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Investigation of EMS effect on *Vicia faba* seed germination and seedling growth under saline condition

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Abstract

The effect of Ethyl-methane Sulphonate (EMS) on seed germination and seedling characters of broad beans (*Vicia faba* L.) was analyzed in this investigation. The seeds were soaked in different percentages and durations of (EMS), then after sowing were irrigated with a range of NaCl concentration. The effect of EMS was observed based on the seed germination (%), and seedling characters such as: total fresh weight, root fresh weight and root length.
Germination percentage generally decreases while concentrations of NaCl increases, however the interaction effect of EMS clearly appeared on concentration 6000 ppm NaCl, which positively increased the germination from 40% in absence of EMS to 60%, when 0.5 or 1% EMS were used. 24 hours soaking in EMS decreased the fresh weight from 33.36 to 13.55 gm per plant, in addition to the decrease of the root length from 22.0cm to 14.2cm per plant. The root nodulation of broad bean is not affected during the increase of NaCl, possibly due to the interactional effect of mutagenesis agent.

Key Words: Broad bean, EMS and NaCl.

Introduction

Vicia faba (2n=12) of family *Fabaceae*, commonly known as Broad bean, is an important pulse crop used as vegetable, silage, forage and stock feed. Faba bean is used green or dried, fresh or canned; it is commonly known as breakfast food in the Middle East, Mediterranean region, China and Ethiopia (Bhat *et al*., 2007). The pulse crops can improve soil fertility by fixing atmospheric nitrogen and increasing inorganic matter of the soil by adding leaves and other plant parts (Bond *et al*., 1985). The salinity of irrigation water as a cause of yield reduction has been the subject of many investigations, the lower the salinity, the better the growth expressed by many parameters such as

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development of leaf area and dry matter (Katerji *et al*., 2002). The yield depression of broad bean confirms the low salt tolerance of broad beans (Katerji *et al*., 2002).

Since genotype of *Vicia faba* is homozygous, because of self pollination, therefore, there is need for its further improvement which can be done by creating additional variability in its genotype by mutation breeding. Inducing mutations serve as a complementary approach in genetic improvement of crops (Joshi *et al*., 2011). Mutation induction programs in different field crops reported numerous successful achievements in developing many induced mutants to new cultivars in different countries (Micke, 1983; Micke, 1988). Statistical reports of The International Agency of Atomic Energy (IAEA), mentioned that more than 30 legume cultivars had been developed from induced mutants either by gamma rays or and chemical mutagens (Micke, 1988). Many researchers have compared the mutagenic efficiencies of different mutagens on different crops. Their results seem to be entirely specific for particular species and even varieties. While many researchers like Dhanayanth and Reddy (2000) and Bhat *et al*., (2005) found chemical mutagens to be more effective than physical ones, many others like Tarar and Dnyansagar (1980), Zeerak (1991) found the reverse case.

Various physical and chemical mutagens agents are used to induce favorable mutations at high frequency in plants (Goyal and Khan 2010), Among the chemical mutagens, ethyl methane sulphonate EMS (Kozgar *et al*., 2011) has been widely used for introducing variability in higher plants

Ethyl methane sulfonate (EMS), is the most commonly used chemical mutagen in plants, its produces random mutations in genetic material by nucleotide substitution; particularly by guanine alkylation. This typically produces only point mutations. It can induce mutations at a rate of $5x10^{-4}$ to $5x10^{-2}$ per gene without substantial killing. However, the use of mutagenic alkylating agents, especially EMS, has become a standard approach for mutagenesis that has been successfully used in the classic forward genetic screens that have defined the field of developmental genetics, as well as in many alternative screening schemes that have since been developed (Bökel, 2008). The ethyl group of EMS reacts with guanine in DNA, forming the abnormal base O-6-ethylguanine. During DNA replication, DNA polymerases that catalyze the process frequently place thymine, instead of cytosine, opposite O-6-ethylguanine. Following subsequent rounds of replication, the original G:C base pair can become an A:T pair (a transition mutation). This changes the genetic information (Bhat *et al*., 2007).

The present investigation aimed to determine the tolerance of *Vicia faba* to NaCl conditions in germination and other growth stages, attempt to obtain mutations through the EMS agents and identify its effect on different growth stages of *Vicia faba*.

