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Research Article

The effect of salinity on some morphological and physiological characteristics of three varieties of (*Arachis hypogaea L.)*

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ABSTRACT:

Peanut belongs to Fabaceae family that because of the high oil quality and protein content is cultivated in 108 countries. In Iran, peanut is cultivated in about 3000 hectares of which 2584 hectares are located in Guilan province. Since salinity is expanding in Iran and peanut is mostly cultivated in the coastal area of Guilan, it is increasingly facing salinity stress. In this study, to examine the effect of salinity (0, 50, 100 and 150 mm NaCl) on the morphological and physiological characteristics of three cultivars of peanuts (Local cultivar of Guilan, ICGV96177 and ICGV03060) a laboratorial experiment was conducted as a factorial arrangement in a completely randomized design with three replications in the Faculty of Sciences, University of Guilan. Morphological characteristics were the seedling growth indices and the physiological characteristics included chlorophyll fluorescence, photosynthetic pigments, the free radicals neutralization, and non-enzymatic antioxidants including total flavonoid content and β-carotene. The results showed that increasing salinity, reduced the size of morphological traits of the seedlings in all cultivars, and also reduced the amount of chlorophylls and the quantum yield of photosystem II and increased F0. The results regarding the neutralization of free radicals and non-enzymatic antioxidants were significantly different, and at a concentration of 100 mM, there was a remarkable increase in the examined antioxidants. Generally, the results showed that under salinity stress, the local cultivar of Guilan was less sensitive to salinity compared to the other investigated cultivars.

Keywords: Peanut, Salinity, non-enzymatic antioxidants, Chlorophyll fluorescence

INTRODUCTION

Peanut from the family of Fabaceae is a topical legume which can feed human and poultry. Also, it can be good alternative for meat (Ahmadi, 1989). All parts of plant such as oil, protein, minerals are usable for human. Peanut's lipid is 42 – 52 percent, and its protein is 25-32 percent. There are many vitamin and minerals in peanut including vitamin A, vitamin B, Riboflavin, Folate, Mg, P, Mn, and some of the antioxidants compounds such as vitamin E and vitamin D (Seyed Sharif, 2007; Karra *et al*., 2013).

Peanut, the same as other oilseeds has a good compatibility to a large number of climates. It prefers low pH (6-6.5), but it can grow in the

areas with the pH of 5.5-7. High salinity is not appropriate for peanuts. Totally, salinity decrees the growth indices of the seedlings as well as the seed size (Smart, 1994). In general, peanut is used to produce oil and peanut butter at many countries. About 70 percent of peanut is produced in Asia (25.5 million tons) (Pandy *et al*., 2003).

In Iran, the most amount of peanut production (8691.8 tons) is occurring in Guilan province in 2583 hectare (Safar Zade, 2009). Peanut is facing salinity stress at most parts of the coastal area of Guilan province. So, salinity stress is a main challenge for the agricultural products of this province (Munns, 2005). Iran has dry and semi dry land and almost 15 percent of the land is under salinity stress. Hence, it is necessary to pay more attention to this problem and the related effects. Plant tolerance range is different regarding salinity stress, and it is important to select appropriate plant to cultivate in these areas (Khan *et al.,* 2003). The aim of this study was to investigate the effect of different levels of salinity on some morphological and physiological characteristic of three cultivars of peanut to identify tolerant varieties under salinity stress.

MATERIALS AND METHODS:

Part one: an investigation of germination reaction and peanut seedling growth:

The investigation of the peanut germination and seedling growth under salinity stress has been conducted at the research laboratory of the University of Guilan. The seeds of three cultivars of peanut (Local cultivar of Guilan, ICGV96177 and ICGV03060) were left at distilled water for 5 minute and then 2 minutes at 90 percent alcohol. At the next step, seeds were putted at 20 percent hypo sodium chloride for 5 minutes. Finally, seeds transferred to the sterilized Petri dishes containing 6 seeds and MS culture with 4 sodium chloride concentration (0, 50, 100, 150 mM) covered by Para film (Ranganayakulu *et al.*,2013). They were left at 65 humidity and 20 centigrade temperature in a germinator. The seeds germinated after 5 days. They were measured every other day at certain time. The seeds with 5mm radical length were assumed germinated seeds (Timothy *et al*., 2011). After 13 days, the length of radicle, length of plumule, length of radicle to length of plumule, seeding fresh weight, seeding dry weight, and also seeding growth were examined (Bajji *et al*.,2002).

