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RESEARCH PAPER

Evaluation of salinity-mediated end-point cytogenotoxicity in Germinating Roots of *Lathyrus sativus* L., Variety Mahatora.: Bio-assay guided biomarker studies

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Abstract

Pulse crops are susceptible to salt stress as per different research reports but how far *Lathyrus sativus* L., responds to increasing salinity has been taken up in this work. Thus, the harmful effects of increasing salinity on plant cells at various phases of chromosomal integrity and nucleolus morphology have been evaluated in *Lathyrus sativus* L., variety Mahatora. *Lathyrus sativus* variety Mahatora seeds were subjected to seed priming with serially diluted concentrations of NaCl (500, 400, 300, 200 and 100 mM respectively) and germination percentage (72 hrs), root length inhibition (7 days) normal and abnormal MI (Mitotic Index) with 2% aceto-orcein staining, nucleolar morphometric cum frequency analysis (0.05% hematoxylin staining), total soluble protein *vs* Peoxidase activity (POX), Electrolyte leakage (EL) from etiolated roots and root metabolic activity/dehydrogenase activity were measured (TTC staining). From 200 mM onwards, significant reduction in germination percentage and root length inhibition resulted and at 300 and 400 mM salt-priming significant reduction in normal MI%, increased Abnormal MI% showing both aneugenic and clastogenic responses were accounted. At 500 mM pre-exposed root tip cells were found to develop gradual blackening and root tip death and very less viable cells with highly necrotic, vacuolated with chromosomal erosions and nuclear dismantling and nuclear blobbing resulted apoptosis in addition to decreased POX and dehydrogenase activity (300–500 mM NaCl-treated test sets). NaCl stands out as a potential cyto-genotoxicant in *Lathyrus sativus* L., variety Mahatora. The maximum tolerance level (200–300 mM) and at 400–500 mM NaCl has been highly cytotoxic as per cytological and biochemical data. From 200 mM onwards, nucleolar volume and frequency were altered and at 500 mM pretreatment complete degradation of nuclear machinery was encountered. Owing to high salinity significant proportions of C-mitosis and polyploidy were accounted which conclusively established that NaCl surely had a disruptive role to play during spindle fibre formation process in dividing root cells that in turn produced somatic diads and subsequent polyploidy formations (At 200 to 300 mM).

Keywords

Lathyrus sativus, Salt priming, germination, apoptosis, mitodepression

Introduction

Plants may suffer harm that disturbs genomic stability because some ions produced by the buildup of sodium and chloride in plant tissues as a result of either root uptake ultimately resulting poisonous manifestations for normal physiology. It is essential to comprehend the cytogenetic underpinnings of salt tolerance in order to create tolerant variants. Rice, wheat, pigeon pea, and tomato are only a few of the crops for which the genetics of salinity tolerance have been

studied around the world (Joshi 2007; Li and Xu 2007; Singh et al. 2012; Chaudhary et al. 2013). There are, however, few data on the impact of salinity on the chromosomal levels of various pulse crops. There have been similar reports of genetic regulation for several salinity tolerance traits in chickpeas and soybeans. The salt stress tolerance of soybean was discovered to be determined by salt tolerance rating (STR) and ion buildup (Hamwieh and Xu 2008; Do et al. 2018), but no such data are available from a climate-resilient pulse crop, i.e. *Lathyrus sativus*. Salt affects the stability of proteins (Ahmad et al. 2015) and the lipid composition of plant membrane (Ibrahim et al. 2015) and causes asymmetric division of plant cells in different organs resulting in deregulation of cellular homeostasis in higher plants (Baranova and Gulevich 2021). However, the genotoxic consequences of salt are poorly understood, and Teerarak et al. (2009), found that few studies address the cytological harms to plant cells when brought on by exposure to NaCl. By using plant root tip cells as test models, which are both inexpensive and easy to use, the present authors have tried to examine the genotoxic effects of toxicants i.e., increasing NaCl by calculating the irregularity of the mitotic index. Numerous studies have demonstrated the value of the mitotic Ana-telophase assay as a tool for assessing the genotoxic effects of toxic chemicals. *Lathyrus* is widely used as cheap animal feed and a source of protein for the underprivileged in India. Although it has been suggested that salt damages the double helix of DNA and contributes to chromosomal aberrations, no precise studies on how high salt concentrations (100–500 mM) cause damage to *Lathyrus sativus* roots and chromosomal aberrations have been published in recent years.

