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RESEARCH ARTICLE - BIOLOGY

Effect of Di Ethyl Sulfate (DES) on Increasing Salt Tolerance for Callus of *Vigna sp.* Using Plant Tissue Culture Technique

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Abstract

Embryos were excised from sterile mature seeds of *Vigna sp.* were cultivated upon MS medium that had vitamins as well as development promoters added. The outcomes demonstrated that the optimal 2,4-D concentration for callus formation was 2 mg/l. The callus was then subjected to various concentrations of a combination of salts consisting of NaCl, $CaCl_2$, in addition to $MgCl_2$ with a ratio of 2: 2: 1. (0, 50, 100, 150, 200, and 250) mM of salt mixture add to the culture medium. Various levels of di ethyl sulfate (DES) mutagenic solution at a concentration of (0.1) mM were used to soak the callus for a period of time (15, 30 or 45) minutes. Revealing callus to (DES) increased the weight, whether fresh and dry of callus compared to callus not treated with (DES). To produce genetic variations, callus was cultivated on MS media containing (0 - 250) mM after undergoing treatment for thirty minutes using (0.1) mM of (DES). The outcomes demonstrated that DES-exposed calluses had higher salt tolerance, which was appeared clearly by the increased fresh weight of callus, in contrast to untreated callus.

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Keywords: *Vigna sp.;* Di Ethyl Sulfate; Salt tolerance; Callus; 2,4-Dichlorophenoxyacetic acid (2,4-D)

1. Introduction

This Salinity, a global issue, significantly reduces crop yield and threatens global food security. It is soluble from low to high levels in the soil atmosphere and hampers water uptake during imbibition and seedling establishment, followed by ion uptake. This stressor reduces agricultural yield worldwide, impacting sustenance and economic productivity [1]. Many plant responses to salt are important for lowering cellular excess osmolarity and ion disequilibrium because they work together in a complex way. Moreover, agricultural plants must strive to produce enough biomass under salty conditions [2].

Salinity reduces plant water use, reducing growth and metabolic activities. It also reduces plant development and yield depending on species, salinity, and salt ionic composition.8 High salt stress reduces seed germination, seedling development, vigour, vegetative growth, blooming, and fruit set, decreasing economic yield and product quality. Many plant species accumulate protein and proline when stressed by abiotic factors. These chemicals help stressed plants alter osmotic pressure and safeguard subcellular structures [3]. Studies show that growing domesticated legume crops is hard because of biotic (diseases and pests), abiotic (heat, frost, drought, and salinity), and edaphic (soil nutrient deficit) problems. Policy issues also complicate the situation, as priority starch staples receive more attention than legumes.

Cowpea is an excellent provider of protein that is less expensive than protein of animals (fishes, meats, and poultries) for those with the lowest incomes, which helps lower-income farmer's battle nutritional deficiency. These include the leaves and the grains of this plant, which are finding a variety of uses, particularly in the field of human nutrition, where they can be utilized to make a variety of foods like Akara, Moin-moin, Koki, Couscous, Red-Red Stew, Ndambe, Thiebou Kathiakh, Cake, Bread, and Cookie, as well as ingredient in the preparation of additional meals for the child [4, 5].

This plant is receiving more study interest than other *Vigna* legumes, which might be a result of the crop's broader spread as well as its usage. Based on morphological or physiological indicators [6, 7]. As well as moleculer markers like amplified fragments long palindromic DNA , numerous research investigations have evaluated the genetic diversities, variations, as well as genetic separation within cowpea genotypes. The main abiotic pressures on agricultural output in arid and semi-arid regions include drought and soil salinity. The amount of water available is a significant issue in such challenging atmospheric conditions, which significantly reduce agricultural yield [6, 8, 9].