Materials and Methods

The experiment was conducted at the Faculty of Agriculture, University of Benghazi, in the season of 2013/2014. 180 seeds of broad beans (*Vicia faba* L.), were soaked in 4 EMS solutions (0. 0.1, 0.5 and 1%) twice (1 and 24 hours), then sowed in plastic bags containing 1:1:1 peat, sand and clay soil. The 180 bags divided in four groups were irrigated with (0.3000, 6000 and 9000 ppm) of NaCl solution.

The experiment consisted of factorial arrangements of treatments (NaCl concentrations at four levels, EMS concentrations at four levels and time of EMS soaking at two levels) in a completely random design. Five replicates were assigned per treatment. Data was analyzed using the Statistical Analysis System, general linear model (GLM procedure, SAS Institute Inc., 2004) and means were evaluated by least significant difference (LSD). The response was assessed in the germination stage and in the end of the experiment which consists of total fresh weight, total dry weight, shoots length, shoots weight, roots length and roots weight.

Results and Discussion

The percent of germination in absence of NaCl was decreased from 80% and 90% to 40% due to the increase of EMS concentrations from 0 and 0.1% to 1% respectively (Table 1), which indicate the considerable effect of EMS on germination. Reduction in seed germination in mutagenic treatments, as explained, due to delay or inhibition in physiological and biological processes necessary for seed germination which include enzyme activity (Chrispeeds and Vaener, 1976), hormonal imbalance (Ananthaswamy *et al.*, 1971) and inhibition of mitotic process (Sato and Gaul, 1967). Germination percentage generally decreased with increasing concentrations of EMS and it was also reported in sesame by Ganesan (1998) and in Okra by Kumar and Mishra (2004).

Reduction in germination percentage might be due to an increase in the production of active radicals responsible for seedling survival (Lethality) and increasing concentration of EMS immediately damaged the physiological activities of seeds (Anbarasan and Rajendran, 2013), The reduction in germination percentage might have been also due to the effect of mutagen on meristematic tissues of the seed. The mutagenic treatments also delayed the germination process (Kumar and Yadav, 2010).

The interaction effect of EMS was clearly apparent in the concentration 6000 ppm NaCl, which positively increased the germination from 40% in absence of EMS to 60%, when 0.5 or 1% EMS used, however the raise in seeds germination wasn't significant according to LSD means separation. Lima *et al.*, (2015) report a mutant with enhanced water stress tolerance, identified from the EMS induced mutant population of rice, it showed enhanced germination and increased maximum root length without increase in its root weight, root volume and total root number on crown.

Table (1) show that with increasing NaCl concentration in absence of EMS, the seed germination decreased from 80% in control treatment to 20% at 9000 ppm of NaCl, which was usually due to salinity effect on germination, Shokohifard *et al*., (1989), have reported that salinity stress negatively affected seed germination, either osmotically through reduced water absorption or ionically through the accumulation of sodium and chloride causing an imbalance in nutrient uptake and toxicity effect. Germination and seedling growth are reduced in saline soils with varying responses for species and cultivars (Bliss *et al*., 1986).

Interaction between high concentrations of NaCl 9000 ppm and high concentration of EMS 0.5 and 1% guide to no seeds germinated. According to Lal (1985) and Almansouri *et al*., (2001) seed germination is usually the most critical stage in seedling establishment. Pulses in general are sensitive and have inadequate control over ion uptake, which leads to high internal salt concentrations and results in plant injury when exposed to high salinity. Talebi *et al*., (2012) also observed decrease in germination, seedling height, root length and emergence under field conditions of M1 generation of Malaysian rice as concentration of applied EMS increased.

The data of total fresh weight in (Figure 1) show a significant difference between the interaction of EMS concentration and the duration of soaking, one hour soaking in 0, 0.1, 0.5 and 1% EMS gave 24.88, 26.79, 23.55 and 24.32 gm per plants, which had no significant differences on total fresh weight according to statistical analysis, however 24 hours soaking made a significant difference which decreased the fresh weight from 33.36 and 32 gm per plant in 0 and 0.1% EMS to 13.55 and 16.84 gm per plant in 0.5 and 1% EMS. Akhtar (2014) observed that by increasing dose of EMS shows lethal effect and morphological parameters such as plant height, number of leaves, leaf area, and relative water contents of leaves reduced significantly. But lower doses of EMS and Gamma radiation improve thermo tolerance capacity significantly.