In this study, the focus has been laid on the architecture of the nucleolus and the hazardous effects of salinity on plant cells at different stages of mitosis. The authors have also tried to look studied the development of cell micronuclei, chromosomal abnormalities, and nuclear anomalies in the roots of *Lathyrus sativus* seeds that were germinating after being pre-exposed to the salt regime. *Lathyrus sativus* L. seeds are readily available, simple to grow throughout the year in lab conditions, and have a bimodal karyotype that displays good chromosome complements (2n = 14) with equal spreads under microscopic view, describing nearly the same types of clastogenic and aneugenic effects as described in popular models like *Allium cepa* L and *Vicia faba* L cells throughout the study (Teerarak et al. 2009). Furthermore, there have been no findings published so far demonstrating the genotoxic effects of salinity on this plant's enzymatic parameters. *Lathyrus sativus* is a fantastic model organism (Cavuşoğlu et al. 2017) for assessing all potential consequences, particularly at the chromosomal level following various levels of stress production caused by high salt (100–500 mM NaCl).

Therefore, the aim of this research is to evaluate the impact of salt stress over root cells through the study of mitotic index (MI), and distinct chromosomal anomalies, utilizing root tip cells of high yielding Mahatora variety. Thus, by analysing the mitotic index (MI) and particular chromosomal abnormalities in root tip cells from the Mahatora variety of *Lathyrus sativus* L., the goal of this work is to ascertain how salt stress affects germinating root cells. Additionally, as per recent opinions prolonged salt stress produces severe metabolic and cytogentoxic stress in *Allium cepa* L., (Singh and Roy 2016; Kielkowsla 2017). In this experimental process the authors have also tried to opine that the study of nucleolar alterations as well as cytogenetic changes as a potential cytological tool (Bio-marker) to evaluate the cytotoxicity of tested chemicals may yield useful information relating to the salt tolerance mechanism and suboptimal concentrations of tolerance as of this crop is also concerned.

Materials and methods

Study area

The study had been carried out at the Plant Cell and Molecular Research laboratory, Undergraduate and Post-Graduate deptartment of Botany, Hooghly Mohsin College, Chinsurah, Hooghly, starting from November 2021 to August 2022.

Certified Mahatora variety of *Lathyrus sativus* L., seeds to conduct this research program was taken from State Seed Testing Laboratory, Govt. of West Bengal, District Agriculture Farm, Kalna Road, Burdawan 713101, India.

Lathyrus sativus L., seeds (Mahatora variety) were sterilised with a 2% mercuric chloride solution for 10 minutes, then repeatedly washed under running water. Three hours later, the seeds were immersed in distilled water. The seeds were then split into six groups (total 10 seed in each set was employed in the study) and placed in petri plates with various sodium chloride concentrations (500, 400, 300, 200, and 100 mM, respectively). Before the final experimental setup, the doses were tested and modified based on the morphological traits the germination of the seeds produced. Before applying the aforementioned dosing regimen, a separate 500 mM NaCl treatment made the seeds black and prevented them from germinating. Even the few seeds germinated after 72 hours of incubation had turned dark brown- to-black and *neither* any nuclear complement nor the chromosomal makeup could be seen under a microscope, Olympus CH20i microscope, Japan); thereby these roots were rejected. Following multiple trial runs, the salt dose for exposure was decided upon, for the final experiment. The seeds were let to sprout for 24 hours after exposure, and after 72 hours of root growth, the root tips were removed, soaked in 70% ethanol for the night in Carnoy's fixation solution, which contains ethanol and glacial acetic acid, and then hydrolyzed with 1N HCl^{14}.The root cap was removed, and the root meristematic tissues were dyed with 2% aceto-orcein, compressed onto slides, and then viewed with an Olympus CH20i compound microscope (Japan) equipped with an IS 500, 5.0 MP CMOS camera. Each duplicate had at least three stained root meristems. A minimum of 500 cells were subjected to each treatment (control and salt treatments) for the analysis. A compound microscope (Olympus CH20i microscope) equipped with CMOS Camera (IS 500, 5.0

MP), attached to a PC, and VIEW 7 image analysis software were used to take pictures of the preparations.

Germination percentage

Every 24 hours up to 168 hours (7 days), the germination potential of seeds that had been pre-treated with NaCl as well as the radicle (embryonic root) length (measured using a millimetre ruler) were examined. Three times the experiment was conducted in identical circumstances. The proportion of germination of seeds for *Lathyrus sativus* L. was calculated (after a 96-hour period). The rate of germination was calculated as % of seed germination = Total No of seeds germinating (72 hrs)/total seeds taken X 100 (Ghosh et al. 2020).

