l to mg/g fresh weight, each value multiplied by (extraction volume/ (sample weight*1000)).

- *Number of stomata and length, and width of stomatal guard cell in the adaxial and abaxial leaves surfaces*: The leaves that selected previously and kept in polythene bags were brought to the laboratory. The leaf epidermal peel slides were made by the methods of lasting impressions. In this method, at least one square centimeter on leaf surface was painted by a thick patch of clear nail polish. Allowed the nail polish to dry completely then taped a piece of clear cellophane tape to the dried nail polish patch by carton sealing tape. Peel out the nail polish patch gently by pulling the corner of the tape and the finger nail polish along with the leaf peel. This is the leaf impression which was taped on slides and labeled as abaxial and adaxial surfaces. Leaf impression was examined under 400x magnifications by light microscope. In order to determine numbers of stomatal guard cell length and width, 25 randomly selected stomata from five leaves were measured microscopically using an ocular micrometer [14].

Statistical analysis

A factorial experiment with randomized complete block design with 3 replicates was used for conducting the study. Data were subjected to analysis of variance using the SAS program. Means were compared by using Duncan's Multiple Range test at 5% level [15].

Results and Discussions

The results in table 3 show that foliar spraying with KCl increased chlorophyll a, chlorophyll b and total carotenoids significantly compared to other treatments, except CaSO₄ treatment for chlorophyll b. There were non-significant differences between salts concentration on the pigments. Regarding the interaction between salt types and concentrations, foliar spraying of 5g/l KCl increased chlorophyll a, b and total carotenoids compared to other treatments, whereas 5g/l K₂SO₄ gave the lowest values. From the results of table 3, it was clearly noticed that photosynthesis pigments were greatly affected by KCl salt, since, the salt KCl achieved the highest concentrations, of chlorophyll a and total carotenoids significantly compared to other treatments. In this respect, Shokr et al. (2014) [16] recorded same results, on snap bean leaves, were K foliar spraying gave the highest chlorophyll a, b and carotenoids significantly upon Ca salt which affects significantly upon the control treatment. The increasing of photosynthesis pigments in plants that sprayed with KCl may due to the soil of the study was low in K (Table 1) so the response was higher, where

Abuthahi and Younis (1988) [17] stated that Iraqi soils are characterized with high content of calcium carbonate and approximately alkaline pH, which is lead to more difficulty in realization of some nutrient elements from the soil as potassium, thereby available potassium becomes insufficient for plant growth [18]. Also foliar spraying with K and Cl salts that absorbed effectively as cations and anion may delay the synthesis of abscisic acid and promoted cytokinin activity [19], causing higher chlorophyll retention. In addition, the negative effect of foliar spraying of KCl with increasing the concentration (Table 3) on chlorophyll content may be attributed to the antagonism between K and Mg [20]. In this respect Kabesh et al., (1985) [21] observed that excess of K supply depress the uptake of Mg and this can induce Mg deficiency. Our results agree with Sarkar and Malik (2001) [4] who stated that the prevalence of K^+ in KNO_3 , increasing photosynthetic activity and effective translocation of assimilates to other plant parts, and the foliar spraying of KNO_3 at moderate rate of 0.5% proved more effective than lower (0.25%) and higher rate (1%) and all the rates of $Ca(NO_3)_2$ supplying equivalent amount of NO_3^- as in KNO_3 for grass pea. Also Srivastava (2010) [12] stated that chloride foliar spraying increase water and chlorophyll contents of plant tissues. However, many studies show that the foliar spraying of calcium is not always effective. The results obtained from this study agree with Rosolem et al. (1990) [22], who did not detect differences in the common bean plant with regard to the foliar spraying at the beginning bloom stage for different calcium sources and rates in terms of chlorophyll and carotenoids content.

Results in table 4 show that salts type had non-significant effects on stomata number on each of adaxial and abaxial surfaces. Regarding stomatal guard cell length in abaxial surface also there were non-significant differences between salts types, whereas each of control and $CaCl_2 \cdot 2H_2O$ treatments had the highest length compared to K_2SO_4 treatment in adaxial surfaces. The width of stomatal guard cell on abaxial surfaces increased significantly when $CaSO_4$ salt was sprayed compared to the control and $CaCl_2 \cdot 2H_2O$ treatments, whereas the differences were non-significant in respect to adaxial leaves surfaces. There were non-significant differences between salt concentrations on the characteristics of stomata on abaxial and adaxial leaves surfaces.