Roots fresh weight as in (Figure 2) beehives as a total fresh weight which decreased from 19.80 and 19.48gm per plant in 0 and 0.1% EMS to 7.23 and 7.97 gm per plant

Table 1. Effect of NaCl and EMS concentrations on the germination % of V*ica fab*

EMS Concentration %	NaCl Concentration ppm			
	0	3000	6000	9000
0	80^{ab*}	80^{ab}	40^{cd}	20^{de}
0.1	90^{a}	50^{bcd}	50^{bcd}	0^{e}
0.5	50^{bcd}	50^{bcd}	60^{abc}	0^{e}
1	40^{cd}	30^{cde}	60^{abc}	0^{e}

*Separation of means by LSD test at the 5% level and means with the same letter are not significantly different.

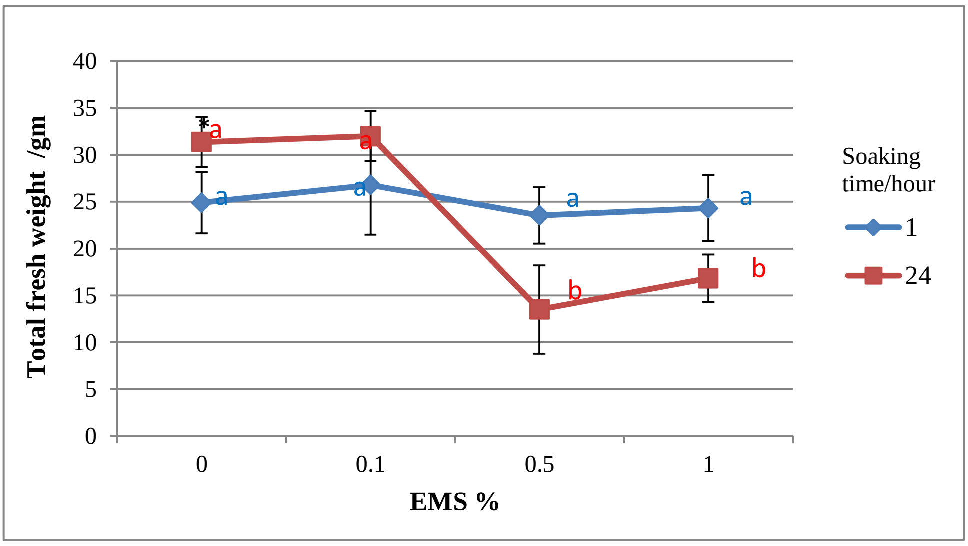

Figure 1. Effect of EMS concentrations and soaking time on the Total fresh weight of *vica faba*
*Separation of means by LSD test at the 5% level and means with the same letter are not significantly different.