In common cases, an interaction or combination of these various abiotic stressors has a significant impact on cultivation, plant growth, and ultimately crop output. On the genetic level, via adaptability (heritable morphological features) or physiologically, through exposure to gradually increasing concentrations of the abiotic stress (acclimation), tolerance to a stress like salt has been achieved [10]. The excessive manufacture of several organic solutes (also known like osmolytes or osmoprotectants), including sucrose, betaines, and proline, is one of the well-known responding for plants which is stress-tolerance. They support osmotic regulation or safeguard the metabolism with subcellular structures [11].

The choice of cell lines that are salt- and drought-tolerant has employed tissue culture. In numerous crops, including potato, tomato, and wheat, these lines are being employed to regenerate plants resistant to adverse environmental circumstances [12, 13]. It can thus be possible to regenerate plants that are capable of withstanding harder environments than non-acclimatized plants of similar species employing in vitro plant culture of cells to distinguish tolerance clones of intolerance individuals as well as acclimative methods [14].

On nutrient medium, callus cultures are clusters of undifferentiated plant cells. The medium's addition of auxin plus cytokinins, which make up the majority of the phytohormone balance, maintains the condition of undifferentiated formation.

Natural plant life forms callus tissue following being wounded, and this cell mass aids in the wound's quick healing. A tissue is injured in an in vitro culture, and the induced callus is then sub cultured on nutritive medium [15].

Diethyl sulfate (DES), a chemical mutagen, has been employed for plant or microbial breeding, but until our research preliminarily explored its usage for this purpose, it had not been utilized to activate quiet pathways. Additionally, although it is employed in mutasynthesis to create novel antibiotics, chemical mutagenesis has not been explicitly highlighted in research publications as a technique to activate quiet pathways [16].

An ethylating agent is diethyl sulfate (DES). Its main applications include serving as an accelerator in the sulfation of ethylene and in several sulfonation processes, as well as a chemical intermediary in the production of ethyl derivatives of phenols, amines, and thiols. DES can potentially expose people through ingestion, inhalation, and skin contact during manufacture and usage [17].

Nomenclature & Symbols			
DES	Di Ethyl Sulfate	NAA	Naphthalene acetic acid
MS	Murashige and Skoog Media	NaCl	Sodium chloride
2, 4-D	Di chlorophenoxy acetic acid	$CaCl_2$	Calcium chloride
BA	Benzyl adenine	$MgCl_2$	Magnesium chloride

2. Material and Methods

2.1. Callus Induction Mediums

A culture media was utilised to generate callus, as seen below. We grew Vigna unguiculata L. embryos in universal tubes using MS media with different amounts of 2, 4-D (0, 1, 2, 3, or 4) mg/l, as shown in Table (1). These tubes were then divided into 10 repetitions and placed in a dark environment at 25 ± 1 °C. The callus induction findings were collected after 30 days, as documented by Ramawat [15].

Table 1: Utilisation of Intermediate Elements for Inducing Callus Formation from *Vigna Unguiculata* Seeds.

No.	Components	Concentration (mg/l)
1	MS	4400
2	Sucrose	30000
3	Glycine	100
4	NAA	0.2
5	BA	0.2
6	Agar-Agar	8000
7	2,4-D	0,1,2,3,4

2.2. Callus cultivation on culture media with varying salt concentrations

Segments of the four-week-old *Vigna unguiculata* L. callus weighing 250 mg were isolated. The pieces were placed on the same medium as the callus induction (MS) medium, adding 2 mg/l of 2,4-D and varying concentrations of NaCl, $CaCl_2$, and $MgCl_2$. The concentrations used were 0, 50, 100, 150, 200, and 250 mM, with NaCl, $CaCl_2$, and $MgCl_2$ in a ratio of 2:2:1, respectively [18]. Description of the composition of soils in Iraq inspired the choice of this ratio.

2.3. Mutation Induction by Chemical Mutagen (Di Ethyl sulfate, DES)

Diethyl sulfate (DES) solution had been made by added 90 ml DES with 10 ml distill water which convert to oil consistency as shown in Fig. 1.