Eq1:

$GP = (N_i/S) \times 100$

GP is germination percentage, N_i is the number of seed that germinated every day, and S is the number of the total seed.

Eq2: $GS = \Sigma Ni/ Ti$

GS is germination speed; Ni is seed that geminate every day, Ti is examination day.

SV also is seed vigor:

Eq3: $SV = (PL + RL) \times GP$

RL: length of radical, PL: length of plumule, GP germination percentage.

Second part: Planting in pots

Examination units contain pots by 20*20 dimension which filled by 3 kg soil. The germinated seeds of the three cultivars of peanut (Local cultivar of Guilan, ICGV96177 and ICGV03060) were located at 5 CM beneath the pot soil. The temperature in the laboratory was 28 degree centigrade (Hajar *et al*., 1993). The pots were irrigated 3 times every week, and salinity treatment started 4 weeks after seed cultivation with 0, 50, 100, and 150 mM salinity level 2 times every week (Hajar *et al*., 1993, Srivation *et al*, 2007). Chemical fertilizers consists of Urea, Ptassium Sulfate, Superphosphate have been used after seed cultivation in the pots (Salwa *et al*, 2010). After 8 weeks, the factors have been examined.

Determination of chlorophyll florescence:

Some leaves were putted at darkness for 30 minute, then the quantum yield of photosystem II, fm and F0 were investigated using a pulse-

amplitude modulated (PAM*)* Fluorometer *(*Mini-PAM; Walz, Effetrich, Germany) (Genty et al, 1989).

Determination of photosynthetic pigments (chlorophyll a, b, and carotenoids):

For the extraction of pigments, 0.5 gram of leaves squished in liquid nitrogen (Davoody Fard *et al.,* 2012). 20ml of 80 percent acetone were added and putted in the centrifuge for 10 minute. The separated part was transferred to other falcons. Then the absorption level was examined at 663, 645, and 470 nm. Finally, chlorophyll a, b, and carotenoids were determined (Arnon, 1967).

Sampling:

After 12 weeks, the leaves of all pots were collected. Half of the leaves were dried at 70 degree in an oven, and the other half transferred to a capsule which had liquid nitrogen at -70 degree to investigate non-enzymatic antioxidants.

Determination of antioxidants activity:

Extraction:

0.5 g of the dry leaves was putted in 1500µL solution which consisted of methanol acetic acid (85 percent) for 15 minutes. Then the solution centrifuged for 10 minute at 10000 rpm and then left at -20 degree for next steps (Bakhshi and Arakawa, 2006).

Antioxidants activity examined using the method of Kontogiorgis and Hadjipavlou-Litina (2005). 50 µL of the extraction and of 950 µL normal solution were transferred in a microtube and left in dark room for 30 minutes. Then the free radicals of the extraction calculated using the following equation:

$$\%DPPH_{sc} = (A_{cont} - A_{samp}) / A_{cont} \times 100$$

Total flavonoids investigation:

For flavonoids examination, 0.1 g of the dry leaves was putted in 3 ml Ethanol and centrifuged at 12000 rpm for 15 minutes. Then, the solution was left at hot water (80 degree) for 10 minutes. The extraction was analyzed in a spectrophotometer at 270, 300, 330 nm and flavonoids content was examined using the method of Krizek et al. (1993).

Determination of β -Carotene:

The method of Yamashita & Nagata (1992) was used for β-carotene examination. So that 0.25 g of the samples transferred to a falcon filled with liquid nitrogen adding 4ml acetone-hegzan. At the next step, the solution putted at darkness for 10 minute. It has been separated in two parts. The upper part of the solution was examined in a spectrophotometer at 453, 505, 645, and 663 nm and B-carotene, chlorophyll b, chlorophyll a were calculated using the following equations:

(β-carotene)= 0.216 A_{663} - 1.220 A_{645} - 0.304 A_{505} + 0.452 A_{453}

RESULTS

The peanut germination and seedling growth:

Investigation regarding the germination and seedling growth of the 3 cultivars of peanut (table 1) indicated that the effect of cultivar on the seedling fresh weight, length of radicle, length of plumule, length of radicle to length of plumule, and also germination speed and percentage were significantly different compared to the control plants ($p \leq 0.01$), however, this effect on the seedling dry weight was not significantly different. Also, the effect of salinity on the length of radicle, length of plumule, length of radicle to length of plumule, and also germination speed and seedling vigor were significantly different compared to the control plants $p<0.01$ but, this effect for seedling dry and fresh weight was not significantly different. The cultivar and salinity interaction on the seedling dry weight, length of radicle, length of radicle to length of plumule and total germination speed and percentage and also seedling vigor were significantly different compared to the control plants $p \leq 0.01$, however, this effect on the seedling dry weight and length of plumule was not significantly different (Fig.1).