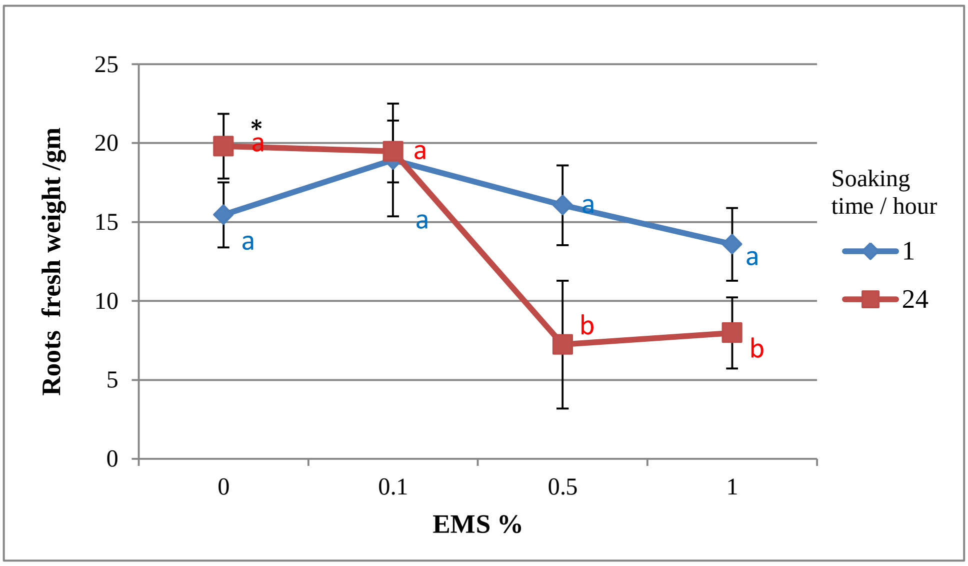

Figure 2. Effect of EMS concentrations and soaking time on Roots fresh weight of *vica faba*
*Separation of means by LSD test at the 5% level and means with the same letter are not significantly different.

Roots fresh weight as in (Figure 2) beehives as a total fresh weight which decreased from 19.80 and 19.48gm per plant in 0 and 0.1% EMS to 7.23 and 7.97 gm per plant in 0.5 and 1% EMS. Kumar and. Yadav (2010) concluded that the mutagenic effectiveness increased with the increase in the dose/treatment of EMS, verifying the effects of EMS on germination, plant height, seed yield and its correlation with meiotic behavior.

High doses of EMS are inhibit the growth of chilli morphological parameters such as (taproot length, longest lateral root length and lateral roots number) due to genotoxic effects (Sri Devi and Mullainathan, 2011), also observed Stickiness, precocious movements, chromosomal bridge, micronucleus and others chromosomal aberrations in chilli root tip cells were treated with various concentration of EMS. Kleinhofs *et al*., (1978) reported a delay in the initiation of metabolism following germination, resulting in uniform delay in mitotic activity, seedling growth, plant height, and ATP and DNA synthesis.

The data presented in (Figure.3) indicate that the concentration of NaCl in irrigated water has a significant effect in decreasing of the length of the roots of the broad bean. The roots length went from 22.0cm per plant in the control treatment to 14.2cm per plant when 3000 ppm of NaCl was added. Shah *et al.*, (2008) noticed the same effect on the roots length of chick beans, also Bibi *et al.*, (2012) found that the plants length tend to decrease with increase in salinity in broad bean. Epstien and Norlyn (1997) reported that salinity effects the growth of plants by decreasing the rate of water uptake due to osmotic effect through ion specific toxic effect or through a nutritional imbalance caused by ion antagonism.