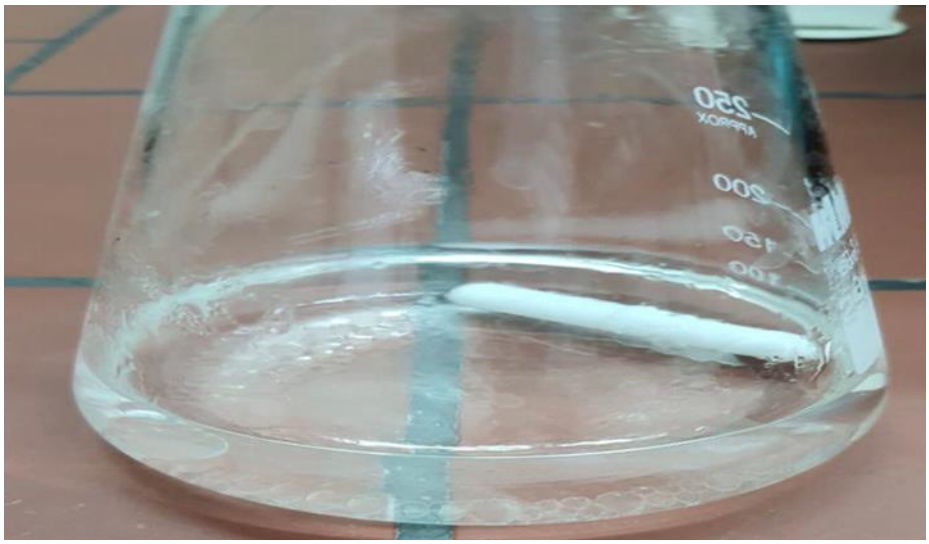

Fig. 1. Oil consistency of Diethyl sulfate (DES) solution.

Then sterilization within a 0.45-m Milliporing filters before being the calluses have been put in sterilized petri plates within the air flow cabinet as well as submerged for 15, 30, and 45 minutes in 0.1 mM DES (Fig. 2) With 7 replications for every therapy over a time period, the callus was next transferred at a

consistent weight of 250 mg and deposited on new callus media identical to what was utilized to create ordinary callus.

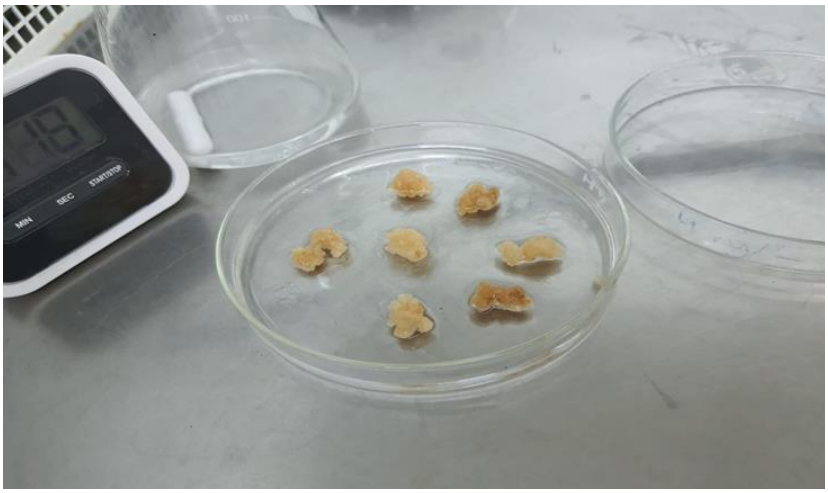

Fig. 2. The callus was soaked in a 0.1 mM DES solution.

The callus's moist and dry weights following 21 days were utilized to calculate the proper DES concentration and soaking time, followed by incubation once again under the identical circumstances.

2.4. Cultivating the DES-treated callus on salt culture media

The callus was soaked in a 0.1 mM DES solution for a period of thirty minutes (the ideal amount of time for the callus to be submerged in a DES solution), then it was cultured on culture media with salt levels of 0, 50, 150, 200, as well as 250 mM with 10 replications of every concentration for salt.

