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Assessment of Physiological and Cytological Responses of Salinity Stressed *Vicia faba* Plants to Royal Jelly Treatment

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Keywords:
Faba bean; Salt stress; Royal jelly; Mitotic index ; Chromosomal abnormalities.

ABSTRACT

Vicia faba L. One of the oldest legume species which considered as the main source of protein for the majority of African and Asian poor people. Salt stress is a major potential constraint for *V. faba* growth and production. The present study aimed to investigate the possible role of Royal jelly in alleviation of hazardous effects of salinity stress on Giza 617 variety of Faba bean. Germination %, fresh and dry weight, mitotic index and chromosomal aberrations were determined. The obtained results revealed that, salinity stress induced by treatment with100 mM NaCl caused a significant reduction in germination percentage, fresh and dry weights. It also induced an inhibition in mitotic index and increasing in the ratio of chromosomal aberrations. Royal jelly treatment showed positive effects on all studied parameters in both stressed and non-stressed plants. The recovery effect of royal jelly was recorded as an increase in mitotic index as well as a reduction in chromosomal abnormalities in stressed plants. The present study pointed to the role of Royal jelly in improving salinity tolerance in Faba bean.

1. INTRODUCTION

Salinity is one of the most prevalent abiotic stresses. It affects numerous biochemical and physiological processes in plant cells and causes a significant reduction in the world's food production (**Shrivastava and Kumar 2015**). Growth inhibition, accelerated development, senescence and death after prolonged exposure are the most common associated signs of salt stress (**Zhu, 2007**). Ultimately, it leads to a reduction in crop yield (**Hasanuzzaman *et al*., 2013**).

Faba bean is one of the earliest types of legumes (**Zong *et al*., 2009**) and ranks as the fourth in terms of total production (**Alharbi *et al*., 2020**). It is considered a major human food crop and an important component of animal feed in the Mediterranean and various African and Asian countries with average protein content 24–30% (**Kumari and Makkouk, 2007; Sahile *et al*., 2009**).

Leguminous species are sensitive to saline conditions and Faba bean tends to be more sensitive to salinity (**Katerji *et al.*, 2003**).
Considering water resources limitation and the world demand for using seawater as an alternative source for irrigation water, it became in focus to find new strategies to overcome hazardous effects of salinity on sensitive plant species (**Sadak *et al.*, 2020**).
Recently, exogenous applications of some plant extracts (**Babu and Maheswari, 2006; Çavuşoğlu and Karaferyeli, 2015**), phyto hormones (**Lateef *et al.*, 2021; Quamruzzaman *et al.*, 2021**) as well as nano-materials (**Zedan and Omar, 2019; Anshu *et al.*, 2019; Elsheery *et al.*, 2020**) are considered as promising strategies to improve salinity tolerance in various species.
Royal jelly (RJ), the exclusive food of the queen honey bee larva, is produced from specialized glands located on heads of bee workers to feed all bee larvae in the beginning stages of their growth and the queen bee through its life course (**Snodgrass, 1984**). The powerful epigenetic impact of RJ in the differentiation of larvae to either the workers or queens was reported (**Ramanathan *et al.*, 2018**). Analysis of its components is well established. Royal jelly is made up of water (50% to 60%), carbohydrates (15%), proteins (18%), lipids (3% to 6%), mineral salts (1.5%) primarily copper, iron, zinc, calcium, potassium, manganese and sodium salts, and trace amounts of polyphenols, flavonoids and vitamins riboflavin B2, thiamine B1, folic acid B9, pantothenic acid B5, B6, B8 biotin, vitamin C and E (**Viuda-M *et al.*, 2008**). The amount of total sugar varies between 7.2% and 21.2% and is primarily made up of fructose and glucose. Together, glucose and fructose make up 90% of all sugars (**Kamakura *et al.*, 2014**). It was regarded as a good source of organic antioxidants that might prevent the negative effects of oxidative stress (**Albert *et al.*, 1999**).
Many studies have been conducted on the responses of human and animal tissues to RJ as well as its usage in the therapy of numerous diseases. It was reported that several RJ proteins inhibit the growth of human breast cancer cells (**Ramadan and Al-Ghamdi, 2012**). Less is documented about the positive effects of RJ against abiotic stresses in plants (**Çavuşoğlu *et al.*, 2017**).
The present study aimed to investigate the physiological and cytological responses of salinity stressed Faba bean plants to the exogenous application of RJ.