The interaction between salts types and their concentrations show non-significant differences between the treatments in respect to stomata number on abaxial and adaxial leaves surfaces. Foliar spraying of 7.5g/l $CaCl_2 \cdot 2H_2O$ increased stomatal guard cell length and width significantly to 26.18 and 23.56 micrometers compared to KCl treatment on abaxial and adaxial leaves surfaces. Stomatal guard cell width increased significantly when $CaSO_4$ sprayed by 5g/l significantly compared to most treatments especially 7.5 g/l $CaCl_2 \cdot 2H_2O$ and the control treatments, whereas non-significant differences between treatments were obtained for adaxial surfaces. It is clear in most treatments that increasing stomatal guard cell length due to decreasing in their width and decreasing in stomata opening as it shown in figure 1 and 2 in most treatments especially $CaCl_2 \cdot 2H_2O \times 7.5$ on abaxial leaves surfaces, which is agree with the results of Raschke and Schnabl (1978) [23] who show that guard cells of *V. faba* do not need to import anions for stomatal opening, but guard cells will import Cl^- (and possibly other small anions) if these ions are available, and it is believed that chloride increases the cell acidity and accelerates enzyme action. Chloride foliar spraying has also been found to increase water and chlorophyll contents of plant tissues [12]. Also each of chloride along with potassium participates in stomatal opening by moving from epidermal cells to guard cells to act as an osmotic solute that lead to water uptake into and a bowing apart of the guard cell pair [24].

The increase in stomata resistance in K salts treatments compared to Ca salts (table 4) may due to Iraqi soils are characterized with high content of calcium carbonate and approximately alkaline pH [17] as it clear in table 1 which is lead to more difficulty in realization of some nutrient elements from the soil as potassium, thereby, available potassium becomes insufficient for plant growth [18]. So K deficiency caused stomatal closure (increase in stomatal resistance) [25]. In sugar beet, Na caused stronger effects on stomatal closing and opening than K did [26]. This tendency was also observed by Tomemori et al. (2002) [27] suggesting that the turgor pressure was more efficiently kept by Na than by K and hence Na could maintain photosynthetic rate higher than K did in spinach, whereas in our experiment Ca salts had more effects on stomata resistant than K salts, the result of this study is agree with that reported by Humble and Raschke (1971) [28] stated

that calcium has a role in closing and opening stomata, in addition to potassium, whereas the results of the study did not agree with Rosolem et al. (1990) [22] who did not detect differences in the common bean plant with regard to the foliar spraying at the beginning bloom stage for different calcium sources stomata characteristics.

Conclusions

From the results obtained in this study it can be concluded that salts source have a significant effects on chlorophylls and total carotenoids pigments in broad bean leaves especially potassium chloride salt, whereas the concentration of the salts had non-significant effects. Most of stomata characteristics response to salt types and their concentrations were non-significant, except the effects of calcium sulphate on stomata guard cell length and width.

Table-3: Effect of K and Ca salts types, concentrations and their interactions on some photosynthetic pigments of faba bean leaves.

<i>Treatments</i>	<i>Chlorophyll a</i>	<i>Chlorophyll b</i>	<i>Total carotenoids</i>
	<i>mg/ g fresh weight</i>		
<i>Salts sources</i>			
<i>Control</i>	1.58 b *	0.85 b	0.98 b
<i>KCl</i>	1.96 a	0.97 a	1.21 a
<i>K₂SO₄</i>	1.49 b	0.82 b	0.94 b
<i>CaCl.2H₂O</i>	1.48 b	0.84 b	0.94 b
<i>CaSO₄</i>	1.58 b	0.87 ab	0.99 b
<i>Concentration (g/l)</i>			
<i>5.0</i>	1.63 a	0.81 a	1.01 a
<i>7.5</i>	1.60 a	0.92 a	1.01 a
<i>Interaction between salts source and concentration</i>			
<i>Control</i>	1.58 bc	0.85 bc	0.98 b
<i>KCl x 5.0</i>	2.18 a	1.03 a	1.26 a
<i>KCl x 7.5</i>	1.74 b	0.91 ab	1.15 a
<i>K₂SO₄ x 5.0</i>	1.34 c	0.66 d	0.88 b
<i>K₂SO₄ x 7.5</i>	1.64 bc	1.00 ab	1.01 b
<i>CaCl.2H₂O x 5.0</i>	1.51 bc	0.73 cd	0.96 b
<i>CaCl.2H₂O x 7.5</i>	1.44 bc	0.95 ab	0.92 b
<i>CaSO₄ x 5.0</i>	1.54 bc	0.84 bc	0.98 b
<i>CaSO₄ x 7.5</i>	1.61 bc	0.91 ab	1.00 b

