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INCREASING SALT TOLERANCE OF *PHASEOLUS VULGARIS* L. CALLUS USING SODIUM AZIDE *INVITRO*.

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ABSTRACT
Embryos excised from sterilized mature seeds of kidney bean (*Phaseolus vulgaris* L.) were cultured on (MS) medium supplemented with vitamins and growth regulators. Results showed that 2,4-D at (3) mg/l was the best for callus production. Callus was then exposed to different levels of salt mixtures, which were (NaCl, CaCl2 and MgCl2) in a ratio of 2:2:1 at concentrations of (0, 50, 100, 150, 200and250) mM added to the culture medium. Different levels of the chemical mutagen sodium azide were used to soak callus, the levels were ranging in (0.05,0.1 or 0.5) mM, for period of time (30,60 or 90) min. Exposing callus to SA raised the callus fresh and dry weight in contrast to non-exposing callus. To produce genetic variation we soaked the callus in SA at concentration of (0.1)mM for (30) min. Callus cultures treated with(0.1) mM of SA for(30) min were cultured on MS medium containing (0 – 250) mM salt mixtures. Results offered that the salt tolerance increased in callus that was exposed to SA, obviously proved by the increase in the callus fresh weight, in contrast to unexposed callus. The results showed that SA treated callus combined with salinity treatments was higher than those treated with screening and selection only.

KEYWORDS: *In Vitro, Phaseolus vulgaris*, Sodium Aazide, Salt tolerance.

INTRODUCTION
The kidney bean (*Phaseolus vulgaris* L.) is one of the most ancient crops of the New World and is currently the most important grain legume for direct human consumption in the world. Dry beans offer a high nutrient- density and are a good source of starch, protein, complex carbohydrates fiber as well as vitamins (Vitamin B6 and folate), phytochemicals and minerals (iron, zinc and phosphorous) (USDA, 2011). Important health benefits include reduced disease risk; diabetes (Villegas *et al*., 2008), coronary heart disease (Kabagambe *et al*., 2005), certain cancers (Deneo-Pellegrini *et al*., 2002; Key *et al*., 1997), enhanced longevity (Darmadi-Blackberry *et al*., 2004), lower total cholesterol, LDL- cholesterol, triglycerides, while increasing HDL-cholesterol (Shutler *et al*., 1989; Winham *et al*., 2007). Dietary Guidelines for Americans recommends 3 cups of beans per week (dry weight ~200 g). Drought and soil salinity are considered major abiotic stresses for agricultural production in arid and semi-arid regions. In these harsh environmental conditions, water availability is a major problem that severely limits crop productivity (Dragiiska *et al*., 1996; Lutts *et al*., 2004; Tounekti *et al*., 2011). Tolerance to a stress such as salinity has occurred genetically through adaptation (heritable morphological traits) or physiologically by exposing plants/plant cells to gradual increases in concentration of the abiotic stress (acclimation) (Farooq *et al*., 2009). Plant tissue culture techniques inters in several applications like plant micropropagation, genetic study, plant improvement ,production of pathogen free plant disease (bacterial and fungal), in addition the production of secondary metabolite *In Vitro* is possible through plant tissue culture. plant cells cultured *In Vitro* are suitable to separate tolerant clones from non-tolerant populations and to study acclimative mechanisms where by making it possible to regenerate plants that can withstand more adverse environments than non-acclimatised plants of the same species (Ochatt *et al*., 1999). Sodium azide is a potent mutagen, SA has potential in tissue culture mutagenesis for inducing biochemical mutants because it is a point mutagen and is not known to be highly mutagenic in mammals. (Kleinhofs *et al*., 1978).

MATERIALS AND METHODS: CALLUS INDUCTION
Explants (sterilized embryos) of *phaseolus vulgaris* L. were excised and cultured in universal tubes containing MS medium (Murashige and Skoog, 1962) with concentration of the auxin 2,4-D (3) mg/l, which incubated in dark at a temperature 23 ± 1°C.