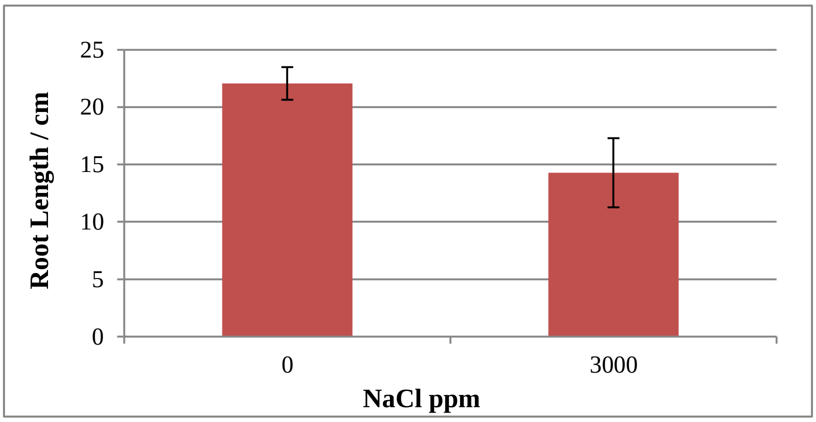

Figure 3. Effect of NaCl concentrations on root length of *vica faba.*

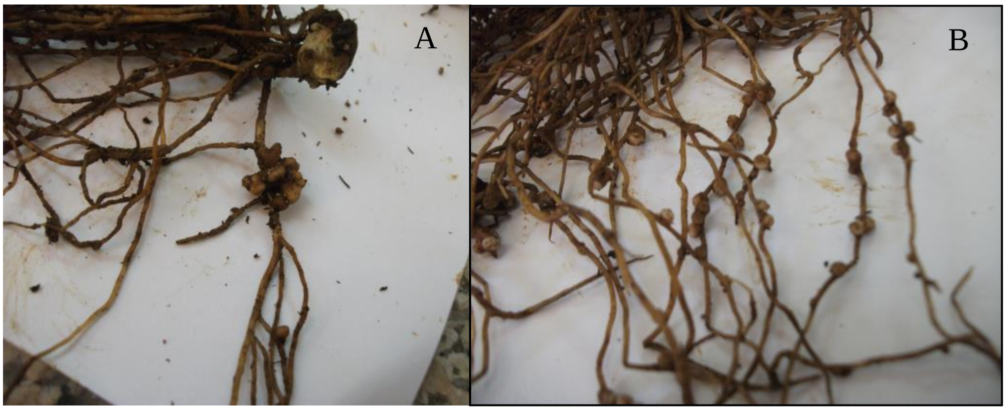

Figure 4. Root nodulation of broad bean **A**. Few nodulation compared with **B.** more nodulation may due to EMS effect.

It was also noticed that the roots nodulation of broad bean is not affected during the increase of NaCl, possibly due to the interactional effect of mutagenesis agent as shown in (Figure.4) While the growth of beans due to salt stress is associated with toxic effect of NaCl and inhibition of nodule formation due to low interaction between rhizobium and plant root for nodule formation under salt stress, this result contradicts Bibi *et al*., (2012) they noticed a decrease in the number and weight of the nodulation in the root of the broad bean due to the increase of NaCl.

Generally, nodulated crop plants do not like saline conditions (Mirza and Tariq, 2011). Yousaf and Sprent (1983) found that under saline conditions nodules partially compensated by producing larger rather than more nodules. The inhibitory effect of salinity on the nodulation is well documented (Cooper *et al.,* 1986; Sprent, 1984). Adverse effects of increasing salinity on nodules number and nodule weight have been well reported (Lauter *et al.,* 1981; Predeepa and Ravindran, 2010).

Conclusion

The irrigation with saline water is decreasing the seed germination and effecting the seedling's fresh weight, root's fresh weight and root length of Vicia faba. By treating the seeds with EMS, the germination increased from 40% to 60%, additionally maintaining the root nodulation, however the high dosage of EMS along with extensive soaking time led to the reduction of the fresh weight from 33.36 to 13.55 gm per plant and shrinkage of the root length from 22.0cm to 14.2cm per plant.

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استقصاء فى تأثير EMS على أنبات بذور ونمو بادرات نبات الفول البلدي تحت ظروف الملوحة.

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الملخص

تأثير مادة ايثايل ميثان سالفونيت EMS على انبات البذور وصفات البادرات في نبات الفول البلدي درست بناءً على النسبة المئوية لإنبات البذور وكذلك صفات البادرات مثل الوزن الرطب الكلي، وزن الجذور الرطب وطول الجذور، حيث نقعت البذور في عدة تركيزات وفترات زمنية مختلفة من مادة EMS ، ثم بعد زراعتها تم ريها بمحاليل مختلفة من كلوريد الصوديوم.
بصفة عامة كلما زاد تركيز كلوريد الصوديوم في مياه الري كلما قلت نسبة الإنبات، ولكن كان لتداخل مادة EMS مع تركيز 6000 جزء في المليون كلوريد صوديوم تأثير إيجابياً ، حيث زادت نسبة الإنبات من 40% بدون EMSإلى 60% حين تم استخدام مادة EMS بتركيز 0.5 و 1%. كما كان لفترة النقع لمدة 24 ساعة في ماد EMS تأثيراً معنوياً في إنخفاض الوزن الكلي الرطب من 33.36 جم إلى 13.55 جم لكل نبات، بالإضافة إلى إنخفاض طول الجذور من 22.0 سم إلى 14.2 سم لكل نبات، كما لوحظ عدم تأثر العقد البكتيرية في جذور نبات الفول بزيادة تركيز كلوريد الصوديوم في مياه الري وربما يعود ذلك إلى تأثير مادة EMS المطفرة.

مفتاح الكلمات: الفول البلدي، مادة EMS، كلوريد الصوديوم.