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EFFECT OF SALINE WATER ON SPROUTS GROWTH TRAITS, PROXIMATE ANALYSIS AND BIOGENIC AMINES OF EGYPTIAN BARLEY

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ABSTRACT

This study was carried out in horticulture Department, faculty of Agriculture, Ain Shams University and The Regional Center for food and feed (RCFF), Agricultural Research Center during 2018. For measuring three types of estimations, experiment included 4 treatments which were the combinations of 2 levels of sterilization and 2 levels of water salinity, in addition to untreated grains (dry grains). In type I, showed that sprout growth characters were not affected significantly by using sterilized grains except for radical and seedling fresh weigh. The highest hypocotyl, radical weight, seedling fresh and dry weight were obtained for non-sterilized grains using saline water. In type II, data revealed that proximate analysis was significantly increased with the using sterilized grains and highest carbohydrate content (79.84%) was found in dry barely grains .Also, the highest moisture, protein and lipid contents were found with barley sprout using tap water and sterilized grains. With type III, findings show detected that six biogenic amines were barley sprouts using saline water.

Key words. Biogenic Amine , Proteins, Salinity, Seed sprouting

INTRODUCTION

Barley is one the major cereal used for the production of several alcoholic and non-alcoholic products such as beers (powders and syrups).Also malt is an important trade commodity throughout the world as it is used in the brewing making process. One of the steps in the production of malt is barley hydration. In the steeping process the grain is soaked in water for 24 to 36 hours in order to increase its moisture to 42-44% (wet basis). Water uptake by the grain may be influenced by factors such as barley composition, after steeping, the grains are removed from water for the germination step under controlled temperature and moisture. The next step is grain drying (**Mayolle et al., 2012**).

Cereals is staple foods providing a major source of minerals, protein and carbohydrate as well as containing a range of phytochemicals which may provide some of the health benefits seen populations consuming diets based on plant foods (**Goldberg, 2003**).Barley has the highest content of non-starch polysaccharides among all cereal grains (**Boros et al.,1996**). Steeping time and temperature is one of the critical processes influencing the modification of barley malt (**Bryce et al., 2010**). Sprouting is a simple technique to germinate the seeds for the improvement of their nutritive value (**Amal et al., 2007**). During germination, some major substances such as starch and protein will decompose to small molecules (**UK Malt 2011**). The enzymes convert the complex compounds of protein into albumin and globulin thus, improve the quality protein. They also improve the lysine content of grains (**Chavan and Kadam., 1989**).Several commercial products such as barley Alasairasalbh available, liquid and added to a variety of fermented and non-fermented foods to enhance the color, enzyme activity, flavor, sweet, nutrition and quality (**Bamforth et al., 1993**). Sprouting of grains affected the enzyme activity, increased total protein and changes in amino acid profile, crude fiber, increased sugars, certain and minerals, and loss of total dry matter (**Resh 2001**).Biogenic amine(BA) are basic nitrogenous compounds formed mainly by decarboxylation of amino acids or by animation and transamination of aldehydes and ketones (**Askar, 1986**). In virtually all foods that contain protein or

free amino acids and are subject to conditions enabling microbial or biochemical activity biogenic amine can be expected. Biogenic amines contain proteins, enzymes, cofactors, water, salt, and bacteria, and therefore represent an ideal environment for Biogenic amine (BA) production from free amino acids by decarboxylating enzymes of microorganisms during ripening (**Novella-Rodriquez *et al.*, 2000**). In non-fermented foods, the presence of biogenic amines above a certain level is considered as indicative of undesired microbial activity, of microbial spoilage. However, the presence of biogenic amines in food does not necessary (**Santos. *et al.*, 1985 and Vidal-Carou , *et al.*, 1990**).

Many researchers studied the nutritional value of wheat grass juice and it health benefit (**Shirude, 2011; Rana *et al.*, 2011 and Dina Anwar *et al.*, 2015**). It was reported that wheat grass can be traced back in history over 5000 years, to ancient Egyptian and perhaps even early Mesopotamian. It is purported that ancient Egyptians found sacred the young leafy blades of and prized them for their positive effect on their health and vitality. Wheat grass can extracts for consume by two different types like squeezed or chewed then throw out. Wheat grass has quickly become “the new age espresso” offered in smoothies and juices, salads, and even in powders and tablets and is one of the cereal grasses mostly used as a health drink. (**Ben- Arye *et al.*, 2002**).