Cytogenetic analysis: Determination of cytogentoxicity (mitotic inhibition by orcein staining)

The root tips of germination-tested seeds were utilised as a source of mitotic cells to examine the cytogenetic changes brought on by NaCl pre-treatment (in serially diluted amounts) in the *Lathyrus sativus* L. root tips. A minimum of 500 cells from each plate were scored, and the mitotic index was computed. A minimum of 500 cells per slide were examined, and the percentages of chromosomal abnormalities, both normal and abnormal (such as Strap nucleus, disorganised metaphase, metaphase puffing C-metaphase, Star metaphase, and Scattered metaphase Binucleus, telomere puffing, tropokinesis, and bridges during the anaphase and metaphase Dead cells, laggards, and Lesions, polyploidy, and an elongated strap nucleus Numerous abnormalities including Binucleus, Micronucleus, Telomere puffing, Metaphase, clumping, Disoriented metaphase, Tropokinesis, Disturbed Anaphase, Disturbed Metaphase, and Metaphase puffing) were seen and manually recorded. In order to analyze all phases at a magnification of 40× and under an oil immersion objective (100×), a compound microscope (Olympus CH20i microscope, Japan) outfitted with a CMOS Camera (IS 500, 5.0 MP) and its attachment to a computer with the aid of VIEW 7 image processing software was employed.

Images were acquired and cytotoxic and genotoxic end-point parameters were calculated using the following formulas:

Mitotic index % = (Number of dividing cells) ÷ (Total no of cells) × 100 (Ghosh et al. 2020).

The Genotoxicity Index (GnI%) was calculated (after de Souza et al. 2022) (Adhikari et al. 2023). The genotoxicity index (GenI) was calculated using the formula = (No of cells showing abnormal chromosomal response + nuclear buds+ nuclear breakage) ÷ (Total no of cells counted) × 100.

Percentage of % of Mitotic Inhibition = (Mitotic index in Control-Abnormal Mitotic index after treatment) ÷ (Mitotic index in Control) X 100 (Adhikari et al. 2021).

Detection of Morphological Characters for cell death: Computation of the Percentage of Dying Cells: We chose nucleus migration from centre to the margin of cell wall, condensation, vacuolation of cytoplasm, and nuclear fragmentations as characteristic hallmarks of dying cells. Nucleus margination is the displacement of the nucleus in a cell wall margin. Percentage of Dying Cells = (No of cells dying or dead cells) ÷ (Total no of cells counted) × 100.

Detection of morphometric changes in nucleolus in germinating root tips of *Lathyrus sativus* L. variety Mahatora using Hematoxylin staining

Seeds of *Lathyrus sativus* L., were allowed to germinate after 24 hrs after priming with different concentrations of test sample (500, 400, 300, 200 and 100 mM respectively) and after 72 hrs of germination the root tips were cut and fixed in FAA (4% formalin: Glacial Acetic Acid: Ethanol=1:2:7) and kept overnight at 4 °C. The very next day the root tips were hydrolyzed in 45% Acectic acid for 45 mints at water bath not allowing the temperature to rise above 85 °C. After acid hydrolysis the root tips were cooled, washed in distilled water and incubated in saturated solutions of iron alum (ferric ammounium sulphate) for 10 minutes followed by staining in 0.5% aqueous hematoxylin solution for 45 minutes. The root tips were then washed and one drop of 0.2% orcein was applied and squashed in 45% acetic acid and observed under a compound microscope (Olympus CH20i microscope, Japan) outfitted with a CMOS Camera (IS 500, 5.0 MP) and its attachment to a computer with the aid of VIEW 7 image processing software.

Studies on nucleolar morphometric changes: treatment groups having cells with different numbers of nucleoli were manually scored and in different groups apart from control groups showing different numbers of nucleoli with or without nuclear membranes tabulated. Nuclolar volume was measured using the formula $4/3\pi r^3$ using stage micrometer, Erma, Japan to measure the nuclear and nucleolar diameters. Among different shaped nuclear morphological alignments four distinct morphometric parameters were chosen as "cytological markers" of endpoint cytotoxicity i.e., (i) big vacuolated nucleus with translucent centres, (ii) Elongated nuclei, (iii) dumbbell shaped nuclei, (iv) nuclei in chain, and (v) micronucleoli (one fourth of diameter than control nuclei) in scattered conditions throughout the cytoplasm.

