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Action of Benzyl adenine on epidermal characters of *Rumex vesicarius* L., a medicinal plant

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Abstract

Background: *Rumex vesicarius* L., also known as Ruby dock or bladder dock, is one of the worldwide wild medicinal and economic important plants. Benzyl adenine (BA) may contribute to increase *Rumex vesicarius* tolerance to prevailing local environmental conditions, and thus improve its productivity. Objective: The aim of this study is to investigate the action of Benzyl adenine (BA) on the epidermis characteristics of *Rumex vesicarius*.
Methods: The seeds of *R. vesicarius* were sown, Then, the plants were treated with 50 and 500 ppm BA solutions. The leaf and stem epidermal peels were prepared. Microscopic photography was utilized using Ernst Leitz Wetzlar GmbH microscope equipped with an external digital video camera with Motic Images plus 2.0 (MIPlus) software and connected to a computer.
Results: The results show that BA used in this study either in low or high concentration, have no obvious effects on some epidermal characters, eg: the cell wall number, cuticle layer, trichome types, stomata shape and types, in both leaf and stem epidermis. On the other hand, BA have some effects on the other epidermal characters, eg: cells dimensions, number of trichomes, stomata number, and lengths of stomata in both leaf and stem epidermis. Our results showed a significant increase in trichomes number in the epidermis of leaf and stem in low or high concentrations plants, also stomata number in the epidermis of the leaf in low or high concentrations plants and increased insignificant in cells' dimensions of stem epidermis with low or high concentrations. Whereas, appeared significant increasing in stomata number in epidermis stem with low or high concentrations, stomata dimensions in the upper epidermis in high concentration plants, lower epidermis, stem epidermis with low or high concentrations plants, and cells dimensions in lower epidermis with low or high concentrations plants.
Conclusion: This study may indicate that spraying the shoot system of *Rumex vesicarius* L. with low or high concentration of BA may cause some differences in the epidermal characters of the leaf and stem. More studies could be investigated to measure the impact of these positive effects on the tolerance of this useful medicinal plant to cope its resistance to the harsh environmental conditions and to improve its economic importance.

Keywords: Benzyl adenine epidermal characters *Rumex vesicarius* L. trichomes stomata

INTRODUCTION

Rumex vesicarius L., also known as Ruby dock or bladder dock, is one of the worldwide wild plants that grow naturally in the Kingdom of Saudi Arabia. It is a species of perennial flowering plants in the Polygonaceae family. This plant has medicinal and economic importance. Its vegetative part is used for various medicinal purposes to treat some diseases. The plant is used to decorate public and private parks and gardens. It is also used as a food material to be used in the preparation of various vegetable salads as well as being an essential pastoral plant (Al-Yahya, 1990; Boulos, 1983; Kirtikar and Basu, 1999; Nadkarni, 1954). Studies have indicated that some plant growth regulators have effects on plant epidermis. They may alter cell forms, sizes, or numbers, induce or inhibit the growth of trichomes, and may affect the density and distribution of stomata. Hulskamp (Hulskamp *et al.*, 1998) indicate that several factors influence the growth of trichomes and regulation of cell differentiation, including plant hormones. Meinke (Meinke, 1992) suggests that the formation of trichomes in the cotyledon of Arabidopsis thaliana is due to plant hormones.

