Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 3, 1-13
2024
Publisher: Learning Gate
DOI: 10.55214/25768484.v8i3.1088

In vitro induction and investigation of salinity tolerance in the callus of Vigna spp. (L)

Wasan Mohammed Alsewidi[1*], Hashim Kadhim. Al-Oubaidi[1]
[1]Biology Department-Collage of Science, Mustansiriyah University, Baghdad, Iraq; wasan.m.alsuide@gmail.com (W.M.A.); macademics9@gmail.com (H.K.A-O.).

Abstract: Cowpeas can be employed for rotational grazing or generating high-quality food when paired with crops such as maize. Cowpea seeds provide a substantial amount of protein and other essential elements. The purpose of this work was to use a mutagen and a high salt concentration to extract a salt tolerance gene from the callus. The callus was induced by adding 2,4-Dichlorophenoxyacetic acid 2,4- D at mg/l and was then subjected to various concentrations of a combination of salts consisting of sodium chloride NaCl and Calcium Chloride.CaCl2, in addition to Magnesium chloride MgCl2, with a rate of 2:2:1 and potion 0, 50, 100, 150, 200, and 250 mM of the salt mixture added 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 30 minutes**.** The results showed that when using quantitative polymerase chain reaction q-PCR, they showed two genes of *vigna*, the first gene in this research. Aldehyde dehydrogenase family 3 (ADF3) demonstrated the explanation of the genes at doses of DES+200, 200, and 250 mM; however, the most elevated level of gene expression at DES+200mM is CT 30.47, with an upregulation of 2.479. The second gene, Protein S2, demonstrated that the gene is highly expressed at DES+200mM, cycle threshold (CT) 31.68, and DES+250mM concentrations and is upregulated at DES+150mM was 128.

Keywords: *Gene expression, Mutagen, Q-PCR, Salt tolerance, Vigna sp.*

1. Introduction

Cowpea might be utilized for rotational grazing or for producing food of excellent quality if combined with crops like maize. Cowpea seeds contain a significant quantity of protein and other nutrients. People worldwide, especially in impoverished countries, consume cowpea seeds because they cultivate them to produce fully formed seeds and underdeveloped pods. Its amino acids complement those in cereals, while its mineral contents—calcium and iron—are greater than those in meat, fish, and eggs, and the iron content is comparable to that of milk. Its water-soluble vitamins—thiamine, riboflavin, and niacin—have levels that are comparable to those in lean meat and fish, making them a healthier alternative to those foods [1].

Therapeutic Uses The edible seeds are utilized as a nutritional protein supply. These were also utilized to kill stomach worms and enhance the stomach. The herb treats burns, wounds, adenitis, measles, and smallpox. Decoction or soup can treat leucorrhoea, menstrual irregularities, intestinal colic, and urine discharges; it also serves as an aphrodisiac, laxative, antipyretic, diuretic, anti-hyperglycaemic, ant nociceptive, galactagogue, and liver tonic, among other uses. It has thrombolytic, antiviral, antibacterial, antioxidant, and free radical-scavenging properties. Antibacterial action against both Gram-positive and Gram-negative pathogens is also seen. It is beneficial for conditions such as constipation, anorexia, epilepsy, and jaundice [2].

After cereals, grain legumes are the most important family of agricultural crop species worldwide [3]. One grain legume that grows in tropical and subtropical regions of the world is the cowpea (Vigna

History: Received: 18 December 2023; Revised: 22 January 2024; Accepted: 16 February 2024; Published: 19 March 2024
Correspondence: wasan.m.alsuide@gmail.com

spp., L.). It is a noteworthy food legume. Many African countries consume delicate leaves, fresh pods, and grains, making it a staple and multipurpose dietary legume [4].

Plant tissue culture, in addition to meeting the demands for large-scale plant multiplication, is essential for other biotechnology applications in plant development [5, 6]. Its value as a device for immediate use in basic research in well-known fields like molecular genetics, plant biology, and biochemistry is significant. Today, there are eight billion unnerving individuals living on the planet.

One of the greatest issues facing the human race in this period is supplying food and goods, most of which are plant-based, although the extraordinary challenges posed by climate change are greater than ever [7, 8]. Global warming is caused by a variety of climatic changes that have a significant impact on agricultural systems around the world. This includes substantial environmental stressors such as salt, exposure to hazardous substances, floods, excessive heat or cold, and drought [9].

Over the past forty years, there has been a significant increase in the study of stresses on plants, with emphasis on the impacts of stress elements such as a shortage of water, extreme heat, salt, exposure to dangerous materials, insufficient or excessive radiation, plant pathogen infection, and insect outbreaks [10, 11].

Plant tissue culturing is the most effective technique for improving and developing salt tolerance in plants. It is convenient to concentrate on because it highlights the physiological and biochemical processes that are vital to the cell as well as the alterations brought about by using salt overload in plant cells and tissue culture [12].

Researchers have introduced molecular techniques to more accurately evaluate the genetic relationships of cowpeas. These techniques include microsatellite markers [13] random amplified polymorphic Deoxyribonucleic Acid (DNA) (RAPD) [14] amplified fragment length polymorphisms (AFLP) [15] restriction fragment length polymorphisms (RFLP) [16]. Several agricultural plant species have had their taxonomic connections resolved and farmed variants well characterized by applying seed storage protein markers: black gram [17] *Capsicum annuum* L. *Solanum*, *Vigna spp*., and wheat [18].

The declining genetic resources of crop types pose a significant threat to agricultural product manufacturing. Due to its lengthy history of domestication— abundant Asian nations had documentation from 2,000 years ago that they planted and produced cowpea—cowpea now faces a comparable threat [19]. As a result, the foundation of cowpea breeding and genetic research is the preservation, exploration, development, and application of plant genetic variety. The foundation for genetic variety and the genetic connections between genotypes of cowpeas are found in genetic diversity research, which also gives information for the creation of cultivars and the preservation and utilization of germplasm resources [20].

The arid agroecologies of the tropics in South Asia, Latin America, and Africa primarily produce the herbaceous annual crop known as black eye pea, or cowpea (Vigna unguiculata (L.) Walpers). It is a legume from the genus Vig, *the tribe Phaseoleae*, and the family Fabacea.