* Means followed by the same letters within columns are not significantly different at $p \leq 0.05$ according to the Duncan test.

Table-4: Effects of K and Ca salts types, concentration and their interactions on some stomata characteristics for faba bean leaves.

<i>Treatments</i>	<i>Stomata Number/mm²</i>		<i>Stomatal Guard Cell Length (micrometer)</i>		<i>Stomatal Guard Cell Width (micrometer)</i>	
	<i>Abaxial surface</i>	<i>Adaxial surface</i>	<i>Abaxial surface</i>	<i>Adaxial surface</i>	<i>Abaxial surface</i>	<i>Adaxial surface</i>
<i>Salts sources</i>						
<i>Control</i>	94.47 a	69.43 a	23.44 a	23.22 a	4.00 b	4.17 a
<i>KCl</i>	83.03 a	72.22 a	23.61 a	21.66 ab	4.47 ab	3.28 a
<i>K₂SO₄</i>	80.85 a	76.13 a	23.61 a	21.50 b	4.67 ab	3.50 a
<i>CaCl₂H₂O</i>	81.62 a	69.98 a	25.81 a	23.34 a	3.98 b	3.67 a
<i>CaSO₄</i>	77.77 a	68.35 a	25.17 a	23.00 ab	4.95 a	4.06 a
<i>Concentration (g/l)</i>						
<i>5.0</i>	86.22 a	76.53 a	23.80 a	22.13 a	4.53 a	3.73 a
<i>7.5</i>	80.09 a	65.89 a	24.86 a	22.95 a	4.29 a	3.73 a
<i>Interaction between salts source and concentration</i>						
<i>Control</i>	94.47 a	6.94 a	23.44 ab	23.22 a	4.00 c	4.17 a
<i>KCl x 5.0</i>	88.87 a	81.10 a	22.33 b	20.33 b	4.11 bc	3.06 a
<i>KCl x 7.5</i>	77.20 a	63.33 a	24.89 ab	23.00 a	4.83 abc	3.50 a
<i>K₂SO₄ x 5.0</i>	87.23 a	86.70 a	23.00 ab	21.44 ab	5.11 ab	3.78 a
<i>K₂SO₄ x 7.5</i>	74.47 a	65.57 a	24.22 ab	21.55 ab	4.22 bc	3.22 a
<i>CaCl₂H₂O x 5.0</i>	80.00 a	72.20 a	25.44 ab	23.11 a	4.11 bc	3.67 a
<i>CaCl₂H₂O x 7.5</i>	83.23 a	67.77 a	26.18 a	23.56 a	3.85 c	3.67 a
<i>CaSO₄ x 5.0</i>	80.53 a	73.33 a	24.78 ab	22.56 ab	5.33 a	4.00 a
<i>CaSO₄ x 7.5</i>	75.00 a	63.37 a	25.55 ab	23.44 a	4.56 abc	4.11 a

* Means followed by the same letters within columns are not significantly different at $p \leq 0.05$ according to the Duncan test.