2. MATERIALS AND METHODS

2.1. Plant material

Seeds of Egyptian commercial *Vicia faba* variety, Giza-716, were received from the Agricultural Research Centre, Kafr El-sheikh, Egypt.

2.2. Royal jelly treatment

The RJ concentration used in the experiments was 50 mg/ L, RJ (Package containing 25 g of freeze-dried RJ) was obtained from a local market in Tanta, EL-Gharbia Governorate, Egypt.

2.3. Experimental design

This experiment was carried out in pots at the farm of the Faculty of Agriculture at Tanta University during 2020/ 2021 winter seasons. Seeds of the Giza-716 variety were placed in pots filled with 5 Kg soil. Pots were divided into four groups with three replicates each. First group was irrigated with tap water as the control treatment (Control). In the second group, pots were irrigated with water containing 100 mM NaCl as salinity treatment. Seeds of the third group were soaked for 24 h before planting in a solution of 50 mg/L of RJ and pots were irrigated with water containing 50 mg/L of RJ. The last group seeds were soaked for 24 h before planting in a solution of 50 mg/L of RJ +100 mM NaCl and pots were irrigated with water

containing 50 mg/L of RJ +100 mM NaCl. Samples were collected for fresh measurements after 30 days of planting or stored at -80ºC for further analysis.

2.4. Seed germinability

Seed germinability was determined as percentage of germinated seed as well as the rate of germination of the seeds of all tested groups. Changes in the germination rate and radical length were documented photographically. Percentage of germination was examined in Petri dishes experiment. Seeds were soaked in distilled water for 12 hours at room temperature in the dark. Soaked seeds were labeled with a germination time of 0 hours. Soaked seeds were split into the four designed categories of the experiment (control, 100 mM NaCl, 50 mg/L of RJ and 50 mg/L of R J +100 mM NaCl) and maintained in the incubator (POL-EKO-APARATURA SP.J) at 25 °C. Germinated seeds were observed and photographed at 24, 48, and 72 hours. Germination percentage was calculated using the equation below:

$$G\ (\%) = \frac{number\ of\ germinated\ seeds}{total\ number\ of\ seeds\ in\ the\ plate} * 100$$

2.5. Fresh and dry weights

Fresh weight (FW) of whole seedling (shoot and root) was estimated after carefully washing of each seedling with distilled water and gently drying with a paper towel. Dry weight (DW) of seedlings was determined by reweighting after oven drying at 105°C for three h.

2.6. Cytological analysis

Root tips (1-1.5 cm) of three days old germinated seeds were used for cytological analysis. They were fixed in ethanol: glacial acetic acid (3: 1) solution overnight at room temperature then stored at 4°C in 70% ethanol until use. The root tips were hydrolyzed in 1 N HCl at 60°C for 5 min. Meristems of root tips were then smashed in a drop of aceto-carmin stain for 1-1.5 min. Slides were examined using a light microscope. All mitotic phases and chromosomal aberrations were counted in at least 3000 examined cells per treatment. Obtained results were used in the calculation of mitotic index (MI) and chromosomal abnormalities using the following formulas:

$$\text{Mitotic index (MI)} = \frac{\text{Total dividing cell}}{\text{total dividing and non dividing cells}} * 100$$

$$\text{Mitotic inhibition} = \frac{\text{MI of control} - \text{MI of treatment}}{\text{MI of control}} * 100$$

$$\text{Percentage of abnormal cells} = \frac{\text{Total abnormal cells}}{\text{Total dividing cells}} * 100$$

2.7. Statistical Analysis

All experiments in this study were carried out using a full factorial split-plot design arranged in randomized complete blocks with salinity as main plots and RJ as subplots. For each treatment, at least three biological replicates were used in all experiments. ANOVA was used to test for significant differences between salinity levels ($_{pSalinity}$), Royal jelly ($_{pRoyal\ jelly}$), and their interaction ($_{pSalinity\ \times\ pRoyal\ jelly}$). For post-hoc analysis, Tukey's honestly

significant difference (HSD) test was used ($_{pSalinity \times pRoyal\ jelly}$ 0.05).