Plant tissue culture is the controlled cultivation of plant cells, tissues, or organs in a sterile and artificial environment with precise control over the surrounding conditions. We developed the methods based on tot-potency, the ability of a cell to generate a complete multicellular organism, and cell theory, which asserts that cells serve as the fundamental units of structure and reproduction in all living things [21].

A fresh source of genetic diversity that can be used to improve plant germplasm is soma clonal variation in plant cell culture, which is a useful method for testing hypotheses regarding the advantages and risks of different chemicals and biological agents because it is a crucial component of plant science and biotechnology. This technique is also important for changing genes, making plants that don't have any pathogens, micropropagation, keeping germplasm safe, and making different medicinal secondary metabolisms [22]. The principles of totipotency and developmental plasticity are two fundamental ideas required for plant regeneration in tissue culture.

Halophytes are a source of high-value products with various commercial uses in the food, pharmaceutical, and cosmetic sectors. Thus, we can use plant tissue culture (PTC) with halophyte species to enhance vegetative and sexual multiplication of those with restricted propagation, boost bioactive

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 3: 1-13, 2024
DOI: 10.55214/25768484.v8i3.1088

chemical synthesis, and propagate fragile and endangered species [23]. By using the economic potential of agricultural and medicinal plants, PTC approaches have made significant contributions to industry and agriculture over the past 60 years [16]. Research on halophytes is expanding, primarily emphasizing culture and its biochemical characteristics.

Mutations are the existence of mobile genetic components like transposons in the genome, which are mobile and repeating. Different environmental factors, such as ultraviolet (UV) radiation or naturally occurring chemical or biotic agents, can also cause mutations in plants. Mutations are the primary cause of genetic variety in all living things [24]. The pace at which spontaneous mutations accumulate is accelerated by the unique environmental circumstances of in vitro culture settings, tissue reprogramming, and disorganized development commonly connected to in vitro culture, particularly in cases where callus development is a factor [25].

Three basic processes make up the use of mutagenesis techniques, both physical and chemical, in mutant breeding. Random changes are made to the plant's DNA in phase 1, the mutants are screened for desirable phenotypes in step 2, and the traits that are wanted are chosen in step 3 [26]. The following sections provide further information on the various mutagenesis methods used in mutation breeding (phase 1). Phase 2 includes applying to the mutants created by these techniques a screening using either the "forward" or "reverse" genetic approaches [27]. Phenotypes drive the forward genetics approach because it entails evaluating the altered plants' phenotype directly (e.g., abnormalities in stem height, number of seeds, chlorophyll and leaf content, etc.). Molecular methods, such as markers based on polymerase chain reaction (PCR), can facilitate the next step of pinpointing the mutation causing the condition.

The technique of employing chemical agents to cause mutations in the plant genome is known as chemical mutagenesis, which includes alkylating compounds, intercalating agents, and base analogs [28]. Table 1 lists the chemical agents often used in plants for haphazard mutagenesis. The chemical mutagens ethyl methane sulfonate (EMS), which is frequently used in mutant breeding, methyl nitrosourea (MNU), 1-ethyl-1-nitrosourea (ENU), diethyl sulfate (DES), and sodium azide (SA). Combinations of MNU and SA are frequently observed [29]. Chemical mutagenesis may be performed utilizing several plant materials, including cell cultures and whole plants grown in a laboratory setting. Seed is the primary plant material used in chemical mutagenesis.

The original PCR serves as the basis for several techniques, one of which is dPCR. In order to digitally represent the pool of DNA molecules, which can have either one copy or no copy in each subsample, a PCR specimen needs to be split into many, many millions of derived subsamples. Microfluidics is the foundation for droplet-based and chip-based dPCR. DPCR may skip the laborious qPCR quantification based on standard curves and instead offer a precise measurement of the DNA or RNA copy number. Moreover, multiplex PCR amplifies DNA materials with undesirable ratios of abundant and uncommon DNA [30].

Contrary to popular belief, they declared that "RT-PCR" should only refer to reverse transcription PCR and not real-time PCR [31]. In a reverse transcription polymerase chain reaction (RTP) template, RNA is used to produce complementary DNA (cDNA). The reverse transcriptase enzyme creates a single-stranded copy of cDNA. After that, one can use a DNA polymerase to amplify it, resulting in double-stranded cDNA suitable for a conventional PCR-based amplification procedure.

The qPCR cycle is detected in real-time, producing a Relative Risk (RR) amplification curve with plateau, exponential, and start phases. This curve represents the fundamental concept of quantitation. The low level of fluorescence at the start of amplification establishes the baseline level of fluorescence. The threshold level, at which the reaction enters exponential growth, is when fluorescence becomes considerably higher than the baseline. The Ct or Cq value, which shows the moment at which your sample surpasses this threshold, forms the core of quantitative analysis. Reagent shortages during the plateau period, for example, have no impact on the reading since the threshold is set during the exponential phase [32].

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 3: 1-13, 2024
DOI: 10.55214/25768484.v8i3.1088

2. Material and Methods

2.1. Callus Induction

After being removed, sterile embryos known as explants from Vigna spp. were cultured in universal tubes using Murashige and Skoog (MS) media, treated with auxin 2,4-D (2) mg/l and maintained at a temperature of 23±1°C in the dark [15]. The four-week-old callus of Vigna (250 mg) pieces were divided and then put on the MS medium, which had been mixed with 2,4-D at a dosage of 2 mg/l and NaCl, CaCl2, and MgCl2 at concentrations of 0, 50, 100, 150, 200, and 250 mM [17]. (NaCl, CaCl2, and MgCl2 ratios are 2:1:1, which is close to the ratio of the soil discovered in Iraq.)

2.2. Di-ethyl Sulfate, a Chemical Mutagen, Induces Mutations

The sterilization procedure was completed using a diethyl sulfate (DES) solution. The calluses were placed in sanitized petri plates within the airflow cabinet and immersed for 15, 30, and 45 minutes in 0.1 mM DES after being placed inside a 0.45-m Millipore filter. The callus was then transferred at a constant weight of 250 mg and placed on a fresh callus medium identical to what was used to produce a typical callus, requiring 7 replications during the course of therapy.

2.3. DES-Treated Callus Cultivation on Salt Culture Medium

The callus was cultivated in a culture medium containing ten replications of each salt concentration at 0, 50, 150, 200, and 250 mM after being immersed in a 0.1 mM DES solution for thirty minutes (the recommended amount of time for the callus to be submerged in a DES solution).