The aims of our study were to evaluate the effect of saline water on sprouts growth traits, proximate analysis and biogenic amines of Egyptian barley sprouts.

MATERIALS AND METHODS

This study was carried out in Horticulture Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt and the Regional Center for Food and Feed (RCFF), Agricultural Research Center (ARC), Giza, Egypt during 2018. Egyptian Barley grains were obtained from the Crops Research Institute Agriculture Research Center, Giza Egypt.

Grains sprouting :

Sprouting of barley grains experiment was carried out in the glass jar as described by **Abdallah (2008)**. The experiment included 4 treatments which were the combinations of 2 levels of sterilization and

2 levels of water salinity, in addition to untreated grains (dry grains). In this respect, the barley grains were divided into three parts, i.e. dry grains, sterilized grains and unsterilized grains. Sterilization was done with treating grains by calcium hypochlorite 2% for 20 minutes. Each of sterilized and unsterilized grains soaked in tap water or in NaCl 1000 ppm solution for sprouting procedure.

Assessments :

Three types of assessments were estimated as follow:

Type I. Sprouting growth:

After three days old of treating grains, samples (ten sprouts) etiolated sprouts were taken from each treatment for measuring sprout characters (length and fresh weight of hypocotyl and sprout).

Type II. The Proximate analysis:

Three days old sprouts from each sprouting treatment and dry grains (oven dried at 60 °C for 72 hr.) were ground to pass through a 40 mesh sieve; the samples were stored until analysis at 5°C. Ash, total protein, lipids and crude fiber contents of samples were determined according to **AOAC (2012)**. Total carbohydrates were determined by subtracting. Energy value was calculated using the method [(9 x fat) + (4 x carbohydrate) + (4 x protein)] as described by **Chinma and Igyor (2007)**.

Type III. Biogenic amine determination:

Six biogenic amines included histamine, tyramine, cadaverine, spermine, putrescine and spermidine were extracted and determined in all tested samples using HPLC (**Mohamed et al, 2013**)

Reagents :

- a. Dansyl chloride solution: 500mg of dansylchloride were dissolved in 100 ml acetone.
- b. Standard solutions: Stock standard solutions of the tested amines: 25mg of each standard pure amine histamine-2HCl, tyramine - HCl, cadaverine - 2 HCl, putrescence -2 HCl and β-phenyl ethylamine were dissolved in 50 ml distilled water individually. 3.
- c. Working standard solutions. Two milliliters of each stock standard solution were pipetted into 100 ml volumetric flask and diluted to volume with 5% trichloroacetic acid (TCA). This solution is prepared freshly (weakly) and stored in a refrigerator. Mobile phase solvents consist of solvent A, Acetonitrile: 0.02 N acetic acid (1:9) and solvent B, 0.02 N acetic acid: acetonitrile: methanol (1:9: 9).

Solvent A and B were used in gradient elution program as follow:

Time min.	Flow rate ml/min	Solvent		Curve
		A %	B %	
0	1	25	75	-
10	1	10	90	6
20	1	5	95	6
25	1	25	75	6

Apparatus:

High performance liquid chromatography (HPLC) (Waters 600) was used to dansylamines determination. The system equipped with delivery system, reverse phase CI8 Nucleosil column 250 x 4 mm, 10 μ m packing,(Macherey - Naggl). The detection was performed using U.V detector (Waters 486) at wavelength 254 nm using linear program of 25 min period and 1 ml / min constant solvent flow rate. Data were integrated and recorded using a Millennium Chromatography (Waters, Milford MA 01757).