Cultivation of callus on culture media contain different concentrations of salts

Four-week-old callus of *phaseolus vulgaris*, was divided into pieces of (300 mg). These pieces were transferred onto the same medium those were used for the callus induction supplemented with 3 mg / l of 2,4-D and with different concentrations of (NaCl, CaCl2, MgCl2 mixture), such as, 0, 50, 100, 150, 200 and 250 mM. (NaCl, CaCl2, MgCl2 at the ratio 2: 2: 1 respectively) and this ratio comparable to the ratio of Iraqi soils (Buringh, 1960).

Induction of mutation through chemical mutagens (Sodium azide)

The protocol of callus treatment with sodium azide as reported in (Al – Oubaidi, 2006) for the development of mutant calli in *Glycine max* L. was employed The callus were treated with varying concentrations of NaN3. The solutions of the Sodium Azide (SA) at the concentrations (0, 0.05, 0.1, 0.5) mM were prepared and filter sterilized through a 0.45 µm Millipore filter and placed in sterile petri dishes inside the air flow cabinet where the callus were soaked in SA for (30, 60,90) minutes. Then we took constant weight of callus by 300 mg and they were placed on fresh same callusing media as used for normal callus and by 10 replications for each treatment and for each period of time. Then they were incubated under the previous conditions, to determine the appropriate concentration of the SA and the appropriate soaking duration for the callus, we depended on the wet and dry weight of callus after four weeks.

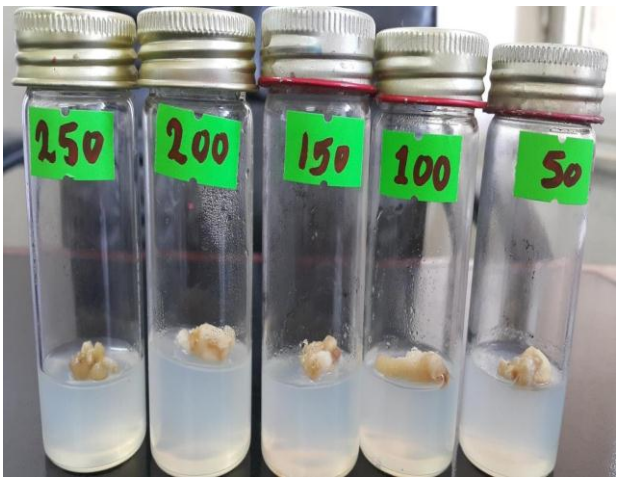
Culturing the callus that were treated with SA on salt culture media

SA solution were prepared at a concentration of 0.1 mM (which is the optimum concentration of SA) and immersion callus in it for 30 minutes (the length of time optimization to submerge the callus with a SA solution), then we took the constant weight of callus (300 mg) and were cultured on culture media containing salt levels (0, 50,150,200 or 250)mM and by 10 replications for each level of salt. Then they were Incubated under the previous conditions and after four weeks depending on the amount of the increase in the fresh weight of callus and drawing the curve in the growth of the callus to compare it with the curve in the first growth of the callus grown on various salt levels that were untreated with SA.

RESULTS AND DISCUSSION

Effect of Salinity Stress on Callus Fresh Weight (mg) from Stressed Callus

The result in the table (1) and fig (1) showed that the salt concentration effect significantly on the callus fresh weight. The increasing of salt concentration reduce the callus fresh weight. the high average of callus fresh weight found on control treatment (453)mg this treatment significantly different than the treatments of 200 mM and 250mM which reached to (312,254)mg respectively, but this treatment (cont) was not significantly different than other treatments (50,100,150)mM. The lowest average of callus fresh weight recorded with the treatment of 250mM which reached to (254) mg.