2.4. Total RNA Extraction and Purification using the Abm Kit

After grinding 50 to 100 mg of callus with a mortar and pestle, we poured a small amount of liquid nitrogen onto the specimen to chill it. We then processed the specimen to a fine powder in liquid nitrogen. The subsurface material was combined by vortexing 500 µl of RB buffer (or PRB buffer) and 5 µl of mercaptoethanol before being incubated for 5 min at room temperature. We added the specimen mixture to a filter column and centrifuged it for 1 minute at 1000 rpm. The filtrates of the cleared mixture were added to a 500-l volume of 100% ethanol and violently shaken. The mixture was then centrifuged at a speed of 14000–16000 rpm for a duration of 2 minutes.

Then, the RB column was transferred to another 2 ml collection tube. In the center of the RB column matrix, 100 µl of DNase was introduced and left for ten minutes. Aliquots of 400 l of wash buffer W1 were introduced to the center of the RB column and centrifuged for one minute at between 14000 and 16000 rpm. After that, 600 µl of wash buffer W2 was added to the middle of the column. The flow-through was then discarded.

Later, 50 µl was added of RNase-free water was added. The column was then allowed to stand for 3 minutes before being centrifuged at 14000–16000 rpm for 3 minutes. After total RNA was converted to cDNA using AccuPower® RT Premix, the RT PCR method was employed to amplify the cDNA. The PCR mixture (total volum 20µl) consists of 5 µl of AccuPower® RT Premix, five µl of each Oligo dt15 primer (100 PM), five µl of 0.1% of DEPC-water and 0.5–1.0 µg/5 µl of template RNA. The RNA template and Oligo dt15 primer annealing mixture were incubated at 70 °C for 5 minutes before being put on ice. The thermocycler process had been utilized to create the cDNA in the following manner: 42 ˚C for 60 min (cDNA synthesis). 95 ˚C, for 5 min (RTase inactivation).

2.5. RT-PCR Reverse Transcriptase Technique for cDNA Synthesis

RT-PCR is the method that uses any one of numerous reverse transcriptases to convert RNA molecules into their complementary DNA (cDNA) sequences. Conventional PCR techniques then amplify the freshly produced cDNA.

Complementary DNA (cDNA) is created via reverse transcription, which synthesizes DNA from an RNA template. To guide the synthesis of the first strand of cDNA, which may be used directly as a

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 3: 1-13, 2024
DOI: 10.55214/25768484.v8i3.1088

template for the Polymerase Chain Reaction (PCR), reverse transcriptases (RTs) employ an RNA template and a primer corresponding to the three ′ ends of the RNA.

Reverse transcription plus PCR (RT-PCR) makes it possible to identify low-abundance RNAs in a sample and produce the matching cDNA, which makes low-copy gene cloning easier [33]. Table 1 displays the sequences of primers used to amplify the genes under study (ADF, Protein S2).

Table 1.
The sequences of primers used for amplification of studied genes (ADF, protein S2).

Genes	Primer sequences 5' ⟶ 3'	Size (bp)
ADF	F-TGTCAGGCAGAAAGGCAGAA	84
	R-TGAGGACAAGACCCAGTGGT	84
Protein-S2	F-CACACAATGAGGTCTTCTCTTGC	70
	R-CTTCTATGGTGCCACTTAACAAGAA	70

2.6. RT-qPCR Reverse Transcription

We measured quantitative PCR for this investigation using SYBR Green I fluorescent dye. We performed the heat cycling protocols using real-time PCR equipment (Rotor-Gen Q, Qiagen, Germany) equipped with the WIZpURE Tm qPCR Master (SYPR) Kit (Wizbiosolution Business, North Korea). The PCR programs were carried out according to the manufacturer's instructions, and the reaction conditions followed the protocol [13]. The conditions of thermo cycling have been mentioned in Figure 1.

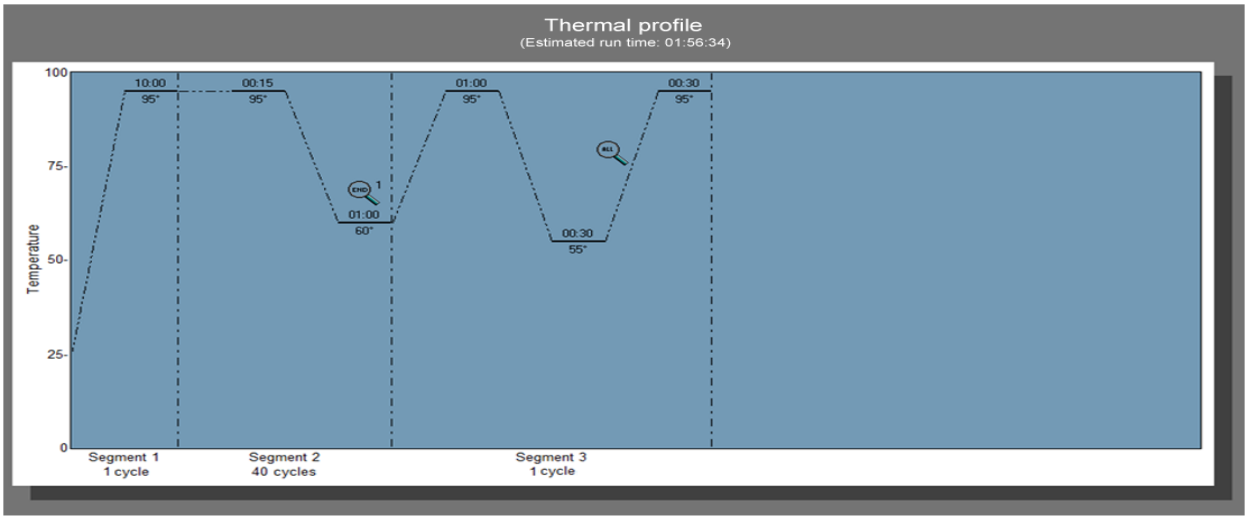

Figure 1.
Conditions of amplification.

2.7. Data Evaluation

The results of three replicates of the Real-time polymerase chain reaction (RT-PCR) study were used to calculate the average expression level of the test genes. Quantitative real-time PCR data using the threshold cycle (CT) technique.