Extraction :

Twenty five grams of barley sprouts was blended with 125 ml of TCA 5% for 3 min using a warning blender then filtration was done using filter paper Whatman No. 1. Ten milliliters of the extracts was transferred into a suitable culture tube with 4g NaCl and 1 ml of 50 % NaOH then extracted three times by 5 ml n-butanol / chloroform (1:1 v/v) stoppered and shaken vigorously for 2.0 min. followed by centrifugation for 5.0 min. at 3000 rpm and the upper layer was transferred to 50 ml separating funnel using disposable Pasteur pipette. To the combined organic extracts (upper layers), 15 ml of n-heptane was added and extracted three times with 1.0 ml portions of 0.2 N HC1, the HC1 layer was collected in a glass stoppered tube. Solution was evaporated just to dryness using water bath at 95°C with aid of a gentle current of air.

Formation of dansylamines :

Two hundred μ L of each stock standard solution (or sample extract) were transferred to a culture tube and dried under vacuum. About 0.5 ml of saturated. NaHCO₃ solution was added to the residue of the sample extract (or the standard). Stoppered and carefully mixed to prevent loss- due to spattering. Carefully, 1.0 ml dansyl chloride

solution was added and mixed thoroughly using vortex mixer. The reaction mixture was incubated at 55°C for 45 min. About 10 ml of distilled water were added to the reaction mixture, stoppered and shaken vigorously using vortex mixer, then the extraction of dansylated biogenic amines was carried out using three times of 5.0 ml portions of diethylether, stoppered, shaken carefully for 1.0 min and the ether layers were collected in a culture tube using disposable pasteur pipette. The combined ether extracts were carefully evaporated at 35°C on hot plate with aid of current air. The obtained dry film was dissolved in 1ml methanol, then 10 µL were injected in HPLC.

Calibration:

Two hundred of each stock standard solution was transferred to glass stoppered tube. Using a current of air on dry bath, at 90°C the solution was evaporated to <200 µL. Dansyl derivatives were prepared above the residue was dissolved in 5.0 ml acetonitrile (1ml = 20 µg or 50 µL = 1µg each of the derivatives). Injection was carried out using 20 µL of each calibrated. The elution pattern of dansyl derivatives was as follows: B-phenylethamine, Putrescine, Cadaverine, Histamine and Tyramine.

Calculation :

Peaks area of each of the eight dansylamines (standard or the examined sample) was obtained from the HPLC Millennium report and the concentrations were calculated according to the follow equation: ppm of each dansylamine = $(P/P) \times \text{dilution factor of sample}$. Where P = peak area of the dansylamine in sample P* = peak area of standard.

Statistical analysis:

The data were statistically analyzed by analysis of variance using completely randomized design. The data were statistically analyzed by analysis of variance using completely randomized design and least significant difference (L.S.D) at 0.05 levels according to the method described by **Snedecor and Cochran (1980)**.

RESULTS AND DISCUSSION

Type I. Sprouting growth

The effect of saline water sterilization of grains and their interaction on barley sprout characters are illustrated in **Table 1**. The statistical data revealed that, the barley sprout growth characters were not significantly affected by using sterilized grains except for radical and seedling fresh weight. Radical and seedling fresh weight produced from non-sterilized seeds recorded the maximum values.

Concerning salt stress effect; hypocotyl and radical length in addition to seedling length did not significantly differ as use saline water for sprouting barley grains compared with tap water. However, hypocotyl weight and seedling fresh weight were increased significantly with use saline water exceeding tap water by about 15.8 and 7.9%, respectively. As for the effect of interaction between grains sterilization and salt stress, data in **Table (1)** indicated that the highest hypocotyl and radical weights as well as seedling fresh and dry weights were obtained with non-sterilized seeds supplied by saline water.

Moreover, the difference between sterilized and non-sterilized seeds under saline water for hypocotyl weight was not significant. Also, sterilized seeds x tap water statistically leveled with non-sterilized seeds x saline water in seedling dry weight.

By and large, using saline water can be improving the barley sprout biomass which encourages the usage of saline water (1000 ppm) in addition to tap water for sprouting barley grains. Similar results on the reduction effect of sprout characters with higher NaCl concentration were reported by **Ghoulam and Fares (2001)**, **Ibrahim (2017)**, **Abd El ghany(2017)** and **Ashish *et al.*, (2012)**.