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Cytokinins (CK) are a class of plant growth substances (phytohormones) that promote cell division, or cytokinesis, in plant roots and shoots. Ether natural Cytokinin present in plants or externally added to the plants usually lead to some morphological and physiological changes and modifications in plant organs, such as regulating the dominance of the seeds of many plants, root formation, vegetative growth and branch formation, floral growth and fruit development, and stimulating cell division and expansion (Devlin, 1975; Krishnamurthy and Bhagwat, 1989; Kushwah *et al*., 2011; Thimann, 1972). They also caused cells' expansion and enlargement in the pith of tobacco (Glasziou, 1957) and the roots (Arora *et al*., 1959). Treating of etiolated leaf discs of bean with kinetin also causes leaf expansion (Miller, 1956; Powell and Griffith, 1960). Kinetin causes an enlargement in the cotyledons of radishes due to its effect on cell expansion and size (Letham, 1966). Wang (Wang *et al*., 1997) states that cytokinin increases the number and dimensions of stomata in the epidermis of the tobacco (*Nicotiana tabacum*), which increases the transpiration rate and decreases resistance to diffusion. It also stimulates the expansion of its leaf discs, enlargement of the middle veins, and other veins, and increases the thickness of the stem due to the increase and expansion of the pith. The use external use of cytokinin for growing plants stimulates the division of different tissue cells such as the epidermis and pith (Devlin, 1975; Krishnamurthy and Bhagwat, 1989). Moreover, BA increases the intensity of the stomata in the upper epidermis of the leaf of *Orthosiphon stamineus* (Stoyanova-Koleva *et al*., 2012). Different anatomical characteristics, including the cuticle thickness, epidermis thickness, thickness of phloem tissue, xylem tissue thickness, number of xylem vessels /xylem row, and diameter of the xylem vessel in the stem of *Moringa olifera* leaflets and stems were positively responded to foliar spray with benzyl adenine (Abou-shlell *et al*., 2017). Stem and leaf anatomy measurements of Soybeans (*Glycine max* L.) were increased, especially (stem and leaf vascular and transporter tissues as a result of treating the plants with benzyl adenine (BA) at 20 and 40 mg/L (Zewail, 2019). *Micromeria pulegium* glandular trichomes in relation to the secretory process were characterized for the first-time using SEM and light microscopy, and their secretion was histochemically analyzed under in vitro conditions and benzyl adenine (Stojičić *et al*., 2016). However, the treatment of developing "Pink Lady" apples with Benzyl adenine has resulted in mature fruits with a thicker cuticle comprising clusters of epidermal cells, which results in thickening of the cuticle layer and possibly enhancing crack repair (Fogelman *et al*., 2015).

There is little information on the role of hormones in the formation of stomata. In a study on the Arabidopsis thaliana plants grown in a nutritious media, Nelson (Saibo *et al*., 2003) stated that adding of 10 µmol GA3 to the media had a positive effect on induction and elongation of stomata in the hypocotyl, and this role could be enhanced by adding auxins. Morini (Morini *et al*., 1991) reports that the number of stomata has increased in the tips collected from in vitro established shoots grown in MS media with BA at 2.7 µM, GA3 at 0.7 µM and IBA at 0.3 µM exposed to 12-h photoperiod. In a study on the tomato (*Lycopersicon esculentum*) leaves, the applying of 100 ppm NAA and 50 ppm GA3 increased the number of stomata on the upper epidermis, while epidermal cells on the upper and lower sides decreased due to their large in size (Martins and Castro, 1999).

The main objective of this study is to find out the action of benzyl adenine (BA) on the epidermis characteristics of *Rumex vesicarius*, which may contribute to increasing its tolerance to prevailing local environmental conditions, and thus improve its productivity.

2. MATERIALS AND METHODS

2.1. Plant materials and experimentation

The seeds of *R. vesicarius* were collected from the King Saud University campus in Riyadh and kept dry in carton boxes at normal room temperature until use. Then the seeds were washed with distilled water and seeded in 3 parallel lines (i.e., three groups) in the soil of the botanical garden, Department of Botany and Microbiology, College of Sciences, King Saud University in Riyadh. Clay and sandy soils were added in a ratio of 1: 2, respectively, at a temperature of 12-22 °C, 66% air humidity, and sunlight. After germination, the plants were thinned to 10 plants per line. The plants were sprayed in the next day with growth regulator solutions. It was reduced the surface tension and resistance through the diffusion added of 1 mL of Tween 20, shaken well, and adjusted pH at 6.5. The spraying was applied using 8 ml/plant/3 days along 25 days as follows. Groupe 1: control, sprayed with distilled water; Group 2: sprayed with 50 ppm Action of benzyl adenine (BA) (low concentration); and Group 3: sprayed with 500 ppm Action of benzyl adenine (BA) (high concentration).

2.2. Preparation of epidermal peel:

After 25 days of treatment, the epidermal peels were prepared from the leaves and stems, according to the method of preparing epidermal peels (Zhu *et al*., 2016).

2.3. Measuring studied parameters:

The trichomes were counted in an area of 1/4 (0.25) mm 2 and the stomata in an area of 1/16 (0.063) mm2 at the magnification power of X10. The cell dimensions were measured at the magnification power of X10, and the stomata dimensions were measured at the magnification power of X40. A total of 25 random reads were recorded for each of the studied traits, then the averages were calculated.