3. Results and Discussion

3.1. Impact of Salinity Stress on V. Unguiculata's Fresh Callus Weight (mg)

Table 2 and Figure 2 results show that the fresh weight of the callus was strongly impacted by the salt concentration effect, with a note that the primary weight of the callus was 250mg/l. The large mean

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 3: 1-13, 2024
DOI: 10.55214/25768484.v8i3.1088

of callus fresh weight observed on the 200 mM treatment (321.7 mg) constructed that regimen significantly distinct from other therapies (cont, 50, 150, 250), except for the 100 mM treatment, which reached 303.3 mg. The 50 mM treatment manufactured the smallest mean callus fresh weight. As the salt concentration increases, the fresh weight of the callus decreases. Between all treatments, the dry weight was not significant.

The medium's salinity affected callus formation; callus growth gradually decreased as the salinity increased [34]. Tissue culture techniques have helped researchers learn more about how cells can tolerate salt by using certain salt-tolerant cell lines as a study system [35, 36].

Our investigation focused on the advances in producing salt-tolerant lines using tissue culture-based in vitro selection. Plants have developed several biochemical and molecular processes to endure and thrive in stressful situations. Selecting agents that allow for preferred survival and expansion of desirable phenotypes has been a successful strategy to promote tolerance [37]. In vitro selection often uses NaCl for salt tolerance. In the culture medium, we can subject the explants to a wide spectrum of selection agents. We only choose specimens that can withstand these conditions over time. During the in vitro selection process, it is possible for cells that are not tolerant to undergo adaptation. Salinity resulting from excessive NaCl, the most prevalent salt stress, leads to a rise in sodium (Na+) concentrations and a decrease in plants' potassium (K+) concentrations. The phenomenon is called the salt-specific or ion-excess impact of salinity [38]. Plants have developed defensive systems to cope with salt stress, enabling them to adapt. These methods include osmotic adjustment, often achieved by accumulating betaine and polyols [39].

Table 2.
The effect of salts levels on callus fresh weight (mg) after four weeks of culture on medium.

Concentration of salt (mM)	Fresh weight(mg)	Dry weight(mg)
Cont.	250.0	30.7
50 mM	227.0	30.3
100mM	303.3	32.0
150 mM	285.7	29.7
200 mM	321.7	31.3
250 mM	259.0	30.3
LSD 0.05	20.78	N.S

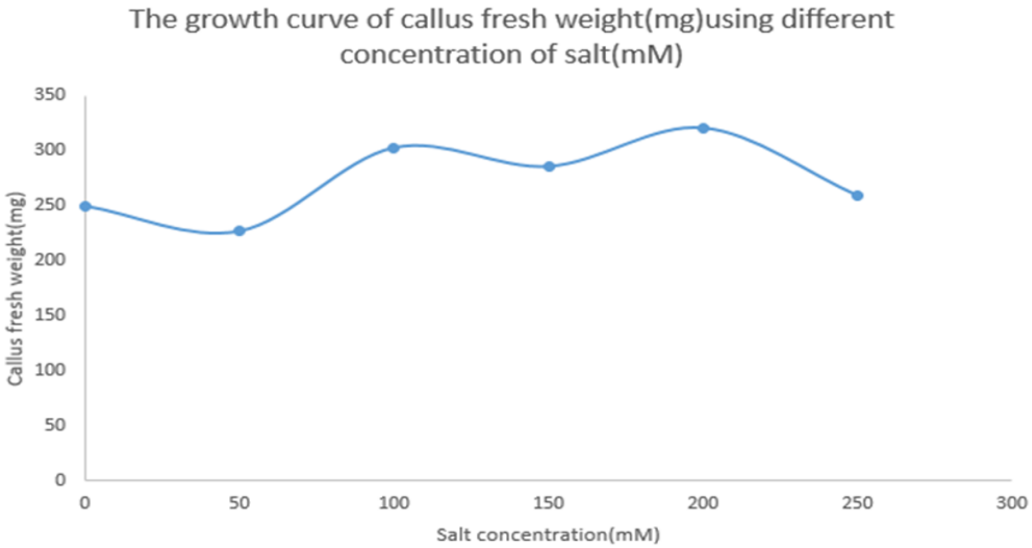

Figure 2.
The callus's growth curve at various salt concentrations (mM).

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 3: 1-13, 2024
DOI: 10.55214/25768484.v8i3.1088

3.2. Effect of Different Treatments of Di Ethyl Sulfate DES on Callus Salt Tolerance

Calluses tolerate the impact of DES on salts after re-cultivation at salt concentrations ranging from 0 to 250 mM. The results in Table 3 and Figure 3 demonstrated that DES assisted in raising salts tolerated in *Vigna* callus cells. It was 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 (250 289.70) mg, respectively, while the treatment 250mM had no significant differences than treatments (100, 150,200) mM, which came to (340.30, 363.70, 363.30) mg respectively. Figure 4 demonstrates that the use of diethyl sulfate DES increased callus weight now as opposed to before the use of diethyl sulfate. The treatment with 250 mM gave the largest average of callus dry weight, reaching 29.30mg, which was substantially different from cont 50 therapies [28].

A rise in the callus's fresh weight (treated with DES) and growth on salt levels may result from variations that led to obtaining cells with the ability to withstand higher salinity. This could indicate that the DES treatment has induced genetic changes in the callus, enabling it to adapt and survive in high salt conditions. These variations may have altered the expression of certain genes responsible for regulating salt tolerance in the callus. We need further research to understand the mechanisms of this increased tolerance and its potential applications in crop breeding in saline environments. 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 Lee, et al. [30] and Vargas, et al. [32] 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 [37].

Table 3.
The four weeks of medium culture, the impact of DES on callus salt tolerance for each dry weight, and fresh weight (mg).