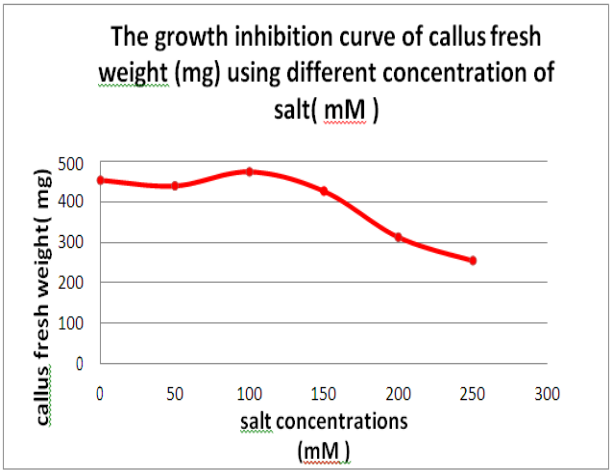


Fig(1) Effect of different concentrations of salt on callus fresh weight (mg)

Also Similar results in other study of salt stress effects on callus fresh weight of *phaseolus vulgaris* L., in general the mean of callus fresh weight decreased significantly at (200mM). The callus formation was affected by the salinity of the medium. Although callus growth was observed in all the salt concentrations, there was a gradual decrease in callus growth as the salinity increased. (Thiagarajan, 2013).

Table(1)The effect of salts levels on callus fresh weight (mg) after four weeks of culture on medium.

Salt Concentrations (mM)	Cont	50	100	150	200	250
Fresh weight mean (mg)	453	439	474	426	312	254
LSD (0.05)	97.1					



Shape(1) The growth inhibition curve of callus fresh weight (mg) using different concentration of salt (mM)

Effect of different treatments of Sodium azide on callus fresh and dry weight

The effect of sodium azide treatment on callus proliferation showed in Table(2), it is obvious that the SA different concentrations effected significantly on the callus fresh weight. The highest average of callus fresh weight found (368.9) mg the callus was soaked in 0.1mM of SAV this treatment significantly different from the treatment of 0.5mM which reached to(325.5)mg. but the treatment of (0.1) mM was not significantly different from the (0.05) mM treatment which reached (331.7)mg. there were no significant different between time treatments.

For the callus dry weight there were no significant different between the treatments of (0.5, 0.1 and 0.05) mM showed in table (2), but for the time treatments the highest dry weight was for the treatment of (30) min which reached to (22.67) mg that mean this treatment significantly different from the others(60 and 90) min which reached to (19.40, 19.53) mg respectively.

It is likely that only higher doses resulted in the restriction of cell growth, which did not affect lower doses after interaction of the mutagens with plant growth regulators. (Khawar and Özcan.2006).

Varying doses of NaN3 showed mutagenic effect on percent seed germination, root and shoot length of *Artemisia annua*. The mutagenic effect was found to be dose dependent. However, the mutagenicity of this mutagen has been reported to depend on its concentration, dilution factors, pH of the phosphate buffer solution and incubation period (Al- Qurainy and Khan, 2009; 2010). Sodium azide (NaN3) was employed on the callus cells to create mutation and further mutant shoots were regenerated from these treated calluses on MS media. (Al-Qurainy *et al*., 2011).

Table (2)Effect of different treatments of Sodium azide on callus fresh and dry weight(mg) respectively after four weeks of culture on medium.

Concentrations of SA	Time			Mean
	30	60	90	
0.5	339.0	303.6	333.8	325.5
0.1	408.0	349.2	349.4	368.9
0.05	332.8	349.6	312.6	331.7
Mean	359.9	334.1	331.9	
LSD (0.05)	Concentrations =37.28 Time = N.S. Concentration × Time =N.S.			
Concentrations of SA	Time			Mean
	30	60	90	
0.5	22.60	17.60	20.20	20.13
0.1	23.80	20.60	21.00	21.80
0.05	21.60	20.00	17.40	19.67
Mean	22.67	19.40	19.53	
LSD (0.05)	Concentrations = N.S. Time = 2.713 Concentration × Time =N.S.			