According to the table 2, the mean comparison of the cultivar and salinity interaction showed that

the highest seeding fresh and dry weight, length of radicle, length of radicle to length of plumule, germination speed and seedling vigor were belong to the local cultivar of Guilan in control plants and the lowest length of radicle, length of radicle to length of plumule, seedling vigor were belong to the cultivar of ICGV03060 at 150 mM salinity level.

Table 1: Analysis of variance for investigated traits(morphological parameters) in *Arachis hypogaea* cultivars under salinity stress

		Average of squares								
	df	Seedling Fresh weight	Seedling dry weight	Length of radicle	Length of plumule	Length of radicle / plumule	sum(Leanth radicle + plumule)	Germinatin percent GP	Seed vigor SV	Germination speed GS
cultivar	2	2/14**	3/45^{ns}	470/350**	12/51**	7/65**	621/52**	2961/19**	8140191/19**	7/04**
salinity	3	2/03^{ns}	1/36^{ns}	149/01**	3/22**	2/93**	198/86**	1205/58**	2897622/74**	0/45**
Cultivar ×salinity	6	0/09**	7/89^{ns}	43/87**	0/15^{ns}	1/24**	44/91**	360/53**	410164/60**	0/31**
error	24	0/01	1/44	4/44	0/34	0/13	6/49	53/42	83057/92	0/02

ns and **: not-significant and significant at p≤0.01 , respectively.

Table 2: Comparison of means of 3 peanut cultivars and salinity interaction effects

cultivar	salinity	Seedling fresh weight	Seedling dry weight	Length of radicle	Length of plumule	Length of radicle / plumule	The sum of radicle to plumule	GP	SV	GS
Local cultivar of guilan	0	1.99a	1.23a	29.1a	6.06a	5.05a	35.17a	100.00a	3516.66a	2.97 a
	50	1.62b	0.94b	18.83b	5.44a	3.15b	24.30b	100.00a	2428.00b	2.10b
	100	1.53b	0.91b	15.44b	5.06a	3.05b	20.50b	100.00a	2050.00b	2.00b
	150	1.45	0.87b	9.33d	4.44b	1.98d	13.80e	89.00c	1260.33d	1.87c
ICGV96177	0	0.92e	0.60bc	11.78c	5.22a	2.28c	17.30c	100.00a	1732.00c	1.23e
	50	1.12d	0.61bc	12.83c	5.00a	2.57bc	17.80c	100.00a	1783.00c	1.67d
	100	1.15d	0.65bc	10.11d	4.78ab	2.09c	15.00e	94.33b	1420.67 d	1.23e
	150	1.08d	0.62bc	7.17e	4.27c	1.71e	11.47f	77.67d	898.00e	1.37e
ICGV03060	0	0.84ef	0.54bc	8.87e	4.33c	2.09c	13.10e	100.00a	1311.00d	1.07f
	50	0.78f	0.46c	5.24f	3.22cd	1.68e	8.47g	67.00e	565.33f	0.53h
	100	0.82f	0.50bc	5.17f	3.11cd	1.74e	8.27g	55.67f	466.67f	0.70g
	150	0.84ef	0.49c	3.95g	2.56d	1.58f	6.50h	50.00f	324.67g	0.53h

Means followed by similar letters in the same column are not significantly different at p≤0.05.

Chlorophyll fluorescence and photo system parameters:

The analysis of variance regarding chlorophyll fluorescence and photosystem factors indicated that the effect of salinity on chlorophyll a, chlorophyll a+b, and Fm were significantly different compared to the control plants (p≤0.01). Also, the effect of salinity interaction on chlorophyll a, chlorophyll a+b, and F0

and quantum yield of photosystem II at p≤0.01 and chlorophyll b at p≤0.05 were significantly different compared to the control plants.

Table 3: Analysis of variance for investigated traits (photosynthetic parameters) in *Arachis hypogaea* cultivars under salinity stress

Changing refrence	Average of squares							
	df	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Carotenoid	F_0	F_m	Quantum yield
cultivar	2	2.7^{*}	0.17^{ns}	4.2^{*}	0.01^{ns}	58.11^{ns}	2981.19*	0.01^{ns}
salinity	3	3.8^{**}	0.22^{*}	5.86^{**}	0.01^{ns}	1019.93^{**}	1174.11^{ns}	0.01^{**}
cultivar× salinity	6	1.56^{*}	0.22^{*}	2.94^{*}	0.01^{ns}	970.81^{**}	3771.63^{**}	0.01^{**}
error	24	0.57	0.07	0.99	0.01	149.52	666.67	0.01

ns and **: not-significant and significant at p≤0.01,respectively.