Salt concentration(mM)	Dry weight(mg)	Fresh weight(mg)
Cont.	250.00	16.00
50	289.70	18.70
100	340.30	23.00
150	363.70	24.70
200	363.30	28.00
250	375.00	29.30
LSD (0.05)	69.45	7.96

Figure 3.
The callus fresh weight was treated with DES and grew on salt levels.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 3: 1-13, 2024
DOI: 10.55214/25768484.v8i3.1088

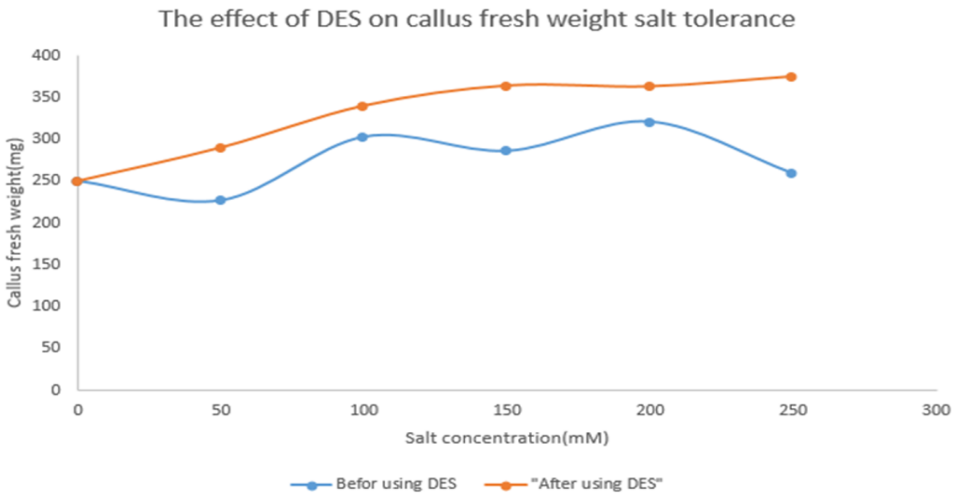

Figure 4.
The impact of DES on salt tolerance in callus fresh weight.

3.3. Gene Expression Analysis for the Callus of Vigna Unguiculata

The specimen's target nucleic acid content is inversely proportional to CT levels. Real-time tests run through 40 amplification cycles. For this study's first gene, compare CT salt concentrations with mutagen DES and CT controls. ADF demonstrated the expression of the genes at concentrations (DES+200, 200, and 250); however, the elevated level of gene expression at DES+200 is CT 30.47, with an upregulation of 2.479, Table 4, Figures 5 and 6.

Table 4.
Control of the ADF gene for the Vigna callus.

ADF3/ Gene	CT	ΔCT	ΔΔCT	Folding
Cont.	29.53	-0.01	0	1
DES+150	29.23	-0.47	-0.46	0.726
150	29.42	0.19	0.2	1.148
DES+200	30.47	1.3	1.31	2.479
200	29.66	0.29	0.3	1.231
DES+250	29.16	-0.31	-0.3	0.812
250	29.91	0.68	0.69	1.613

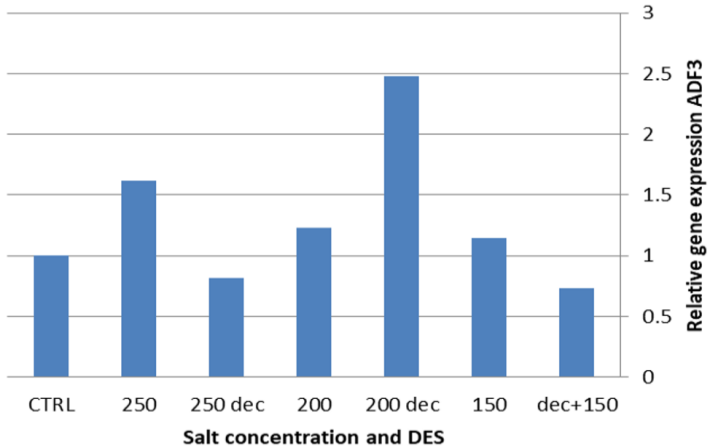

Figure 5.
The correlation among relative gene expression and salt concentration + DES.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 3: 1-13, 2024
DOI: 10.55214/25768484.v8i3.1088

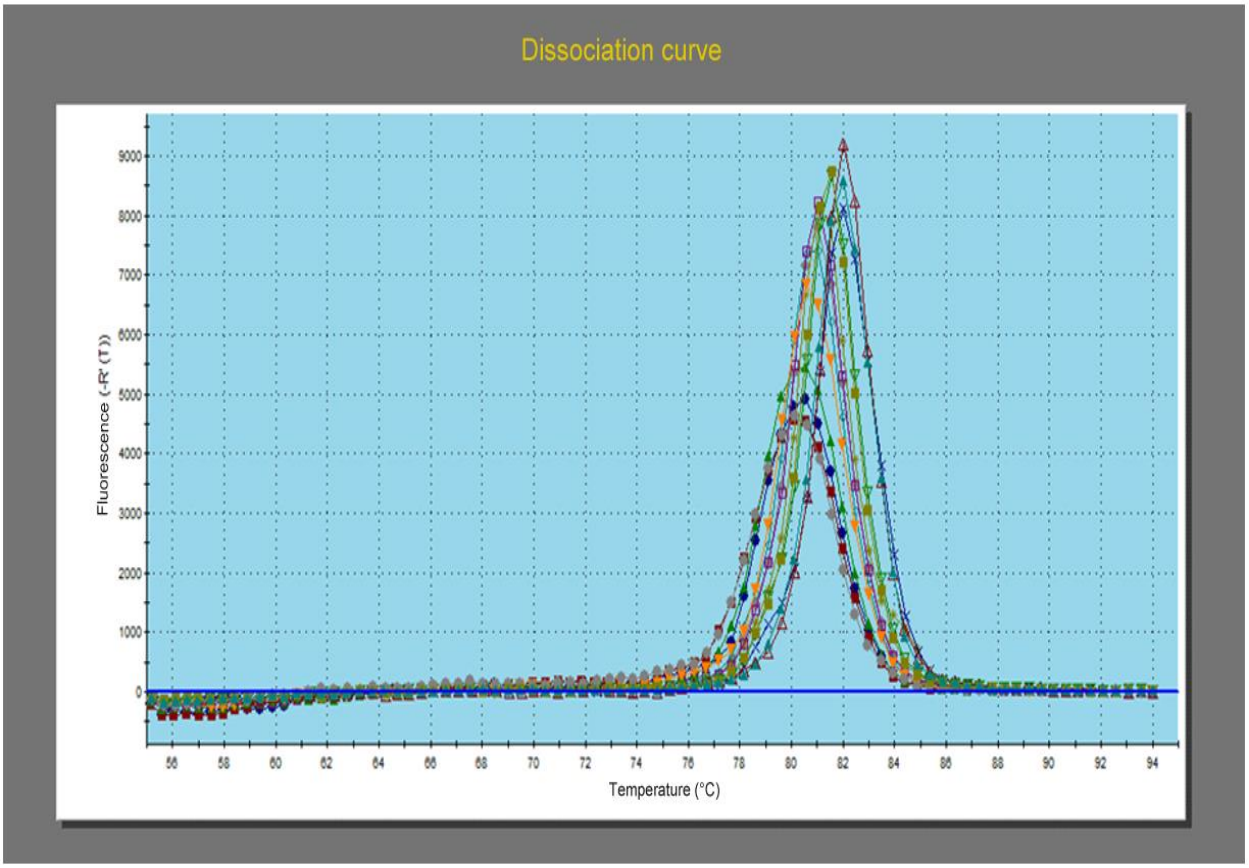

Figure 6.
Dissociation curve of ADF gene of *vigna SP*.