2.4. Imaging techniques:

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Microscopic photography was utilized using ERNST LEITZ WETZLAR GMBH microscope equipped with an external digital video camera (Moticam 2000 / 2.0M Pixel USB 2.0 Order No: SP100382) with Motic Images plus 2.0 (MIPlus) software and connected to a computer.

2.5. Statistical analysis

Completely Randomized Design(CRD) was used (Stell *et al*., 1980). Analysis of variance was performed on the collected data using SAS 9.1, SAS Inc., North Carolina, USA (Anon., 2002), and the least significant difference (LSD) test was used to discern differences among the mean values of the treatments and accessions.

3. RESULTS

3.1. Epidermal Characters in the control

3.1.1. Leaf Epidermis

The upper epidermis cells are polyhedral in shape, their vertical walls on the surface area of cellulose, thin, straight, and un-perforated. Their average dimensions are 137.2 µm in length, and 78.5 µm in width (Table 1).

Table 1: The features in the epidermal cells of *R. vesicarius* leaves measured in µm.

Averages of parameters in leaf epidermis												
Treatments ppm	cells dimensions (μm)				Trichomes No /0.25 mm^2		Stomata No /0.63 mm^2		stomata dimensions (µm)			
	UE		LE						UE		LE	
	L	W	L	W	UE	LE	UE	LE	L	W	L	W
Control	137.2	78.5	130.8	72.8	2.9	2.3	20.0	19.5	39.4	27.5	41.5	26.0
BA *50*	136.5	78.4	92.0	53.9	3.6	4.8	21.0	22.0	39.2	27.7	27.2	21.7
BA *500*	102.8	64.2	81.7	52.8	5.2	5.1	20.8	23.0	33.8	24.0	26.0	22.0
LSD at 5%.	9.66	5.47	9.29	5.69	0.5	0.43	0.81	0.83	1.23	1.1	1.26	1.11
LSD at 1%	13.66	7.71	13.1	8.03	0.7	0.61	1.14	1.17	1.73	1.55	1.78	1.57

L: Length; W: Width; UE: Upper epidermis LE: Lower epidermis; BA: benzyl adenine average indicators in the leaf epidermis cells

The cuticle is thin, smooth, but striped around the stomata only. Trichomes are unbranched glandular with a neck of two-row cells and multicellular head with an average number of 2.9 / 0.25 mm^2 (11.6 trichomes/1 mm^2). Stomata are oval, single, scattered, with renal guard cells. They are located at the level of epidermal cells and some are at a higher level. They are of the anisocytic stoma type or anomocytic stoma but rare. Average number of stomata are 20 stomata / 0.063 mm2 (320 stoma / 1 mm^2). The average dimensions of stomata are 39.4 µm in length and 27.5 µm in width (Fig. 1 A, D, E, F).