According to table 4, the results indicated that the highest amount of chlorophyll a, b, a+b were 4.25, 1.24, 5.5 (Mg/gfw)related to the local cultivar of Guilan at 50 mM salinity level and the lowest amount of chlorophyll a, a+b and also Fm, were belong to the local cultivar of Guilan at 150 mM salinity level.

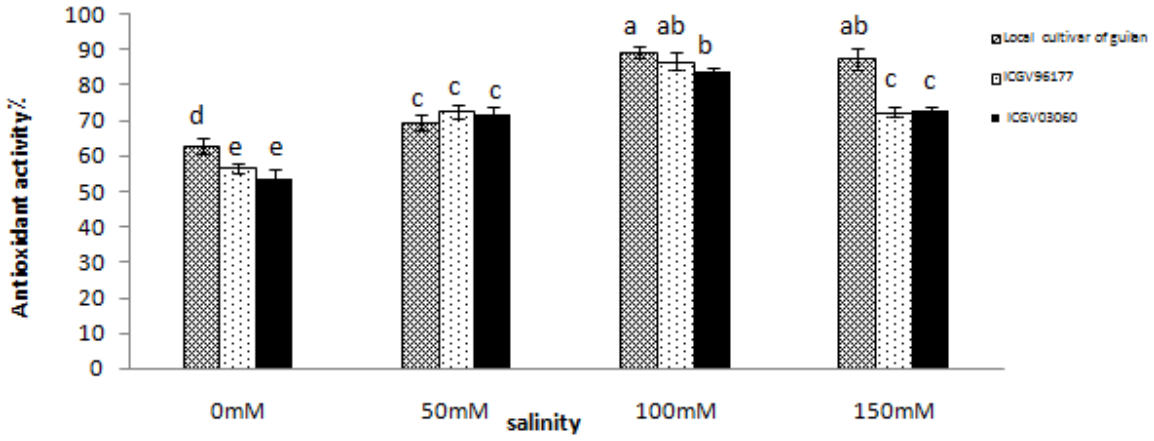
Table 4: Comparison of means of 3 peanut cultivars and salinity interaction effects.

salinity	cultivar	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	carotenoid	F_0	F_m	Quantum yield
Local cultivar of guilan	0	3.62ab	0.91b	4.53b	0.08a	185.33a	896a	0.79b
	50	4.25a	1.24a	5.5a	0. 16 a	167.33b	843.33b	0.80a
	100	3.28 b	0.85 b	4.14b	0.09 a	149d	855.33b	0.82a
	150	1.27 f	0.28 d	1.55 e	0.01 a	184.33a	884.33a	0.80 a
ICGV96177	0	2.87c	0.75 b	3.63bc	0.11a	143d	816c	0.82a
	50	1.55e	0.34 d	1.89d	0.02 a	159c	870.33a	0.81 a
	100	2.83c	0.84 b	3.68bc	0.13 a	189a	888a	0.79 b
	150	1.89e	0.63bc	2.53 c	0.10 a	177.67 b	801d	0.77 c
ICGV03060	0	2.63cd	0.76b	3.4 bc	0.18a	169.66b	873.67a	0.80a
	50	2.46 d	0.61bc	3.09bc	0.06 a	155.33b	818c	0.81 a
	100	2.39d	0.55 c	2.94bc	0.07 a	157.66 c	839.33b	0.81 a
	150	1.64e	0.45 c	2.09c	0.02 a	192a	834b	0.77 c

Means followed by similar letters in the same column are not significantly different at p≤0.05

Neutralization of free radicals:

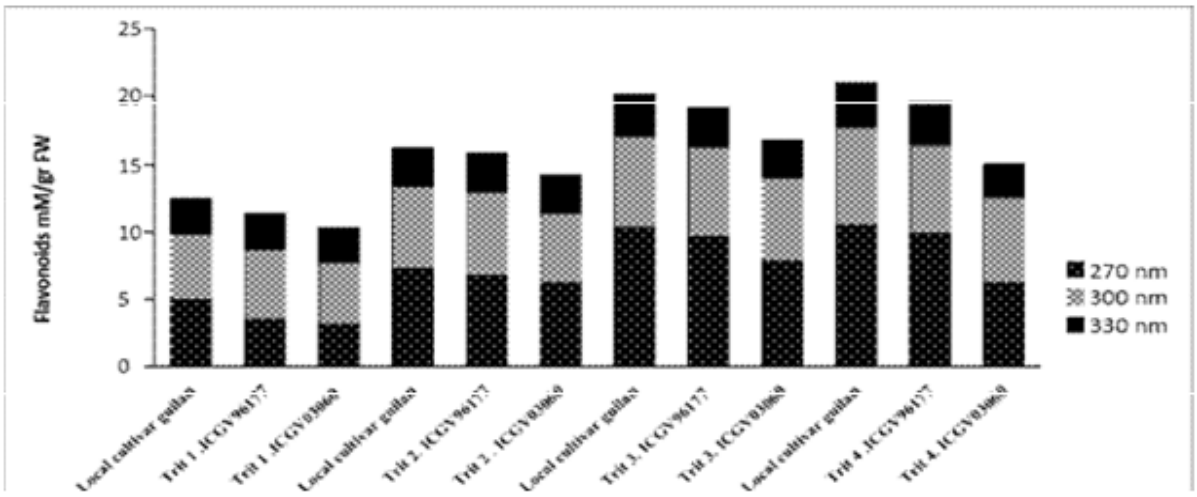
The results regarding the antioxidant activity of the leaves of different cultivars of peanut indicated that this activity was considerably different during increasing the salinity level from 0 to 150 mM. The highest amount of antioxidant activity was 62.81 percent in the local cultivar of Guilan (Fig. 1).



Means followed by similar letters in the same column are not significantly different at p≤0.05
Fig 1: Antioxidant activity of 3 cultivar of peanut leaves under salinity stress

Total flavonoids content

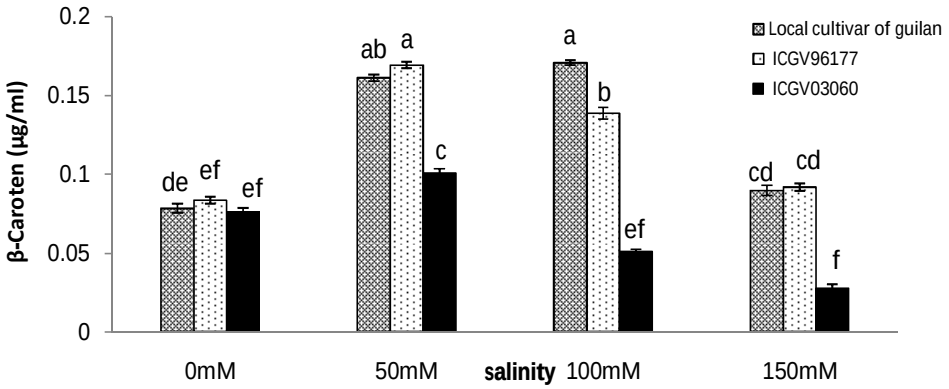
The results of total flavonoid content of 3 peanut cultivars indicated the highest flavoniod content was in the leaves of local cultivar of Guilan and the lowest in the cultivar of ICGV03060. Flavoniods content increased at 100 mM salinity level and it was almost similar at 150 mM but, in the cultivar of ICGV03060, this content increased at 100 mM and after that it decreased at 150 mM salinity level.



Means followed by similar letters in the same column are not significantly different at p≤0.05
Fig 2: The content of flavonoids of 3 cultivars of peanut leaves under salinity stress.

β -carotene content:

The results indicated that the lowest amount of β -carotene was belonging to ICGV03060. By increasing salinity level, the β –carotene content increased in the local cultivar of Guilan until 100 mM at highest level, then the content decreased at 150mM salinity level. In the two other examined cultivars, the β –carotene content increased until 50 mM, and then it decreased at 100mM as well as at 150 mM salinity level.



Means followed by similar letters in the same column are not significantly different at p≤0.05

Fig 3: The content of β -carotene of 3 cultivars of peanut leaves under salinity stress.

150mM level of salinity in ICGV03060 cultivar,

DISCUSSION

Respectively. The result of this study is in

The germination and seedling growth:

Seed germination mostly decreases under salinity stress. Salinity stress may also postpone seed germination because of the Ion toxicity as well as the reduction of soil water potential (Ashraf and Hariis, 2004), and harmful chemical activity in the seedlings (Ejazrasll and Rehman, 1997). Salinity can also prevent seedling growth (Wang *et al*, 2010). The result of the current study showed that the highest percentage of seedling growth was belonging to the local cultivar of Guilan and ICGV96177, and the lowest percentage was belonging to ICGV03060.