We demonstrated high expression of the second gene, Protein S2, at DES+200, CT 31.68, and DES+250 concentrations, and upregulation at DES+150 and 128 (Table 5) (Figures 7 and 8).

Table 5.
Regulation of protein s2 gene for a callus of Vigna SPP.

Protein S2\Gene	CT	ΔCT	ΔΔCT	Folding
Cont.	28.54	0.38	0	1
DES+150	29.47	7.38	7	128
150	28.46	-1.29	-2	0
DES+200	31.68	2.06	2	3
200	28.34	-0.38	-1	1
DES+250	28.71	-0.64	-1	0
250	22.09	-6.53	-7	0

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 3: 1-13, 2024
DOI: 10.55214/25768484.v8i3.1088

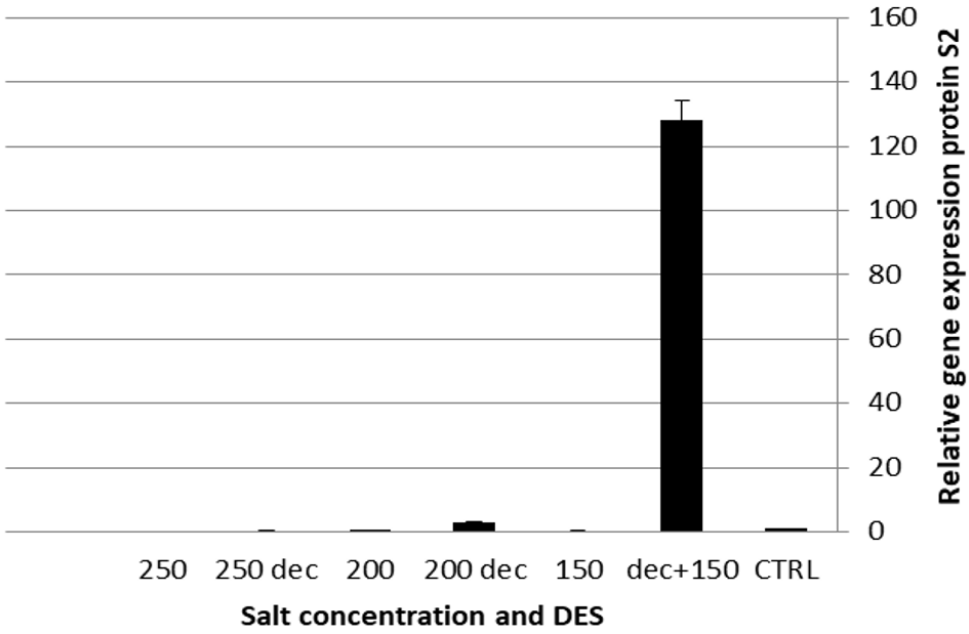

Figure 7.
The relationship between folding (Regulation) and salt concentration.

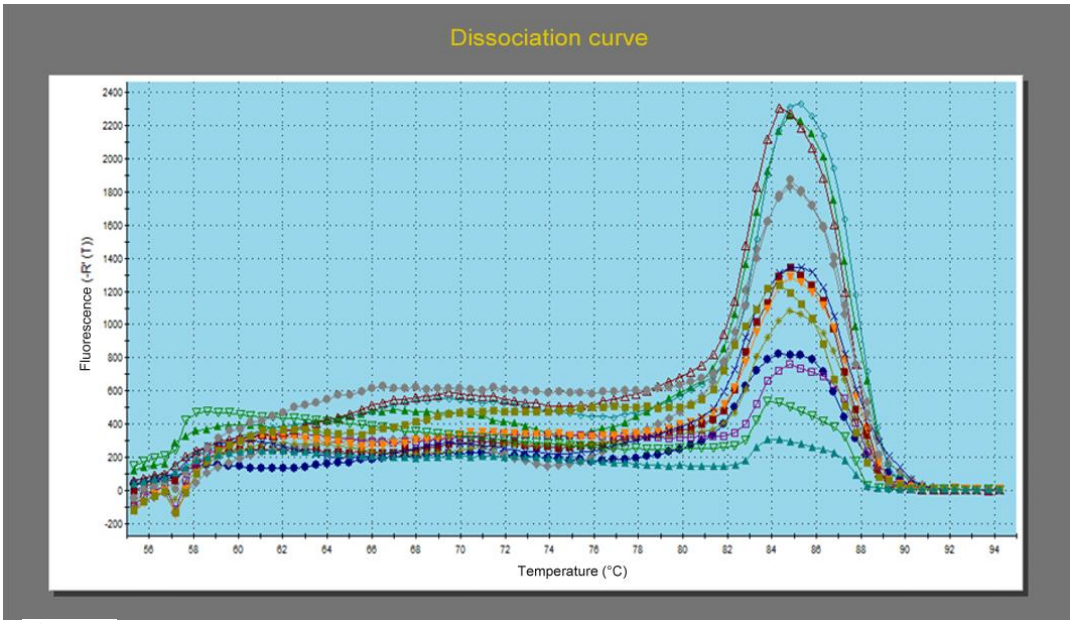

Figure 8.
Dissociation Curve of protein S2 gene of *Vigna SP.*

Reverse transcription qPCR has gained popularity as a method for gene expression analysis because of its high sensitivity, great repeatability, outstanding specificity, excellent throughput, and reliable internal reference genes to correct target gene data and provide accurate findings [35]. In rice seedlings treated with NaCl and mannitol 14, the consistent expression levels of 18S rRNA and 25S rRNA were the highest of all reference genes. Additionally, it was shown that Arabidopsis thaliana and other herbaceous plants consistently expressed the 26S rRNA gene when exposed to 10% Polyethylene glycol (PEG) [36].