The callus is thereby incubated under identical conditions as before, and after a period of 3 weeks, according to the quantity of development in the callus' fresh weight, a curve was drawn showing the callus' growth for comparison to the curve showing the callus' growth when grown on different levels of salt without DES.

3. Results and Discussions

3.1. Salinity Stress the Influence on Fresh Weight of Stressed Callus (Mg)

The large mean of callus fresh weight observed on the 200 mM treatment (321 mg) constructed that regimen significantly distinct from other treatments (cont, 50, 150, 250), except for the 100 mM treatment, which reached 303 mg. The 50 mM treatment manufactured the smallest mean callus fresh weight. The findings in Table 2 as well as Fig. 3 indicated that the salt concentration was an important influence recent weight gain upon callus. The fresh weight for the callus reduces as the salt concentration rises.

Table 2. Over a four-week period of medium culture, the effect of salt concentrations on callus fresh weight (mg)

Salt concentration (mM)	Cont.	50	100	150	200	250
Fresh weight mean (mg)	250	227	303	286	321	259
LSD (0.05)			20.78			

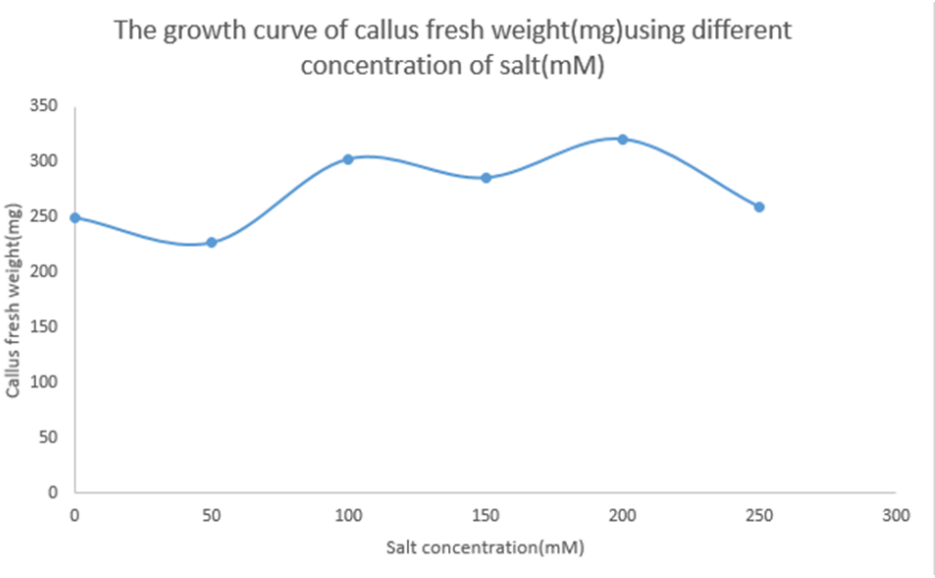

Fig. 3. The callus's growth curve at various salt concentrations (mM).

3.2. Various Diethyl Sulfate Treatments' Impact on Callus Fresh and Dry Weight

Table 3 and Fig. 4 illustrates the impact of diethyl sulfate treatment on callus growth and it appears clear which the callus newly weighed was greatly affected by the various DES levels. Usual one fresh weight of the callus was greatest when it had been immersed in 0.1 mM of DES for 30 minutes (414 mg) and lowest while it was immersed for 15 and 45 minutes (335, 378) mg.

Table 3. The mean fresh weight for times of socked callus in DES

Time of socked callus in 0.1mM DES	Mean
15 Min	335
30 Min	414
45 Min	378
LSD	N. S

Fig. 4. Various diethyl sulfate treatments' impact on callus fresh weight.

