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Growth, Yield, and Biochemicals of Dill (*Anethum graveolens*) and Fennel (*Foeniculum vulgare*) Plants Under Salinity Stress

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ABSTRACT

This study was conducted during 2015 and 2016 seasons Faculty of Agriculture and Natural Resources, Aswan University**,** to compare two species under salinity stress; Dill **(***Anethum graveolens*) and Fennel (*Foeniculum vulgare***)**. Also, the study aimed to identify the biochemical changes to understand tolerance mechanisms. In pots experiments, plants were exposed to three levels of salinity stress 1000, 2000, 3000 ppm as well as control.Data showed that significant reductions were observed in shoot dry weight and seeds yield in Dill plants, but not in Fennel plants. Both species showed significant increases of Na^+ accumulation in plants shoot under stress. The accumulated Na^+ was much higher in Dill than that in Fennel at high salinity stress level (3000 ppm). Fennel had higher K^+ content under control and 1000 ppm treatment. Intensity of proteins band relatively decreased with increasing salinity stress level. Moreover, some protein bands disappeared under salinity stress including130 and 10 KDa for Fennel plants, and 175, 95, 80, 48, and 24 KDa for Dill plants. These results indicated that Dill suffered more damages under salinity stress because of accumulated Na^+ whether on growth and yield level or biochemical level, while Fennel had high tolerance ability to stress. Moreover, both species showed acclimation mechanism to salinity stress through biochemical changes that could be required to avoid dangerous affects and to alleviate salt stress. We recommend using saline irrigation water for cultivation of Fennel plant under South Valley and Aswan conditions.

INTRODUCTION

Salinity stress is strongly influencing food security by causing severe damages to growth and productivity of plants, especially in arid and semi-arid regions (Rasool *et al*., 2013). According to FAO (2011), about 30% of the irrigated land on earth is facing such salinity. In arable soils, sea water and the irrigation water that contains sodium chloride (NaCl) considers the main sources of the accumulated salts (Tester and Davenport , 2003). Improving crops tolerance to salinity stress is a major challenge for plant breeders toward sustainable global food production (Flowers, 2004). Several researches have explored the molecular and physiological mechanism of salinity tolerance, however the key traits that confer such tolerance still unknown (Bartels and Sunkar, 2005; Silva *et al*., 2014).

Salt tolerance in plants is a complicated mechanism included morphological, physiological and biochemical processes (Tabaei-Aghdaei *et al*., 2000; Munns *et al*., 2006). The alteration in cell phenotypes to survive and grow in the presence of high levels of NaCl is correlated with biochemical and physiological changes which in turn involve in gene expression alteration (Singh et al., 1985). Many previous researchers studied the variation in SDS-PAGE protein patterns of plants in response to salinity. Moreover, the response to salinity stress through protein changes might be a reason for salinity tolerance in this condition (Lusardi *et al*., 1991; Arefian *et al*., 2014). This variation includes the over-expression; new expression and suppression of some proteins (Lusardi *et al*., 1991). In Wheat, adaptation to salinity depends on the expression of salt-induced proteins (Dell and Spada, 1992). In *Phaseolus vulgaris,* (Zeid, 2004) reported that changes in gene expression either by the repression or induction of some new genes are required for salt-stress tolerance. In rice, decreasing in protein in leaves and chlorophyll is as a result of treatment with salinity (Harinasut *et al*., 1996). In *Cicer arietinum,* the salinity induced changes in protein banding patterns (Johnson *et al*., 2012). Some protein bands intensities decreased under salt stress (Dell and Spada, 1992; Smart, 1994).

Fennel and Dill are highly aromatic and flavorful herbs with medicinal uses belonging to family *Apiaceae*, *Umbelliferae* (Constance, 1971; Pimenov and Leonov, 1993). In previous study, we examined the differences in salinity tolerance at germination stage and at early growth stage among five *Apiaceae* species; Caraway, Celery, Dill, Fennel and Parsley. Fennel and Dill showed high tolerance to salinity stress at germination stage by no significant decreases in germination rate. At early growth stage, Fennel showed high tolerance meanwhile Dill was sensitive to salinity stress (Soliman and El-Shaieny 2014). In this study, comparison experiment was conducted between Fennel and Dill plants under different salinity levels. The main objective was to clarify the effect of salinity stress growth on yield and biochemical composition.