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 3: 1-13, 2024
DOI: 10.55214/25768484.v8i3.1088

Different plant species frequently use the tissue culture technique with some clones to generate genetically stable and beneficial variants. Thus, regenerated plants experience soma clonal variations, most likely brought on by stressors imposed throughout in vitro culture [37].

Stable-expressed reference genes must be identified for data normalization of target genes under certain testing circumstances in RT-qPCR gene expression investigations related to the risk that applying inadequate, unvalidated reference genes could result in questionable interpretation of the results [38]. Aldehyde dehydrogenase family three and organ-specific protein S2 were researched to assess the expression of genes linked to the stress salt response in Vigna *SP*. The buildup of a fluorescent signal allows the identification of both genes that exhibit positive responses. The number of cycles necessary for the fluorescent signal to cause a change in gene expression is known as the CT (cycle threshold).

4. Conclusion

qPCR showed high levels of gene expression for salt tolerance when DES mutagens had a salt concentration of 200 mM. At a concentration of 200 mM, the DES mutagen significantly enhances the expression of genes associated with salt tolerance. These findings suggest that the DES mutagen could be a potential tool for improving salt tolerance in crops, which is particularly relevant in regions with high salinity levels. Further investigation is necessary to identify the specific genes upregulated by the DES mutagen and comprehend the underlying mechanisms responsible for enhancing salt tolerance. Furthermore, it is critical to evaluate the DES mutant's performance under various salt concentrations in order to determine its optimal range for maximum efficacy. This information will be critical for future research and potential applications in agriculture and crop improvement.

Funding:

This study received no specific financial support.

Institutional Review Board Statement:

Not applicable.

Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

Competing Interests:

The authors declare that they have no competing interests.

Authors' Contributions:

Both authors contributed equally to the conception and design of the study. Both authors have read and agreed to the published version of the manuscript.

References

[1] E. Kebede, "Grain legumes production and productivity in Ethiopian smallholder agricultural system, contribution to livelihoods and the way forward," *Cogent Food & Agriculture*, vol. 6, no. 1, p. 1722353, 2020. https://doi.org/10.1080/23311932.2020.1722353

[2] M. Alemu, Z. Asfaw, Z. Woldu, B. A. Fenta, and B. Medvecky, "Cowpea (Vigna unguiculata (L.) Walp.)(Fabaceae) landrace diversity in Northern Ethiopia," *International Journal of Biodiversity and Conservation*, vol. 8, no. 11, pp. 297-309, 2016. https://doi.org/10.5897/ijbc2016.0946

[3] P. P. Kumar and C. S. Loh, *Plant tissue culture for biotechnology. In Plant Biotechnology and Agriculture.* Amsterdam, the Netherlands: Elsevier, 2012.

[4] O. L. Gamborg, "Plant tissue culture. biotechnology. milestones," *In Vitro Cellular & Developmental Biology-Plant*, vol. 38, pp. 84-92, 2002. https://doi.org/10.1079/ivp2001281

[5] A. Wijerathna-Yapa and R. Pathirana, "Sustainable agro-food systems for addressing climate change and food security," *Agriculture*, vol. 12, no. 10, p. 1554, 2022. https://doi.org/10.3390/agriculture12101554

[6] F. Valoppi *et al.*, "Insight on current advances in food science and technology for feeding the world population," *Frontiers in Sustainable Food Systems*, vol. 5, p. 626227, 2021. https://doi.org/10.3389/fsufs.2021.626227

[7] M. Pessarakli, *Handbook of plant and crop stress.* Boca Raton, FL, USA: CRC Press, 2019.

[8] S. Fahad *et al.*, "Crop plant hormones and environmental stress," *Sustainable Agriculture Reviews*, vol. 15, pp. 371-400, 2015.

[9] M. Ashraf and L. Wu, "Breeding for salinity tolerance in plants," *Critical Reviews in Plant Sciences*, vol. 13, no. 1, pp. 17-42, 1994.

[10] B. Vogelstein and K. W. Kinzler, "Digital pcr," *Proceedings of the National Academy of Sciences*, vol. 96, no. 16, pp. 9236-9241, 1999. http://dx.doi.org/10.1073/pnas.96.16.9236

[11] T. Murashige and F. Skoog, "A revised medium for rapid growth and bio assays with tobacco tissue cultures," *Physiologia Plantarum*, vol. 15, no. 3, pp. 473-497, 1962. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

[12] P. Buringh, *Soils and soil conditions in Iraq. Ministry of Agriculture.* Baghdad, Netherlands: Neenman and Zonen Press, 1960.

[13] J. Wen *et al.*, "Adaptive regulation of testis gene expression and control of male fertility by the Drosophila hairpin RNA pathway," *Molecular Cell*, vol. 57, no. 1, pp. 165-178, 2015. https://doi.org/10.1016/j.molcel.2014.11.025

[14] W. G. Yoo *et al.*, "Reference genes for quantitative analysis on Clonorchis sinensis gene expression by real-time PCR," *Parasitology Research*, vol. 104, pp. 321-328, 2009. https://doi.org/10.1007/s00436-008-1195-x

[15] K. Singh *et al.*, "26S rRNA-based internal control gene primer pair for reverse transcription-polymerase chain reaction-based quantitative expression studies in diverse plant species," *Analytical Biochemistry*, vol. 335, no. 2, pp. 330-333, 2004. https://doi.org/10.1016/j.ab.2004.08.030

[16] C. Fatokun, D. Danesh, N. Young, and E. Stewart, "Molecular taxonomic relationships in the genus Vigna based on RFLP analysis," *Theoretical and Applied Genetics*, vol. 86, pp. 97-104, 1993. https://doi.org/10.1007/bf00223813

[17] H. Krishna *et al.*, "Somaclonal variations and their applications in horticultural crops improvement," *3 Biotech*, vol. 6, pp. 1-18, 2016. https://doi.org/10.1007/s13205-016-0389-7