Germination leading to radicle exit from the seeds occurs to uptake water from the environment, increase metabolism activities. If the osmotic potential of the environment increases because of osmotic stress, seed can no longer uptake water for seedling growth. So, osmotic potential of the environment can affect the seedling growth as well as the length of radicle and plumule (Baalbaki *et al*., 1990).

According to table 2, when salinity stress increased, the length of radicle and plumule decreased. Although, the reduction ratio of the length of plumule to the length of radicle was not significant, the highest and lowest amount of the length of radicl and length of plumule observed at control in the local cultivar of Guilan and at 150 mM level of salinity in ICGV03060 cultivar, respectively. The result of this study is in agreement with the result of Noor et al (2001) who found the length of radicle is more sensitive than the length of plumule under salinity stress. Seedling vigor decreased by increasing salinity level, so that at 150 mM salinity level was the lowest and in control plants was the highest. ICGV03060 had the lowest seedling vigor compared to other examined cultivars.

Chlorophyll fluorescence and photosystem parameters:

Chlorophyll fluorescence is one of the main factors showing the amount of photosystem damage and the impact of the environmental stresses, and identifies the level of tolerance of plants facing various stresses. In fact, the amount of chlorophyll fluorescence indicates thylakoid membranes health and efficiency of electron transition from photosystem II to photosystem I. The efficiency of photosystem II is presented by Fv/Fm (ratio of changing fluorescence to maximum level and the amount of Fv/Fm is much higher in tolerant varieties in stress conditions compared to the sensitive ones (Garriga *et al.*, 2014). The current results showed that in general, salinity stress increased the primary fluorescence (F0) and decreased

maximum fluorescence (Fm) and finally decreased the maximum quantum yield of photosystem II (Zhao *et al*., 2007). In fact, salinity decreased the electron transmission speed in photosynthetic electron transport chain.

According to this result, salinity decreased the amount of chlorophyll a, chlorophyll b, chlorophyll a+b. Reduction of the photosynthetic pigments can damage photosystem activities, and this result was in agreement with the findings of Neocels and Vasilakakis (2007).

Neutralization of the free radicals:

The result of this study indicated that with increasing the salinity level, neutralization of the free radical percentages will increase. This reduction at higher salinity level showed had negative effect on the plant antioxidants and decreased phenolic content as well as antioxidants capacity (Sidsel Fiska *et al*., 2009).

Total flavonoid content:

Flavonoids are consisting of flavons, flavonols, Isoflavonoid and anthocyanins etc. (Halliwell, 1995). According to the current results, the amount of flavonols increased by increasing the salinity level in the local cultivar of Guilan and ICGV96177 (Fig. 2). The highest total flavonoid content was belonging to the local cultivar of Guilan and the lowest in ICGV03060 cultivar. In fact, plants produce flavonoid of the leaves to prevent the oxidative reaction. Flavonoid content increased from 0 to 100 mM salinity level, and then it decreased at 150 mM. Chpar zadeh and Zarandi (2010) also reported that low levels of salinity increase flavonoid production and at high levels of salinity flavonoid content will decrease. In stress conditions, the synthesis of flavonoids is a mechanism to prevent Reactive Oxigen Species (ROS) synthesis (Selmar, 2008).

β -carotene content:

Carotenoids consist of carotenes (beta and alpha carotenes), and xanthophylls (lutein, flavoxantyn, luteoxanthine, zeaxanthin, violaxanthin etc.) and lycopene. Carotenoids can react with the free radicals and prevent the oxidation activities. β -carotene exists in chloroplasts and link to photosystem I, II (Havaux, 1998).

According to the result of this study, increasing the amount β -carotene as an antioxidant at lower salinity level (100mM) can be related to its role in decreasing the amount of ROS and the reduction of β –carotene content could be related to the decomposition of β –carotene at high salinity level (150mM) (Fig. 3).

CONCLUSION:

The current result regarding the germination reaction, seedling growth, photosynthetic pigments and antioxidants parameters indicated that all cultivars of peanut had reaction to different levels of salinity and under salinity stress, the local cultivar of Guilan was more tolerant than the other examined cultivars. The cultivar of ICGV96177 located at the second level and the cultivar of ICGV03060 was more sensitive than the other two cultivars facing salinity stress.

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