MATERIALS AND METHODS

Plant materials and growth conditions

This experiment was conducted in the Agricultural Experimental Farm, Aswan University, Aswan, Egypt during 2015 and 2016 seasons. Two species belonging to family *Apiaceae* were used; Dill (*Anethum graveolens*) and Fennel (*Foeniculum vulgare*). Seeds were sown in 15-cm plastic pots filled with clay and sand (1:1) in the end of November 2015. After two months, the plants were exposed to salinity stress using NaCl with levels of 1000, 2000, or 3000 ppm as well as control. On May 2016, the plants were harvested and air dried. The plant growth was estimated as dry shoot weight, and the yield was estimated as seeds weight per plant. Dried shoot samples (0.2 g) was

digested with 10 ml sulfuric acid (H_2SO_4) at 200°C for 2 h. After cooling, 5 ml 30% hydrogen peroxide (H_2O_2) was added and then heated again at 200°C for 2 h. The digested samples were completed to 50 ml using distilled water, and Na^+ and K^+ contents were measured using Flame spectrophotometer (Kalra, 1998).

SDS-protein electrophoresis

Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique was used to separate the total protein fractions and it was performed according to the method of Laemmli (1970), as modified by Studier (1973). Total proteins were extracted from fresh leaves of Dill and Fennel plants, which were taken from the control and the three treatments with NaCl (1000, 2000 and 3000 ppm). Protein fractionations were performed exclusively on vertical slab (19.8 cm × 26.8 cm × 0.2 cm) gel using the electrophoresis apparatus manufactured by LABOCONCO. The bands were detected and analyzed using Total Lab software.

RESULTS AND DISCUSSION

Table 1 showed that growth and yield of Dill plants represented by dry shoot weight and seeds weight decreased significantly under salinity stress, especially at 3000 ppm treatment. On contrast, Fennel plants showed no significant changes in both growth and yield under salinity stress compared to control. This is consistent with our finding in previous study at early growth stage as Fennel showed high tolerance while Dill was sensitive to salinity stress (Soliman and El-Shaieny, 2014). This result confirmed that Fennel is tolerant to salinity stress compared to Dill plant.

Table 1. The Means±SE (Standard error) and *F* value of Dill and Fennel yield (seeds and shoot yield) under different level of salinity stress. Tukey-Kramer HSD method was used to compare all pairs of means.

Salinity level	Dill		Fennel	
	Seed (g/plant)	Shoot (g/plant)	Seed (g/plant)	Shoot (g/plant)
control	0.162±0.021^{a}	0.348±0.036^{ab}	1.060±0.379^{a}	1.014±0.222^{a}
1000 ppm	0.102±0.017^{ab}	0.500±0.046^{a}	1.824±1.090^{a}	2.306±1.314^{a}
2000 ppm	0.088±0.022^{bc}	0.478±0.106^{a}	1.574±0.970^{a}	1.034±0.313^{a}
3000ppm	0.020±0.008^{c}	0.158±0.017^{b}	0.882±0.187^{a}	1.928±0.357^{a}
F value	11.03***	6.62**	0.33	0.84

****, *** represent significance at probability levels of 1 and 0.1%, respectively**

Table 2 showed that Na^+ content increased significantly under stress, and the accumulated Na^+ was much higher in Dill shoot compared to Fennel at 3000 ppm treatment. On the other hand, K^+ content was much higher in Fennel shoot than Dill at control and salinity stress of 1000 ppm. The differences in K^+ content were not significant at 2000 and 3000 treatments. Dill plant showed fluctuation in K^+ content under salinity stress while K^+ content decreased significantly under stress in Fennel. Salinity stress tolerance is associated with inhibiting Na^+ accumulation and maintaining higher K^+ and K^+/Na^+ ratio in the cytoplasm of mesophyll cells (James *et al.*, 2006). The abnormal K^+/Na^+ ratio and accumulated salts inhibit enzymes activity and protein synthesis (Taiz and Zeiger, 2002). In this study, the accumulated Na^+ content increased twice in Fennel and three times in Dill shoots at 3000 ppm treatment. Although the significance increases in Na+ content under stress, Fennel dry weight was not affected. This result suggested the tolerance ability of Fennel to cope with Na^+ accumulation. The balance between K^+ and Na^+ may be involved as a mechanism of tolerance to salinity stress.

Table 2. The Means±SE (Standard error) of Sodium (Na^+) and potassium (K^+) contents in Dill and Fennel under different level of salinity stress, in addition to the differences (*F* value) between species and among treatments.