Table (1): Effect of saline water and grains sterilization and their interaction on 3-day etiolated barley sprout characters (combined data of two experiments).

Sterilization (ste)	NaCl conteat	HL (cm)	RL (cm)	SDL (cm)	HW (mg/)	RW (mg)	SFW (g)	SDW (mg)
sterilized grains	Tap water	3.46 ^a	4.76 ^a	8.23 ^a	0.37 ^b	0.64 ^b	1.01 ^b	0.24 ^a
	1000ppm	3.50 ^a	4.53 ^a	8.03 ^a	0.42 ^{ab}	0.59 ^b	1.01 ^b	0.23 ^b
Mean		3.48 ^A	4.65 ^A	8.13 ^A	0.39 ^A	0.61 ^B	1.01 ^B	0.23 ^A
Non sterilized	Tap water	3.36 ^a	4.50 ^a	7.86 ^a	0.39 ^b	0.63 ^b	1.02 ^b	0.23 ^b
	1000ppm	3.76 ^a	4.46 ^a	8.23 ^a	0.46 ^a	0.71 ^a	1.17 ^a	0.24 ^a
Mean		3.56 ^A	4.48 ^A	8.05 ^A	0.43 ^A	0.67 ^A	1.10 ^A	0.23 ^A
Average	Tap water	3.41 ^a	4.63 ^a	8.13 ^a	0.38 ^b	0.63 ^a	1.01 ^b	0.23 ^a
	1000ppm	3.63 ^a	4.50 ^a	8.05 ^a	0.44 ^a	0.65 ^a	1.09 ^a	0.23 ^a

Different letters in the same column differ significantly between treatments at ($P < 0.05$). HL= Hypocotyl length, RL= radical length, SDL= seedlings length, HW= Hypocotyl weight, RW= radical weight, SFW= fresh seedlings weight, SDW= dry seedlings weight

Type II. The Proximate analysis

Effects of saline water and grain sterilization and their interaction on the proximate content of barley sprouts are illustrated in **Table (2)**. As a result of sterilization effect, moisture, protein, lipid, and ash contents as well as energy significantly increased and carbohydrate content decreased with using sterilized.

Except for fiber content, all other proximate contents markedly affected by saline water treatment, Herein, high moisture content and energy of barley sprout was observed using saline water, 1000 ppm surpassing tap water and dry grains. Also, protein content was increased significantly after sprouting barley grains wheather with using tap or saline water (16.08 and 15.94% respectively) over dry grains (13.93%). Similar trend was shown for lipid content which increased by using tap water (3.35%) but it is not significantly differ with 1000 ppm saline water (3.28%). The highest carbohydrate and ash content was found in dry barley grains and decreased by sprouting seeds by tap or saline water. For about interaction between sterilization of grains and saline water effects, data in **Table (2)** showed that the highest moisture, protein and lipid contents were

found with barley sprout using tap water and sterilized grains. Due to there is no nitrogen source added externally to the water and saline solution used for irrigation during sprouting. This crude protein % increase was therefore not a likely true increase (**Chavan and Kadam 1989, Abdallah 2008 and Dung *et al* 2010**). Protein, lipid and fiber values increased in barley sprouts than barley grain. The fiber increased with increasing NaCl concentration, while ash decreased. These observations may be due to decrease in carbohydrate content (**Ibrahim, 2017**). The dyed barley sprouts returned increase energy value than grains. These observations may be an idea to reduce the carbohydrate content.

Finally, seeds sterilization process is a good procedure for enhancing the proximate content of barley sprouts. Although, using tap water for sprouting caused an increment on the proximate content, however, it is not significantly differ with saline water. Thus, tap and saline water can be used for sprouting barley seeds with no preferable for any of them

Table (2): Effect of sprouting using 1000 ppm saline water on the proximate analysis (g/100g) of 3 day old etiolated barley sprout.