2. RESULTS AND DISCUSSION

3.1. Seed Germinability

Changes in the rate of germination as the changes in radical length were documented photographically (Figure 1). A noticeable reduction in radical length was recorded under salinity condition in comparison with control condition. Treatment with RJ induced a noticeable improvement in radical length in both stressed and non-stressed plants. The lowest germination % (75%) value was recorded under salinity treatment (Figure 2). Germination % was significantly affected under salinity condition, where it dropped from 100% under control condition to 75% under NaCl treatment (Figure 2). Treatment with RJ induced an increase in the germination percentage of stressed plant compared with non-treated with RJ (Figure 2).

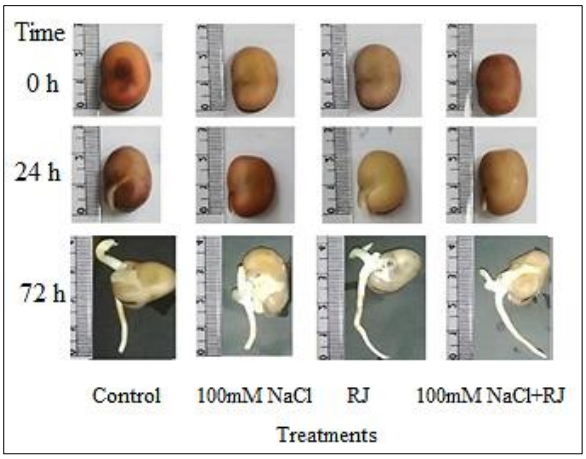

Fig.1. Images of germinated seeds under all experimental conditions at 0, 24 and 72 h intervals. Germination experiment was carried out in the dark at 25 °C.

Salt stress can perform its preventive effect in many ways. It may interfere with seed germination by changing the water status of the seed so that water uptake is inhibited (**Ali, 2000**). Salinity stress negatively affects the formation and growth rate of lateral root, rate of cell division, synthesis of nucleic acids and protein synthesis (**Prakash *et al.*, 1988; McCue and Hanson 1990**). Recovery effect of RJ on stressed plants could be due to its role in adjustment of salt osmotic potential. Recovery effect of RJ on salinity stressed plants was reported in *Allium cepa* (**Çavuşoğlu *et al.*, 2017**).

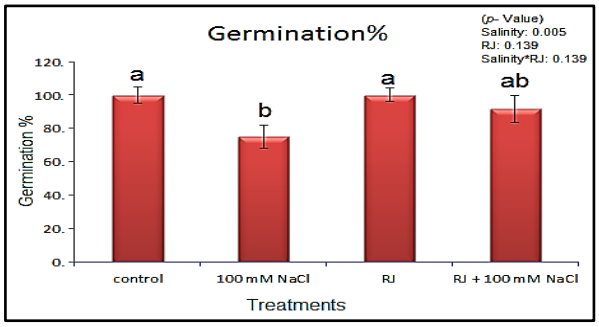

Fig.2. Changes in germination% of *V. faba* seeds under control and all experimental conditions. Columns with different letters are significant.

3.2. Growth parameters

Growth parameters such as fresh and dry weights and root spread showed various changes under different experimental conditions (Figure 3). Great reduction in shoot growth was induced under salinity treatment (Figure 3B). On another hand, RJ treatment showed a good recovery for stressed plants in comparison with un-treated stressed plants (Figure 3 D).