DES's impact on salts tolerate in calluses following re-cultivation at salt concentrations between 0 and 250 mM. The results shown in Table 3 and Fig. 5 demonstrated that DES assisted in rising salts tolerate in *Vigna* callus cells. It had been clear when the therapy of 250 mM resulted in achieving its greatest average callus fresh weight of 375 mg, which was significantly different from the treatments (cont,50) mM which reached to (250, 290) mg respectively, while the treatment 300mM had no significantly differences than treatments (100, 150 ,200) mM which reached to (340, 364, 363) mg respectively.

Table 3. Following 4 weeks of medium culture, the impact of DES on callus salt tolerance for each dry weight as well as fresh weight (mg).

Salt Concentrations (mM)	Cont.	50	100	150	200	250
Fresh weight mean (mg)	250	290	340	364	363	375
Dry weight mean (mg)	16	19	23	25	28	29
LSD (0.05)	For fresh weight=69.45			For dry weight=7.96		

Figure 5: The callus fresh weight treated with DES and grew on salt levels.

Clearly, the Fig. 6 demonstrated that the use of diethyl sulfate DES resulted in an increase in callus weight now as opposed to then prior to the use of diethyl sulfate.

The treatment with 250 mM gave the largest average of callus dry weight, reaching 29mg, which was substantially different from cont,50mM treatments.

A rise in the callus's fresh weight (treated with DES) and grew on salt levels may be as a result of variations led to obtain cells with the ability to withstand higher salinity. Or the increase in fresh weight may be caused by the Somaclonal variation due to the re-culturing process for callus, and this was confirmed by [19] that the re-culturing callus several times can become an important source of genetic variations toward salinity tolerance or improve the qualities of the plant [20].

It has been reported that exposure to DES causes mutations, chromosomal aberrations, and other genetic alterations in various organisms [21].

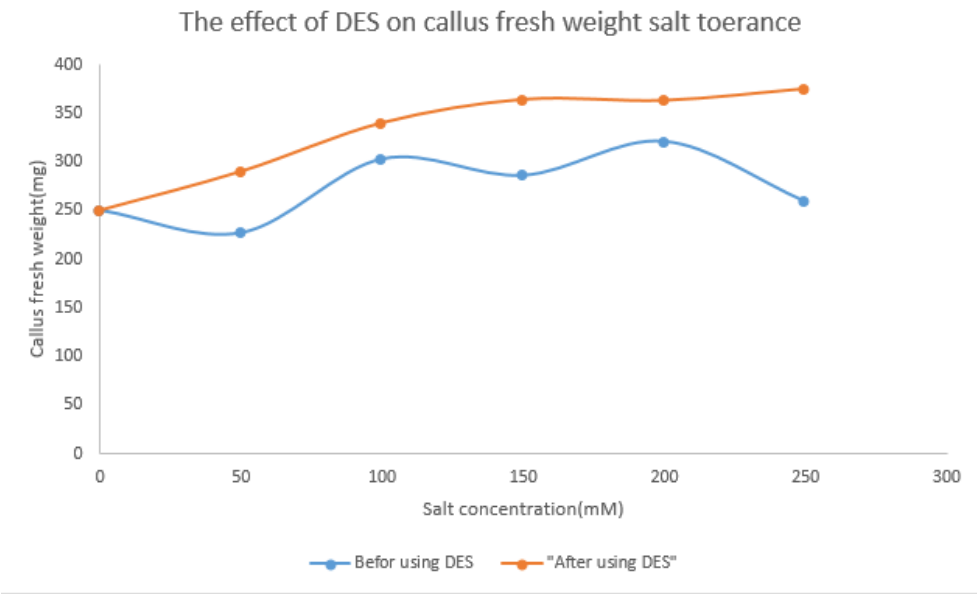

Fig. 6. The impact of DES on salt tolerance in callus fresh weight.

4. Conclusions

The capacity of the callus cells to tolerate salt was improved by treating the callus with 0.1 mM of DES and re-culturing at various salt concentrations.

5. Recommendations

Ethyl nitroso-urea ENU mutagens, physical factors including droughts, and nanoparticles are used to alter Cowpea *Vigna* Sp.

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