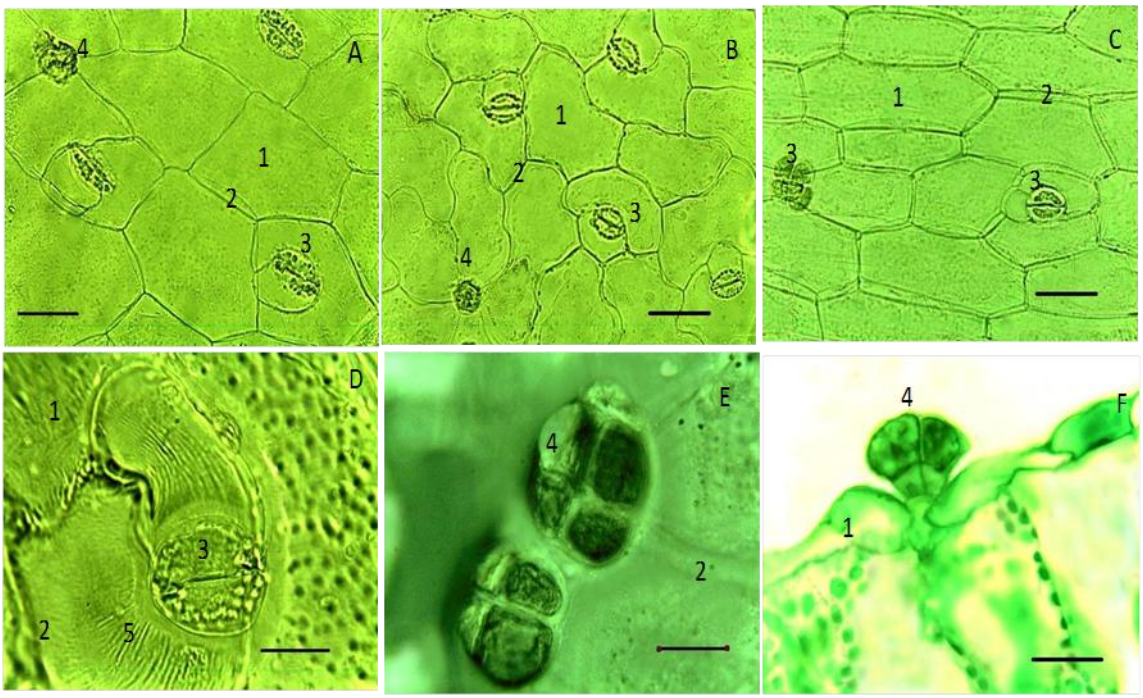

Figure (1) epidermal peels from the epidermis of leaf and stem of *R. vesicarius* (control): (A) a peel in the upper epidermis, (B) a peel in the lower epidermis, (C) a peel in the stem epidermis, (D) surface view of the stoma. (E) surface view of glandular trichomes, (F) part of a cross-section of a leaf showing the glandular trichomes (scale in a, b, c are 50 µm and in d, e, is 25 µm).(1) epidermal cell, (2) cell wall, (3) stoma, (4) glandular trichome, and (5) striped dermis around the stoma.

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The characteristics of the lower epidermis are resembling those of the upper epidermis, but its cells have slightly wavy walls, and their average dimensions are 130.8 µm in length, and 71 µm in width.
Average number of trichomes is 2.3 trichomes / 0.25mm2 (9.2 trichomes/ 1mm2). The average number of stomata is 19.5 stomata / 0.063 mm2 (312 stoma / 1 mm 2), the average dimensions of which are 41.5 µm in length and 26 µm in width. (Figure 1-B).

3.1.2. Stem epidermis consist of rectangular cells, vertical walls on the cellulosic surface, thin, straight and un-perforated. Their average dimensions are 133.1 µm in length and 59.1 µm in width (Table 2).

Table 2: The features in the epidermal cells of *R. vesicarius* stem measured in µm.

Averages of parameters in stem epidermis						
Treatments ppm	cells dimensions (µm)		Trichomes No / 0.25 mm2	Stomata No / 0.63 mm2	stomata dimensions (µm)	
	L	W			L	W
Control	133.1	59.1	2.0	7.2	40.5	28.5
BA 50	143.0	61.1	3.4	4.0	25.8	21.0
BA 500	133.7	62.9	4.1	5.0	24.1	17.1
LSD at 5%.	11.86	0.56	0.36	0.56	1.12	1.06
LSD at 1%	16.22	0.79	0.51	0.79	1.57	1.49

L: Length; W: Width; BA: benzyl adenine
average indicators in the stem epidermis cells

The dermis is thin and smooth. The trichomes are like their counterparts in the leaf, but they are of few with average number of 2 trichomes / 0.25 mm2 (8 hairs / 1 mm2).

The stomata are similar to the ones in the leaf, but they are also few. The average number of stomata is 7.2 stomata/ 0.063mm2 (72 stomps / 1mm2), whereas the average dimensions are 40.5 µm in length and 28.5 µm in width (Figure 1-C).

3.2 Effect of low concentration of Benzyl adenine (50 ppm BA)

3.2.1 Leaf epidermis

The upper epidermis shows similar results as it is in control, but the average of cell dimensions was 136.5 µm in length and 78.4 µm in width. The average number of trichomes was 3.6 trichomes. The average number of stomata was 21 stomata, and the average dimension was 39.2 µm in length and 27.7 µm in width (Figure D-2).
The lower epidermis also revealed similar results to its counterpart in the control; however, its average dimensions were 27.7 µm in length and 53.9 µm in width. The average number of trichomes was 4.8. The average number of stomata was 22 stomata, and its average dimensions were 27.2 µm in length and 21.7 µm in width. (Figure E-2).

3.2.2. Stem epidermis

The characteristics of the epidermis in the stem did not differ from its counterparts in the control, except that the average dimensions of the cells were 143 µm in length and 61.1 µm in width. The average number of trichomes was 3.4 trichomes. The average number of stomata was 4, and its average dimensions were 25.8 µm in length and 21 µm in width (Figure F-2).