Salinity level	Na+ content (mg/g DW)			K+ content (mg/g DW)		
	Dill	Fennel	*F* value	Dill	Fennel	*F* value
control	17.2±0.5	16.8±0.5	0.26	13.5±0.4	17.3±0.6	30.5***
1000 ppm	30.2±1.0	25.1±0.8	17.03**	10.8±0.3	13.3±0.4	21.31**
2000 ppm	23.7±0.8	28.9±0.9	18.71**	14.4±0.5	13.9±0.5	0.66
3000 ppm	50.8±1.6	35.9±1.1	58.9***	12.2±0.4	11.2±0.4	4.00
F value	197.43***	84.14***		15.3***	32.42***	

****, *** represent significance at probability levels of 1 and 0.1%, respectively**

To study the effect of salinity stress on protein banding patterns of Fennel and Dill plants, we extracted total proteins from plants with different concentrations of salinity stress, and the proteins were separated by SDS-PAGE technique. For Fennel, the protein banding patterns exhibited several variations (Fig. 1 and Table 3), whereas the bands 130 and 10KDa appeared in the control and 1000 ppm treatment and disappeared under salinity stress at levels of 2000 and 3000 ppm. Moreover, we observed decreasing in band intensities in the different treatments compared with the control.

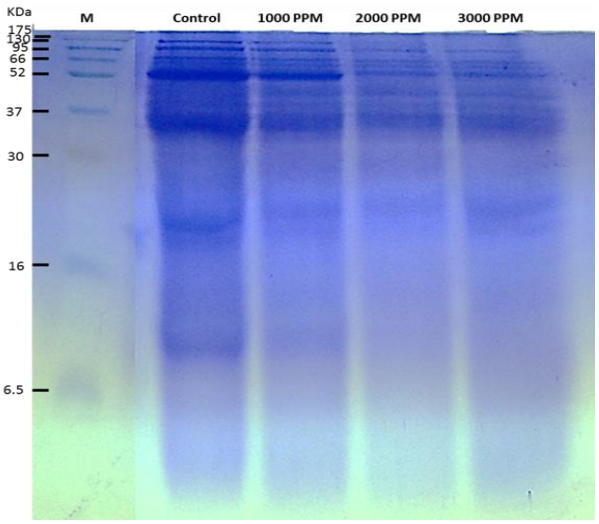

Fig.1 SDS-PAGE total protein extracted from Fennel plants; the control and three concentrations of NaCl (1000, 2000 and 3000 ppm)

The intensity of bands decreased with increasing salinity stress level, and all bands showed the lowest level of intensity at 3000 ppm treatment. For Dill plants

(Fig. 2 and Table 4), the bands with molecular weights of 175, 95, 80 and 48 KDa appeared only in the control and disappeared under salinity stress (1000, 2000 and 3000 ppm). More addition, the band 24 KDa appeared in both control and 1000 ppm treatments and disappeared in order to treatment with 2000 and 3000 ppm. Also, band intensities relatively decreased in under salinity stress compared to the control. Salinity stress effects on biochemical and physiological status of plants which influencing gene expression (Singh *et al*., 1985). Salt stress was associated with decrease or disappearance of some protein bands, and induction of a new protein band (Zhang *et al*., 2013). In this study, we found changes in protein concentration and disappearance of bands in order to salinity stress treatments.

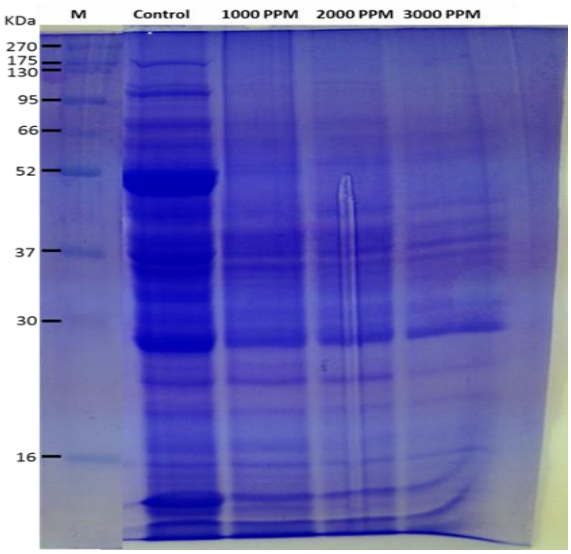

Fig.2 SDS-PAGE total protein extracted from Dill plants; the control and three concentrations of NaCl (1000, 2000 and 3000 ppm)

The presence of salt with different concentrations could make alteration in protein concentration and could inhibited protein synthesis. Similar results were observed in bean (Keshavarz and Sanavy, 2015), in rice and soybean cultivars (Lutts *et al*., 1996; Misra *et al*., 1997) and in chickpea (Johnson *et al*., 2012). Also, salt stress affects gene expression regulation whereas changes in protein synthesis could be due to alteration in regulation at transcription level and/or at post-transcription level, or due to alteration in protein degradation rates (Mohammed *et al*., 2012). Moreover, many of previous studies reported alteration in SDS-PAGE protein banding patterns in response to salinity. This alteration includes over-expression, new synthesis and suppression of some proteins (Al-Naggar et. al., 2008). Suppression in protein bands, as an indicator of inhibited protein synthesis, was much clear in Dill plants compared to Fennel. This result suggested that Dill suffered more damages as a result of accumulated Na^+ than that in Fennel plant. Moreover, variation in protein concentration and/or bands disappearance could be required to avoid dangerous affects and alleviate salt stress.