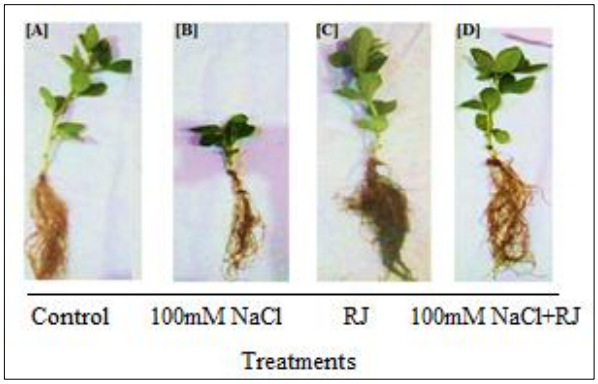

Fig.3. Illustration of changes in growth parameters of *V. faba* plants under all experimental conditions.

In respect of seedling fresh and dry weights, obtained results showed a serious of changes under all examined treatments (Figure 4). Salinity stress (100 mM NaCl) induced significant reductions in both of fresh and dry weights (Figure 4A, B) in comparison with control treatment. Application of RJ caused an improvement in FW and DW, where the highest values of FW and DW were recorded in seedling treated with RJ with significant increase in comparison with stressed and non-stressed plants for FW (figure 4A) and in comparison with stressed plants for DW (Figure 4B). Salinity stress inhibits cell division that may cause a reduction in shoot length, root length, leaf number in Fabaceae and the Lamiaceae plants (**McCue and Hanson, 1990**) as well as all growth parameters and metabolic activities in *Capsicum annuum* (**Kord and Khalil, 1995**).Ionic toxicity caused by salinity had an impact on the plants' ability to absorb water, which in turn reduced photosynthesis and may impacts on growth parameters as fresh and dry weights of rice plant (**Puvanitha and Mahendran, 2017**). RJ induced an improvement in plant growth under both saline and non-saline environments. This subsequently increased both fresh and dry weight of *Allium cepa* (**Çavuşoğlu *et al.*, 2017**). The high content of proteins, carbohydrates, lipids and ash in royal jelly, as well as sugars such as (glucose, sucrose and fructose), minerals such as potassium, magnesium, iron,

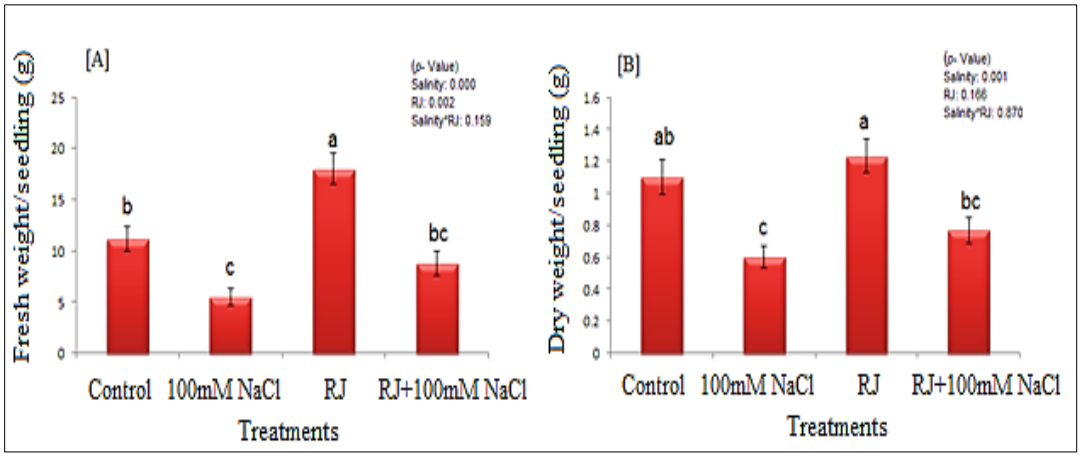

Fig.4. Changes in fresh (A) and dry weigh (B) of Giza-716 seedlings (*V. faba*) under control and all experimental conditions. Values with different letters are significant.

calcium, and silicon, vitamins such as vitamins B1, B2, B5, B6, B8, B9 and B12, hormones and essential amino acids (**Townsend and Lucas, 1966**) which, may contribute in rising of plant biomass and explains the growth improvement in plants treated with RJ.

Abada and Ahmed (2015) reported that applying stimulants like royal jelly and amino acids in place of chemical fertilizers could increase the yield and quality of berries in several grapevine species. The combination of foliar applications of royal jelly and yeast on cucumber plants produced the maximum fruits output (**Nassef and El-Aref, 2016**).