3.3. Effect of high concentration of benzyl adenine (500 ppm BA)

3.3.1. Leaf epidermis

The upper epidermis in the leaf was similar to its counterpart in control plants, except that its average cell dimensions were 102.8 µm in length and 64.2 µm in width, the average number of trichomes was 5.2 trichomes. The average number of stomata was 20.8. The average of dimensions was 33.8 µm in length and 24 µm in width. (Figure G-2).
The lower epidermis showed similar results as it is in control, but their average dimensions were 81.7 µm in length and 52.8 µm in width. The average number of trichomes was 5.1 trichomes, whereas the average number of stomata was 23 stomata. Their average dimensions were 26 µm in length and 22 µm in width (Figure H 2).

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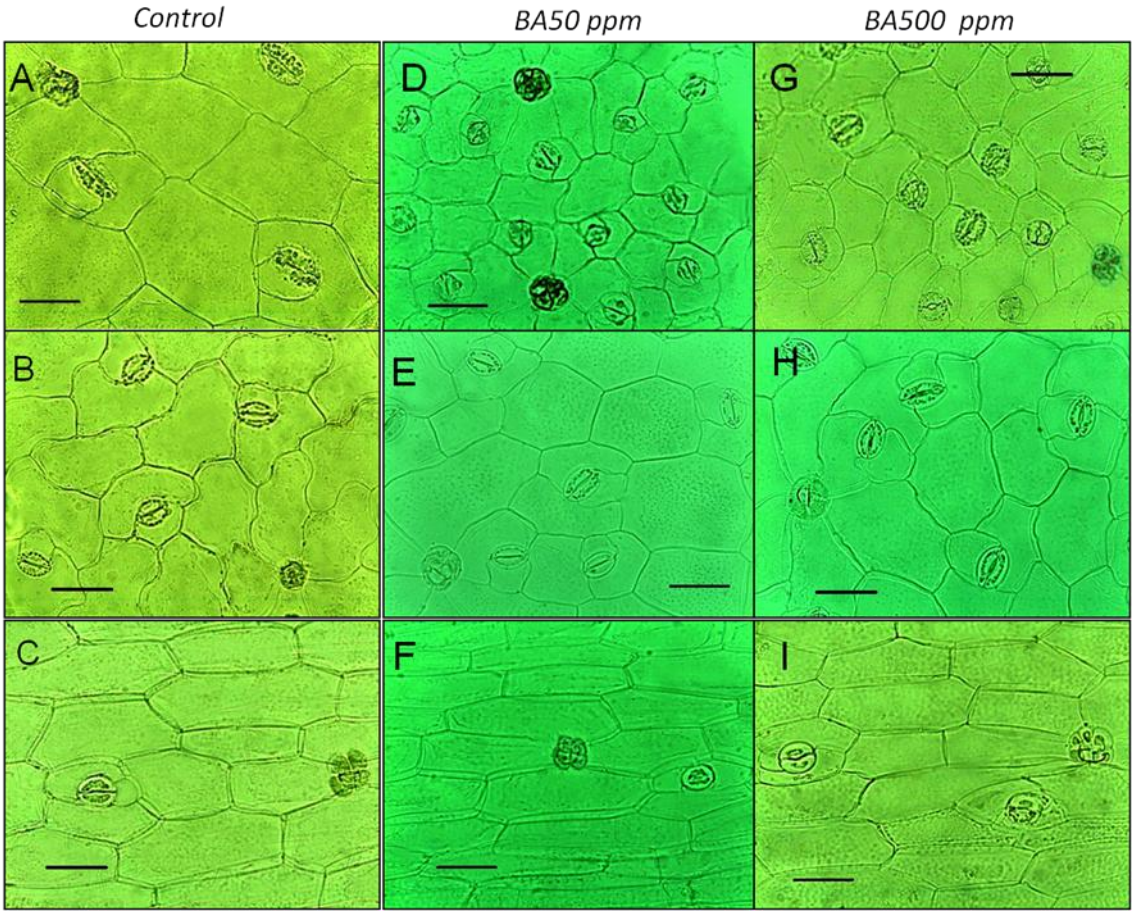

Figure (2) Epidermal peels from the epidermis of the leaf and stem of *R. vesicarius* in the control and Benzyl adenine (BA) treatments: (A) a peel in the upper epidermis of the leaf, (B) a peel in the lower epidermis of the leaf, (C) a peel in stem epidermis (Control). (D), (E), and (F) peels in the 50 ppm BA treatments, (G), (H), and (I) peels in 500 ppm BA treatments. (Scale 50 µm). (1) epidermal cell, (2) cell wall (3) stoma, and (4) glandular trichome.