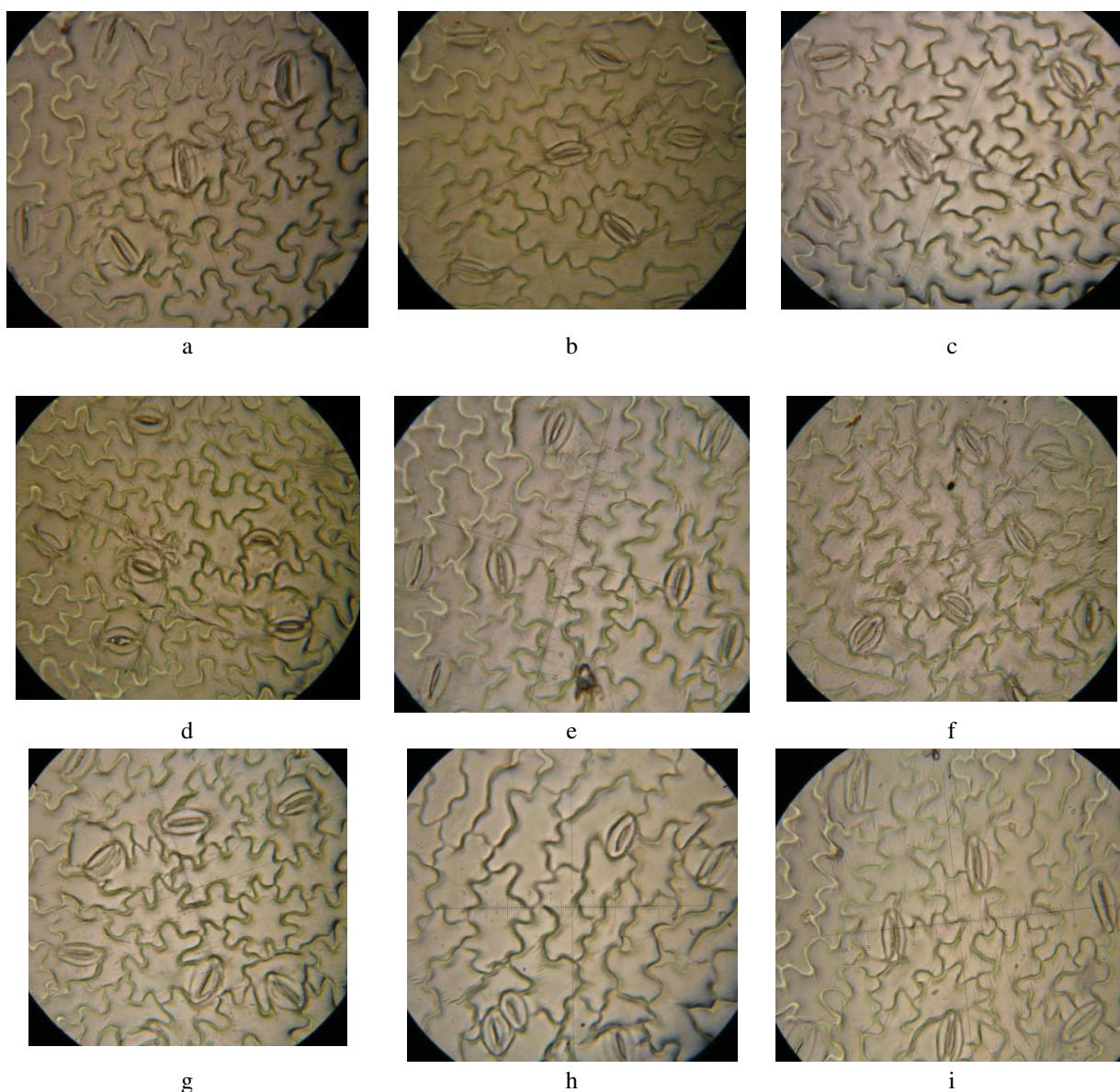


Figure-1: Stomata of upper (adaxial) *Vicia faba* L. leaves surfaces 400X for plants of (a) control (b) KCl -5% (c) KCl -7.5% (d) K₂SO₄ -5.0% (e) K₂SO₄ -7.5% (f) CaCl₂·2H₂O -5.0% (g) CaCl₂·2H₂O -7.5% (h) CaSO₄ -5.0% and (i) CaSO₄ -7.5% treatments.