3.3. Cytological analyses

Cytological analysis was performed to examine the possible recovery effect of RJ on salinity-stressed plants in respect to mitotic index and chromosomal abnormalities. The total number of

proliferating cells and the numbers of cells at various mitotic stages were scored in root tips. The mitotic index and mitotic phase (%) of *V. faba* root tips were calculated and shown in (Table 1). Treatment with 100 mM NaCl induced a significant reduction, about 13%, in mitotic index in comparison with control treatment (Table 1). This reduction is due to the inhibition of cell division. The reduction in cell division under salinity stress is related to osmotic pressure, increased lipid peroxidation, cellular membrane damage and inhibition of DNA, RNA and protein synthesis (**Anuradha and Rao, 2001**). While mitotic index of treated plants with RJ recorded the highest value, about 33.14%, with significant increase in comparison with the control and other treatments. Treatment of stressed seeds with RJ showed non-significant decreases compared with control conditions. However, the mitotic index of stressed seeds treated with RJ increased significantly compared with non-treated stressed seeds. In addition to inhibition of mitotic activity induced by salt treatment, salinity negatively effects on plant growth and delays plant development (**Tabur and Demir, 2010**).

Data shown in Table (2) illustrate the effect of salinity stress and RJ on chromosomal aberrations in Giza-716 variety. The highest ratio of chromosomal aberrations was recorded under salinity stress (100 mM NaCl) in comparison with control and other treatments. Treatment with RJ induced non-significant increase in chromosomal aberrations. Increasing the chromosomal aberration with RJ treatment was reported as a result of its stimulation effect (**Çavuşoğlu *et al*., 2017**). The increase in chromosomal aberrations under RJ treatment was directly proportional to the increase in MI, Treatment with RJ in combination with salinity stress led to a significant reduction in chromosomal aberrations in comparison with non-treated stressed seeds (Table 2). Royal jelly was considered a scavenger of free radicals due to some of its components such as flavonoids and phenolic ingredients with strong antioxidant properties of hydroxyl radical scavenging (**Nabas *et al*., 2014**; **Kocot *et al*., 2018**).

Analysis of chromosomal abnormalities (Table 2) revealed that the most recorded aberrations were; Micronucleus, C-metaphase, laggard and bridges. Micronucleus disappeared under control and RJ treatment, while they appeared at a rate of 12% under salinity stress, while treatment with RJ in combination with salinity stress reduced them to 7%. C-metaphase, Laggard, bridges and vagrant chromosome were the highest scored aberrations under salinity conditions, the percentage of chromosome loss reached to 14% under salinity stress, while it reduced to 4% under treatment with RJ. Treatment with RJ induced a significant recovery for several induced aberrations under salt stress such as fault polarization in telophase irregular prophase, uncoiling chromosomes and chromosome breaks. Increasing of chromosomal abnormalities under salinity stress was reported in root-tip cells of barley (**Tabur and Demir, 2010**).

The majority of chromosomal aberrations caused by salinity stress were caused by colchicine type action that inhibited the spindle fiber formation subsequently causing a metaphase arrest (**Ramel, 1969**). The disruption of the spindle apparatus may be responsible for the irregular spreading of chromosomes (**Gömürgen, 2005**). Laggards might be the result of spindle fiber failing to organize normally (**Patil and Bhat, 1992**). The creation of chromosome bridges during anaphase and telophase may be caused by chromosome segment inversions, or it may

be caused by chromosomal stickiness and the subsequent inability of free anaphase separation due to unequal translocation (**Amer and Farah, 1985**). Micronuclei most eventually created from stray chromosomes and fragments (**Briand and Kapoor, 1989**).

4. CONCLUSION

Obtained results demonstrated the negative effects of salinity stress induced by irrigation with 100mM NaCl on Giza-716 variety of Faba bean. Salinity stress caused a significant reduction in germination percentage, fresh and dry weights. It also induced an inhibition in mitotic index and increasing in the ratio of chromosomal aberrations. Royal jelly treatment showed positive effects on all studied parameters in both stressed and non-stressed plants. The recovery effect of royal jelly was recorded as an increase in mitotic index as well as a reduction in chromosomal abnormalities in stressed plants. The present study pointed to the role of Royal jelly in improving salinity tolerance in Faba beans.