[18] S. A. Bustin *et al.*, "The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments," *Clinical Chemistry*, vol. 55, no. 4, pp. 611-622, 2009. https://doi.org/10.1373/clinchem.2008.112797

[19] S. Coulibaly, R. Pasquet, R. Papa, and P. Gepts, "AFLP analysis of the phenetic organization and genetic diversity of Vigna unguiculata L. Walp. reveals extensive gene flow between wild and domesticated types," *Theoretical and Applied Genetics*, vol. 104, pp. 358-366, 2002. https://doi.org/10.1007/s001220100740

[20] C. Sheridan, "Coronavirus and the race to distribute reliable diagnostics," *Nature Biotechnology*, vol. 38, no. 4, pp. 382-384, 2020. https://doi.org/10.1038/d41587-020-00002-2

[21] S. B. Davenport, S. M. Gallego, M. P. Benavides, and M. L. Tomaro, "Behaviour of antioxidant defense system in the adaptive response to salt stress in Helianthus annuus L. cells," *Plant Growth Regulation*, vol. 40, pp. 81-88, 2003.

[22] D. Samsampour, F. Sadeghi, M. Asadi, and A. Ebrahimzadeh, "Effect of nitric oxide (NO) on the induction of callus and antioxidant capacity of Hyoscyamus niger under in vitro salt stress," *Journal of Applied Botany and Food Quality*, vol. 91, pp. 24-32, 2018.

[23] J. Fang, C.-C. T. Chao, P. A. Roberts, and J. D. Ehlers, "Genetic diversity of cowpea [Vigna unguiculata (L.) Walp.] in four West African and USA breeding programs as determined by AFLP analysis," *Genetic Resources and Crop Evolution*, vol. 54, pp. 1197-1209, 2007. https://doi.org/10.1007/s10722-006-9101-9

[24] N. Ghalmi, M. Malice, J.-M. Jacquemin, S.-M. Ounane, L. Mekliche, and J.-P. Baudoin, "Morphological and molecular diversity within Algerian cowpea (Vigna unguiculata (L.) Walp.) landraces," *Genetic Resources and Crop Evolution*, vol. 57, pp. 371-386, 2010. https://doi.org/10.1007/s10722-009-9476-5

[25] C. D. Li, C. A. Fatokun, B. Ubi, B. B. Singh, and G. J. Scoles, "Determining genetic similarities and relationships among cowpea breeding lines and cultivars by microsatellite markers," *Crop Science*, vol. 41, no. 1, pp. 189-197, 2001. https://doi.org/10.2135/cropsci2001.411189x

[26] A. Ghafoor, Z. Ahmad, and R. Anwar, "Genetic diversity in Pisum sativum and a strategy for indigenous biodiversity conservation," *Pakistan Journal of Botany*, vol. 37, no. 1, pp. 71-77, 2005.

[27] R. Gu, Q. Liu, D. Pei, and X. Jiang, "Understanding saline and osmotic tolerance of Populus euphratica suspended cells," *Plant Cell, Tissue and Organ Culture*, vol. 78, pp. 261-265, 2004. https://doi.org/10.1023/b:ticu.0000025666.84313.92

[28] J. Jankowicz-Cieslak, C. Mba, and B. J. Till, "Mutagenesis for crop breeding and functional genomics," *Biotechnologies for Plant Mutation Breeding: Protocols*, pp. 3-18, 2017. https://doi.org/10.1007/978-3-319-45021-6_1

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 3: 1-13, 2024
DOI: 10.55214/25768484.v8i3.1088

[29] S. M. Jain, "Tissue culture-derived variation in crop improvement," *Euphytica*, vol. 118, pp. 153–166, 2001.
[30] L. S. Lee, B. J. Till, H. Hill, and J. O. A. Huynh, *Mutation and mutation screening. In: RJ Henry and A Furtado (Eds.), Cereal Genomics: Methods and Protocols*. Totowa: Humana Press Inc, 2014.
[31] J. M. Leitao, Q. Y. Shu, B. P. Forster, and H. Nakagawa, *Chemical mutagenesis*. Wallingford, UK: Cabi, 2012.
[32] L. Vargas, M. Victor, and N. Ochoa-Alejo, "An introduction to plant tissue culture: advances and perspectives. In Plant Cell Culture Protocols, eds. Víctor M. Loyola-Vargas and N. Ochoa-Alejo." New York: Springer, New York, 2018, pp. 3–13.
[33] M. Gomez-Samblas, S. Vílchez, J. C. Racero, M. V. Fuentes, and A. Osuna, "Quantification and viability assays of Toxoplasma gondii in commercial "Serrano" ham samples using magnetic capture real-time qPCR and bioassay techniques," *Food Microbiology*, vol. 46, pp. 107-113, 2015.
[34] C. Mba, R. Afza, S. Bado, S. M. Jain, M. R. Davey, and P. Anthony, *Induced mutagenesis in plants using physical and chemical agents*. Chichester, UK: Wiley-Blackwell, 2010.
[35] B. Gulzar, A. Mujib, M. Qadir Malik, J. Mamgain, R. Syeed, and N. Zafar, *Plant tissue culture: Agriculture and industrial applications. In Transgenic Technology Based Value Addition in Plant Biotechnology*. Amsterdam, The Netherlands: Elsevier, 2020.
[36] K. N. Rao and C. G. Suresh, "Bowman–Birk protease inhibitor from the seeds of Vigna unguiculata forms a highly stable dimeric structure," *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, vol. 1774, no. 10, pp. 1264-1273, 2007.
[37] A. Riaz and A. Gul, "Plant mutagenesis and crop improvement," *Crop Production and Global Environmental Issues*, pp. 181-209, 2015.
[38] H. Tan, M. Tie, Q. Luo, Y. Zhu, J. Lai, and H. Li, "A review of molecular makers applied in cowpea (Vigna unguiculata L. Walp.) breeding," *Journal of Life Sciences*, vol. 6, no. 11, p. 1190, 2012.
[39] T. Thiagarajan, H. Recinos, and A. Tillett, "Effect of salinity on callus formation and organogenesis of red kidney beans (Phaseolus vulgaris L.)," *European Scientific Journal*, vol. 9, no. 33, pp. 1857-7881, 2013.

Edelweiss Applied Science and Technology
ISSN: 2576-8484
Vol. 8, No. 3: 1-13, 2024
DOI: 10.55214/25768484.v8i3.1088