Sterilization (ste)	Treatment	Moisture	protein	Carbohydrate	Lipids	Fiber	Ash	Energy (Kcal/g)
	Dry seed	3.34 ^c	13.93 ^c	79.84 ^a	2.95 ^c	2.44 ^a	0.847 ^a	401.63 ^a
Sterilization	Tap water	4.4 ^a	16.50 ^a	76.99 ^c	3.5 ^a	2.36 ^a	0.65 ^b	405.46 ^b
	1000ppm	4.18 ^b	16.16 ^a	77.60 ^c	3.39 ^{ab}	2.46 ^a	0.387 ^c	405.14 ^c
	Mean	3.98 ^A	15.53 ^A	78.14 ^B	3.28 ^A	2.42 ^A	0.628 ^A	404.08 ^A
	Dry seed	3.34 ^c	13.93 ^c	79.84 ^a	2.95 ^c	2.44 ^a	0.847 ^a	401.63 ^a
Non- sterilization	Tap water	4.13 ^b	15.66 ^b	78.33 ^b	3.19 ^{bc}	2.50 ^a	0.317 ^c	404.67 ^d
	1000ppm	4.07 ^b	15.72 ^b	78.53 ^b	3.17 ^{bc}	2.19 ^a	0.38 ^c	405.53 ^a
	Mean	3.85 ^B	15.10 ^B	78.90 ^A	3.10 ^B	2.38 ^A	0.514 ^B	403.94 ^B
	Dry seed	3.34 ^C	13.93 ^B	79.84 ^A	2.95 ^B	2.44 ^A	0.847 ^A	401.63 ^C
Average	Tap water	4.12 ^B	16.08 ^A	77.66 ^B	3.35 ^A	2.43 ^A	0.483 ^B	405.06 ^B
	1000ppm	4.27 ^A	15.94 ^A	78.07 ^B	3.28 ^A	2.33 ^A	0.383 ^B	405.33 ^A

Means in each column followed by the same letter are not significantly different at the 5% level ($p \leq 0.05$).

Type III. Biogenic amine determination:

The results of biogenic amine contents of barley 3 days old etiolated barley sprouts and grains are given in **Table (3)**. Six biogenic amines were investigated in the experiment, i.e. spermine, spermidine, putrescine, cadaverine, histamine and tyramine. The highest spermine and histamine contents (20.11 and 2.70 mg/kg, respectively) were detected in barley sprouts with using the saline water treatment. However, other biogenic amines were detected in big amounts for tap water treatment, as compare with the saline water treatment.

Barley grains contain 22.45mg of spermine, 1.21 mg of putrescine, 0.13mg of cadaverine, 0.62 mg of histamine, 0.14 mg of tyramine and 0.04mg of spermidine. Most of these amines were slightly increased after sprouting seeds either using tap or the saline water. Common seeds such as wheat, barley, rice, oats, corn, sorghum, soybean and legume grains contain polyamines (**Simon-Sarkadi and Holzapfel, 1995 and Shalaby and Ragab, 1997**). The presence of polyamines in such grains may play an important role in the physiology of resting and germinating seeds which are produced when specific amino acids undergo decarboxylation by specific decarboxylase enzymes. **Shalaby (2000)** reported that the development of biogenic amines noticed during the germination of legume seeds may be due to physiological changes occurred in the tissues during sprouting and/or the activity of bacterial decarboxylase enzymes. Also **Shalaby (2000)** showed that cadaverine and putrescine increased during the germination period in bean, lupine and chickpea seeds.

Biogenic amines as natural antinutritional factors are important from the hygienic point of view, since they have been implicated in a number of food poisoning episodes, and they are able to initiate various pharmacological reactions (**Shalaby, 1996**). Histamine, putrescine, cadaverine, tyramine, tryptamine, B-phenyl ethylamine, spermine and spermidine are considered to be the most important biogenic amines occurring in foods. Putrescine, spermidine, and cadaverine have not an adverse health effect, but they may react with nitrite to form carcinogenic nitrosamines and also can be proposed as indicators of spoilage (**Hernandez- Jover, et al., 1997**). Tryptamine can induce blood pressure increase, therefore causes hypertension, however there is no regulation on the maximum amount of tryptamine (**Shalaby., 1996**). However, **Parents et al. (2001)** and **Nout (1994)**

pointed out that the maximum and daily intake of histamine and tyramine should be in the range of 50-100 mg/kg and 100-800 mg/kg, respectively, that over 1080 mg/kg tyramine becomes toxic.