The effect of SA on callus salt tolerance

After the re-cultivation on salt levels 0-250 mM of the mixture of salt. The results were recordedin table (3) showed that SA helped with increasing salt tolerance in *phaseolus vulgaris* callus cells. It was obvious that the treatment of 150 Mm gave highest average of callus fresh weight reached to (649) mg this treatment significantly different than all other treatments. The least average of callus fresh weight recorded with the treatment 250mM which gave (436) mg fig (2). obviously the shape (2) showed that the use of sodium Azaid led to increase the callus fresh weight compared to what it was before the

use of sodium Azaid. For the callus dry weight the treatment of 150 mM gave highest average of callus dry weight reached to (54.7) mg this treatment significantly different than all other treatments.

(Maniu and Mihailescu, 1998) used sodium azide for *In vitro* mutagenesis in barley. Sodium azide is an S stage (synthesis phase) mutagen (Sander and Nilan, 1974, Nilan and Pearson, 1975). It was determined that SA acts through a promutagen or organic metabolite. This metabolite has been isolated and identified as azidoalanine (Owais and Kleinhofs, 1988). Sodium azide also produce DNA single strand breaks (Veleminsky *et al*., 1985), it readily induces base substitutions, but not "frameshifts" or deletions (Niknejad *et al.*, 1972). It preferentially induced G: C → A: T transitions at the second codon position (Koch *et al*., 1994). Although sodium azide causes mutation at gene level but few chromosme aberrations have also been detected (El-Den, 1993). (Del Campo *et al*., 1999) also reported the induction of chromosomal aberrations during replication S phase (synthesis phase) by Sodium azide.

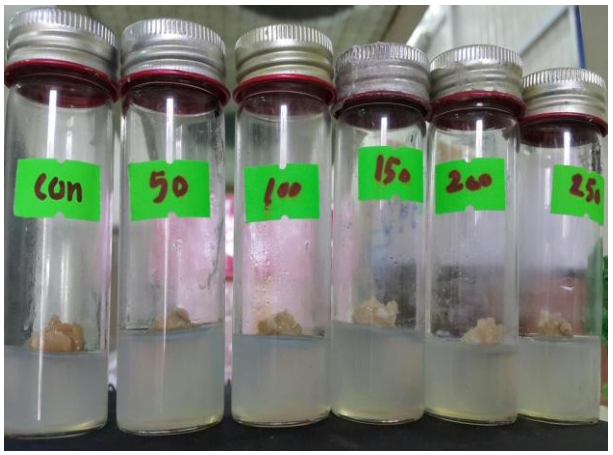
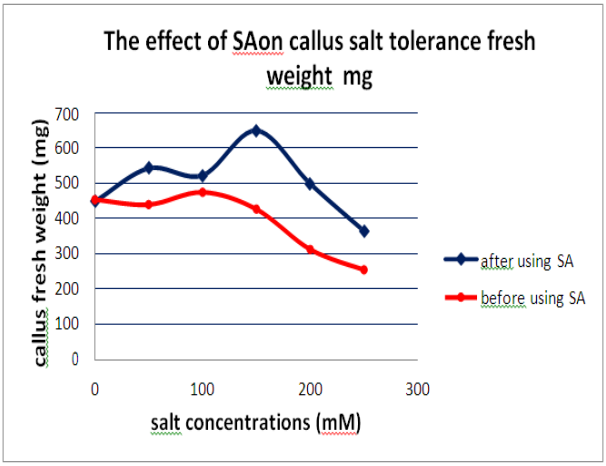


Fig (2) The effect of SA on callus salt tolerance

Table (3) The effect of SA on callus salt tolerance for both fresh and dry weight (mg) after four weeks of culture on medium

Salt Concentrations (mM)	Cont	50	100	150	200	250
Fresh weight mean (mg)	448	542	522	649	498	436
Dry weight mean (mg)	35.7	41.3	43.0	54.7	43.0	34.3
LSD (0.05)	For fresh weight =105.9 For dry weight =10.35					



Shape (2) The effect of SA on callus fresh weight salt tolerance

CONCLUSIONS

The callus treated with (0.1) mM of SA and re-cultured on different salt levels led to increase the ability of salt tolerance in the callus cells.

RECOMMENDATIONS

Use of other chemicals such as EMS mutagen , physical like ionizing radiation and nano particles to create variations in red kidney bean.

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