3.3.2. Stem epidermis

The epidermis in the stem was similar to the epidermis of its counterparts in the control plants. Its average dimensions were 133.7 µm in length and 62.9 µm in width. The average number of trichomes is 4.1 trichomes. The average number of stomata was 5 stomata, with an average dimension of 24.1 µm in length and 17.1 µm in width (Figure I-2).

4. DISCUSSION

The comparison of the epidermal characteristics of the plants treated with the low concentration of benzyl adenine (50 ppm BA) and the plants treated with a high concentration of benzyl adenine (500 ppm BA) with the epidermal characteristics of the plants in control revealed the following results. The dimensions of the epidermal cells of the leaves of plants treated with both low and high concentrations of BA decreased. However, this decrement was not significant in the upper epidermis of the leaf (Table 1). Moreover, the dimensions of the epidermal cells of the stem of plants treated with both low and high concentrations of BA increased, but it is an insignificant increment (Table 2). These results were indicating that the two concentrations of (BA) may inhibit the growth and elongation of epidermal cells in the leaves of *R. vesicarius* but not in the stem. Our results are consistent with the results of the previous studies that reported the cytokinin has a role in increasing growth in plant parts (El-Ghamery and Mousa, 2017; Ibrahim *et al*., 2018; Lee *et al*., 2014; Solórzano-Cascante *et al*., 2018). However, they do not agree with the results of studies that mention that cytokines have a role in increasing the size of the cells (Arora *et al*., 1959; Glasziou, 1957; Letham, 1966; Powell and Griffith, 1960).

The shapes of cell walls that are vertical on the surface of both leaf and stem epidermis in plants exposed to both concentrations of (BA) were not affected and did not undergo an apparent change in the integrity of the walls or their undulation (Fig. D, E, F, G, H, I 2).

It became clear that the increase in the number of trichomes in both leaf and stem epidermis for the plants exposed to low and high concentrations of BA (Table 1). This increase can be any evidence that the treatment of *R. vesicarius* plants with the two concentrations of BA (50 and 500 ppm) influence the formation of the trichomes in the early stages of epidermis formation, and these results are in alignment with suggestions Meinke (Meinke, 1992).

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The number of stomata in the leaf of plants that exposed to both concentrations of BA was increased (Table 1). This result is consistent with the results of Stoyanova-Koleva (Stoyanova-Koleva *et al*., 2012) and Wang (Wang *et al*., 1997) who reported that cytokinins increases the number of stomata in the epidermis. However, the number of stomata in decreased in the stem epidermis of the plants treated with the two-BA concentrations (Table 2). This may be due to the slight increment in the dimensions of the cells, or the dimension of the stomata itself, or that the growth (BA) regulator has an inhibition effect on the initiation of the stomata.

The dimensions of stomata were not significantly affected in the upper epidermis of the leaf under a low concentration of BA. However, the dimensions of stomata decreased significantly in the lower epidermis of the leaf in response to the high BA concentration. Moreover, stomata dimensions of both upper and lower epidermis decreased significantly in response to low and high concentrations of BA (Table 1). The dimensions of epidermal stem cells decreased significantly in the plants that exposed to the low and high concentration of BA (Table 2). These results conflict with (Wang *et al*., 1997), who reported that cytokinin has a role in increasing the dimensions of stomata. However, these results may indicate that the growth regulator (BA) plays a role in inhibiting the elongation of the guard cells and thus the small size of the stomata or their survival as in the control epidermis of the plant.

CONCLUSION

The results revealed that the spraying of *R.vesicarius* plants with the growth regulator (BA) did not have apparent effects on some epidermis characteristics such as the cuticle layer, type of trichomes, shape, and type of stomata, and the multiplicity of cell walls in the surface view and undulation. However, it had effects on other characteristics such as the dimensions of the cells, the number of trichomes, the number of stomata per unit area, and the stomata dimensions. These findings may contribute to increasing the tolerance of R.vesicarius plants to environmental conditions and improving its productivity.

DATA AVAILABILITY

Data needed is available in this paper.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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