Since, barley sprouts contain biogenic amines but it is lower than previous ranges, sprouts can be considered a safe food and germination of seeds either using tap or saline water, and did not cause harmful effect on the health of the food. Besides, using sterilized grains for sprouting caused big decrement in the amines content of barley sprout, while, spermidine content were not detected (**Table 3**). Over all, sterilization of seeds play an important role to decrease the amines content which can be recommended treatment for decline the biogenic amines content of sprouts.

Table (3): Effect of sprouting using saline water on the biogenic amine content of 3 days etiolated barley sprouts

Sterilization	Treat	SPM	PUT	CAD	HIS	TYR	SPD	Total
Tap water	STW	0.22	ND	ND	ND	ND	ND	0.22
	NTW	11.64	1.1	25.3	0.81	1.58	0.06	40.51
Mean		5.93	1.10	25.3	0.81	1.58	0.06	20.36
	STW 1000	6.2	0.2	0.84	1.16	0.1	ND	8.51
Saline water	NTW 1000	34.02	1.52	0.84	4.24	0.24	0.03	40.9
Mean		20.11	0.86	0.84	2.70	0.17	0.03	24.71
	Sterilized	3.21	0.20	0.84	1.16	0.10	ND	4.36
Average	Non- sterilized	22.83	1.31	13.07	2.52	0.91	0.04	40.70
	Barley grain	22.45	1.21	0.13	0.62	0.14	0.04	24.61

ND = not detected, STW=sterilization grain and using tap water, NTW= Non sterilization grain and using tap water, STW1000= sterilization grain and using tap water (NaCl)1000 ppm, NTW1000= Non sterilization tap water (NaCl)1000 ppm, SPM= Spermine, PUT= Putrescine, CAD= Cadaverine, HIS= Histamine, TYR= Tyramine and SPD= Spermidine.

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تأثير استخدام المياه المالحة على نمو النبت والتحليل التقريبي والأمنيات الحيوية فى الشعير

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أجريت هذه الدراسة في قسم البساتين بكلية الزراعة جامعة عين شمس والمركز الإقليمي للأغذية والأعلاف مركز البحوث الزراعية خلال عام 2018 في ثلاث تجارب منفصلة.

أولاً: التنبيت باستخدام المياه المالحة للحبوب المعقمة وغير المعقمة وتفاعلها على تنبيت الشعير.

ثانياً: تأثير استخدام المياه المالحة للحبوب المعقمة وتفاعلها على المحتوى التقريبي لانبات الشعير.

ثالثاً: تأثير الانبات باستخدام 1000 جزء من المليون من المياه المالحة والحبوب المعقمة على محتوى الأمنيات الحيوية لانبات الشعير فى الظلام لمدة 3 أيام. التجربة الأولى: تم اظهار البيانات بأن هناك نمو لم تتأثر بشكل كبير باستخدام الحبوب المعقمة باستثناء الجذور تم الحصول على وزن شتلة طازج وأكبر وزن من نوع السويقه ، والرايكيالية ، والشتلات ، وقد تم الحصول على وزن طازج وجاف للبذور غير المعقمة باستخدام المياه المالحة فيما يتعلق بالتجربة الثانية التي كشفت أن التحليل التقريبي قد ازداد بشكل ملحوظ مع استخدام الحبوب وأعلى نسبة من الكربوهيدرات (79.84%) تم العثور على أعلى نسبة الرطوبة والبروتين والدهون مع تنبت الشعير باستخدام مياه الصنبور والحبوب المعقمة. وتظهر التجربة الثالثة أن ستة أمنيات حيوية تم التحقيق فيها ، وتم الكشف عن محتويات في انبات الشعير باستخدام المياه المالحة.

الكلمات الدالة : نبت حبوب الشعير، التركيب الكيماوي، الأمنيات الحيوية.