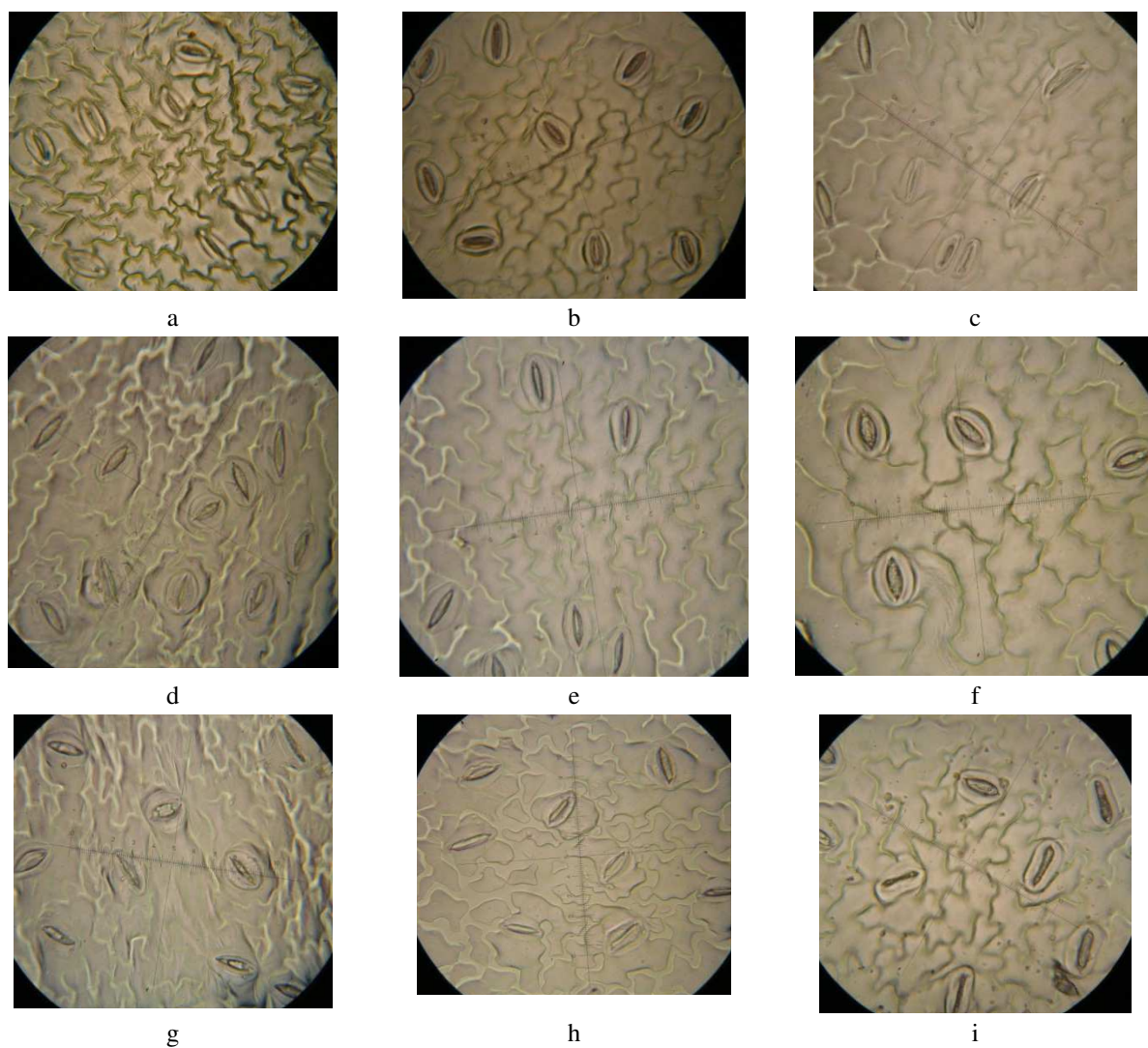


Figure-2: Stomata of lower (abaxial) *Vicia faba* L. leaves surfaces 400X for plants of (a) control (b) KCl -5% (c) KCl -7.5% (d) K₂SO₄ -5.0% (e) K₂SO₄ -7.5% (f) CaCl₂·2H₂O -5.0% (g) CaCl₂·2H₂O -7.5% (h) CaSO₄ -5.0% and (i) CaSO₄ -7.5% treatments.

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