Table 1. Mitotic index, percentage of mitotic phases, of Faba bean root tip cells under different treatments

Treatments	No. of examined cells	No. of dividing cells	Prophase	Metaphase	Anaphase	Telophase	Mitotic index (%)
Control	3402	895	66.44	5.88	11.25	16.44	26.30±1.7 [b]
100 mM NaCl	3353	419	59.38	4.87	8.83	26.50	12.49±0.6 [c]
RJ	3279	1087	70.34	7.95	12.07	9.25	33.14±1.5 [a]
RJ + 100 mM NaCl	3198	795	65.84	6.17	14.08	16.00	24.9±1.9 [b]

Table 2. Types and percentage of chromosomesal abnormalities in the cells of Faba bean root tip under different treatments: control, 100 mM NaCl, RJ and RJ + 100 mM NaCl

Treatments	No. of examined cells	No. of dividing cells	No. of abnormal cells	Abnormalities %	Types of chromosomal aberration (%)											
					Micro-nucleus	C-metaphase	Laggard	bridges	vagrant chromosome	chromosome loss	fault polarization in telophase	irregular prophase	uncoiling chromosomes	chromosome break	spindle disturbance	Multiple nuclear erosion
Control	3402	895	36	4.08±0.9 [b]	0	8	4	9	0	2	1	5	0	5	1	1
100 mM NaCl	3353	419	122	29.27±10 [a]	12	20	17	21	8	14	8	8	2	12	0	0
RJ	3279	1087	81	7.43±1.9 [b]	0	19	12	15	5	4	7	12	0	0	6	0
RJ + 100 mM NaCl	3198	795	57	7.24±2.0 [b]	7	11	7	12	2	4	5	6	3	0	0	0

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تقييم الاستجابات الفسيولوجية والسيتولوجية لنباتات الفول البلدي تحت اجهاد الملوحة للمعاملة بغذاء ملكات النحل

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مجلة العلوم الزراعية والبيئية المستدامة

الكلمات المفتاحية:

الفول البلدي ، إجهاد الملوحة ، غذاء ملكات النحل ، دليل الانقسام، التحورات الكروموسومية

الملخص

يعد الفول البلدي من أقدم أنواع البقوليات. وهو المصدر الرئيسي للبروتين لمعظم الشعوب الأفريقيه والأسيويه. ويعتبر إجهاد الملوحة أحد المحددات الرئيسية لنمو وانتاجيه الفول. وقد هدفت الدراسة الحالية إلى دراسة تأثير غذاء ملكات النحل في التخفيف من التأثيرات الضاره لإجهاد الملوحة على الصنف جيزة 617لنبات الفول البلدي.وقدتضمنت الدراسه تقدير كل من نسبة الإنبات و الوزن الرطب والجاف والتحورات الكروموسومية ودليل الانقسام الميتوزي. أظهرت النتائج المتحصل عليها أن إجهاد الملوحة بأستخدام 100 ملي مولر من كلوريد الصوديوم أدي الي انخفاض كل من نسبة الإنبات والأوزان الرطبة والجافة للبادرت كما ادي ايضا إلي تثبيط معدل الانقسام إحداث أعلى نسبة للتحورات الكروموسومية. بينما أظهرت المعاملة بغذاء ملكات النحل تأثيراً إيجابياً على كل من نسبة الإنبات والوزن الرطب والجاف للبادرات سواء تحت ظروف الاجهاد وعدم الأجهاد .وقد ظهر التأثير الايجابي لغذاء ملكات النحل كزيادة معدل الانقسام معنويا وكذلك انخفاض نسبة التحورات الكروموسومية تحت ظروف الأجهاد الملحي.و قد أشارت الدراسة الحالية إلى دورالمعامله بغذاء ملكات النحل في تحسين تحمل نباتات الفول البلدي لأجهاد الملوحه.