Table (3) Densitometric profile for total protein profiles of control and three concentrations of NaCl on Fennel plants

Band size KDa	Control	1000 ppm	2000 ppm	3000 ppm
130	**1**	**1**	**0**	**0**
95	1	1	1	1
66	1	1	1	1
58	1	1	1	1
52	1	1	1	1
45	1	1	1	1
40	1	1	1	1
35	1	1	1	1
33	1	1	1	1
24	1	1	1	1
20	1	1	1	1
10	**1**	**1**	**0**	**0**

Table (4) Densitometric profile for total protein profiles of control and three concentrations of NaCl on Dill plants

Band size KDa	Control	1000 ppm	2000 ppm	3000 ppm
175	1	0	0	0
115	1	1	1	0
105	1	1	1	1
95	1	0	0	0
80	1	0	0	0
72	1	1	1	1
60	1	1	1	1
57	1	1	1	1
52	1	1	1	1
48	1	0	0	0
40	1	1	1	1
38	1	1	1	1
37	1	1	1	1
36	1	1	1	1
35	1	1	1	1
34	1	1	1	1
32	1	1	1	1
30	1	1	1	1
27	1	1	1	1
24	1	1	0	0
21	1	1	1	1
19	1	1	1	1
17	1	1	1	1
15	1	1	1	1
13	1	1	1	1
10	1	1	1	1
9	1	1	1	1
6	1	1	1	1
5	1	1	1	1

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النمو والمحصول والكيموحيوية في الشبت والشمر تحت ظروف الإجهاد الملحي

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أجريت التجربة في أصص وعرضت النباتات إلي ثلاث مستويات من الإجهاد الملحي بتركيزات ١٠٠٠ و ٢٠٠٠ و ٣٠٠٠ جزء في المليون بالإضافة إلي الكنترول (بدون معاملة). لمقارنة نوعين من النباتات تحت ظروف الاجهاد الملحي وهما الشبت والشمر أثناء موسمى ٢٠١٥، ٢٠١٦ فى منطقة مزرعة كلية الزراعة والموارد الطبيعية بمحافظة أسوان . أيضاً هدفت الدراسة الي توضيح التغيرات الكيموحيوية لفهم آلية المقاومة. وتوضح النتائج حدوث إنخفاضات معنوية في الوزن الجاف للمجموع الخضري ومحصول البذور لنبات الشبت، ولكن لم تظهر هذه الإنخفاضات في نبات الشمر. ولقد زادت كمية الصوديوم المتراكم في المجموع الخضري في كلا النوعين تحت ظروف الإجهاد، وقد كانت الكمية المتركمة من الصوديوم أعلي في الشبت مقارنة بالشمر عند المعاملة بتركيز عالي من الإجهاد الملحي (٣٠٠٠ جزء في المليون). واحتوت أنسجة الشمر علي كمية أكبر من البوتاسيم مقارنة بالشبت تحت ظروف الكنترول والمعاملة بمستوي ملوحة ١٠٠٠ جزء في المليون. بالنسبة كثافة حزم البروتين انخفضت نسبياً مع الزيادة في مستوي الاجهاد الملحي. علاوة علي ذلك بعض البروتينات قد اختفت عند المعاملة بالملوحة مثل حزم البروتين ١٣٠ و ١٠ KDa في نبات الشمر، وحزم البروتين ١٧٥ و ٩٥ و ٨٠ و ٤٨ و ٢٤ KDa في نبات الشبت. هذه النتائج توضح أن نبات الشبت يعاني أضراراً بالغة عند التعرض للملوحة وذلك علي مستوي النمو والمحصول والتغيرات الكيموحيوية نتيجة تراكم الصوديوم في الأنسجة، بينما نبات الشمر أظهر قدرة عالية علي مقاومة الاجهاد الملحي. علاوة علي ذلك فان كلا النوعين أظهروا آلية الأقلمة للاجهاد الملحي من خلال التغيرات الكيموحيوية والتي تعتبر ضرورية لتجنب وتخفيف الأضرار الخطيرة الناتجة من الإجهاد الملحي. ومن خلال هذه الدراسة نوصي بإستخدام مياه الري المالحة في زراعة نبات الشمر تحت ظروف جنوب الوادي وأسوان.