Determination of total soluble soluble protein

The germinating roots (of all treatment groups and control after 96 hr of germination) were cut with sharp razor and were crushed with 10 mL of cold 0.05 M potassium phosphate buffer (pH 7.8) in a porcelain mortar that has already been chilled for 10 minutes. The homogenate was centrifuged at an ultracold 13,000 rpm for 30 minutes at 4 °C after being filtered through Whatman's No. 1 filter

paper and transferred to an eppendorf tube. The supernatant from the centrifugation was then subjected to spectrophotometric analysis (O.D. changes in comparison to blank and control) for biochemical analysis to determine the amount of total soluble protein (Bradford 1976) analysis. All readings are made in triplicate under experiment set ups, in the laboratory *in vitro*.

Determination of total soluble protein content

The total soluble protein content of the root homogenates of the control and treatment sets were performed after (Bradford 1976) where bovine serum albumin (BSA) taken as a standard. Triplicate trials of each set and control were done.

Determination of peroxidase (POX) activity

POX activity was measured (Özceylan and Aki 2020) using spectrophotometric analysis to measure variations in POX activity in different treatment sets. For two minutes, the spectrophotometer measured at 300 nm to determine the POX kinetic reaction in SHIMADZU UV-1800 UV-VIS Spectrophotometer. For each group, the largest changes in the absorbance values taken every 10 seconds over a 2-minute period are identified. These variations have been calculated, converted to mg/mL/min POX enzyme activity, and given as mg/mg protein level. Three repetitions of each POX activity measurement were carried out.

Determination of membrane permeability/ Electrolyte leakage after NaCl treatment on etiolated roots

Ions that were leaking into deionized water from tissue were used to measure membrane permeability or electrolyte leakage (EL). Test tubes containing 10 mL of deionized water and segments of fresh root samples (processed and controlled sets of 100 mg root tissues in each tube) were used. The tubes were immersed in water that was 32 °C-heated for 6 hours. Following incubation, the bathing solution's electrical conductivity (EC1) was measured using an electrical conductivity metre (Systronics M-308, Kolkata, India). After that, the samples were autoclaved for 30 minutes at 121 °C to totally destroy the tissues and liberate all electrolytes. The final electrical conductivity (EC2) of the samples was then calculated after they had been cooled to 25 °C. The formula EL%=EC1/EC2X100 was used to convert the EL into a percentage (Adhikari 2021).

Evaluation of root metabolic/mitochondrial activity

The best method for determining a cell's viability is TTC (2,3,5-Triphenyl tetrazolium chloride) staining. *Lathyrus sativus* L. seeds were subjected to 24 hours of treatment with various NaCl solution concentrations. The same procedure was followed while using pure water as the positive control and 0.1% hydrogen peroxide as the negative control. In 0.5% (w/v) TTC stain for five hours in the dark, all the roots were submerged. After that, distilled water was used to cleanse the roots. Using a spectrophotometer and 95% ethanol as a blank, absorbance was measured at 490 nm. The test O.D.s had been translated into percentages representing the following rise or fall in metabolic activity, and the positive control (hydrogen peroxide O.D.) was taken to represent 100% metabolic/respiratory activity (dehydrogenase) activity, out of root mitochondrial activity (Ghosh et al. 2020).

Statistical analysis

All the values are presented as Mean±SD (standard deviation, n = 6). Statistical analyses were performed with paired t-test and ANOVA is used first, then, if necessary, a post-hoc Dunnett's multiple comparison test. P values below 0.05 were regarded as significant. Using the GRAPH PAD PRIZM-version 6 computer program, analysis of variance (ANOVA) was used to examine differences between the groups in the statistical study.

Results

Effect of increasing molarity of NaCl priming on germination and growth of *Lathyrus sativus* seeds after 96 hrs

Evident from the figure plates it is evident that after 96 hrs of germination applying gradually increasing concentrations of NaCl (seed priming for 24 hrs) there were qualitative and quantitative inhibition of germination vis-a-vis embryonic root length inhibition with increasing salinity. In 500 mM NaCl primed seeds almost all the seed coats became blackened showing significant inhibition of radical formation; however, only a single seed with visible symptoms of germination was seen and a stunted root with gradual wilting-like morphological signs was prominent. In 400 mM NaCl primed seeds almost all the seed coats became blackened showing significant inhibition of radical formation; however, 4 seeds with visible symptoms of germination were accounted but the root with gradual brownish to blackening tips with morphologically stunted growth was prominent. In 300 mM NaCl primed seeds half of all the seed coats became although a gradual increase in germination profile with an increase in root length could be observed. However, the roots here were with visible symptoms of less brownish tips were visible. At 200 and 100 mM primed seeds there was marked difference could be accounted, whereasin the 200 mM treated group almost 85% seed germination with significant root length growth was seen. Interestingly 100 mM NaCl priming produced vigorous germination with a robust increase in root length with shoot formation was accounted which was almost similar to control sets (Fig. 1A–F).

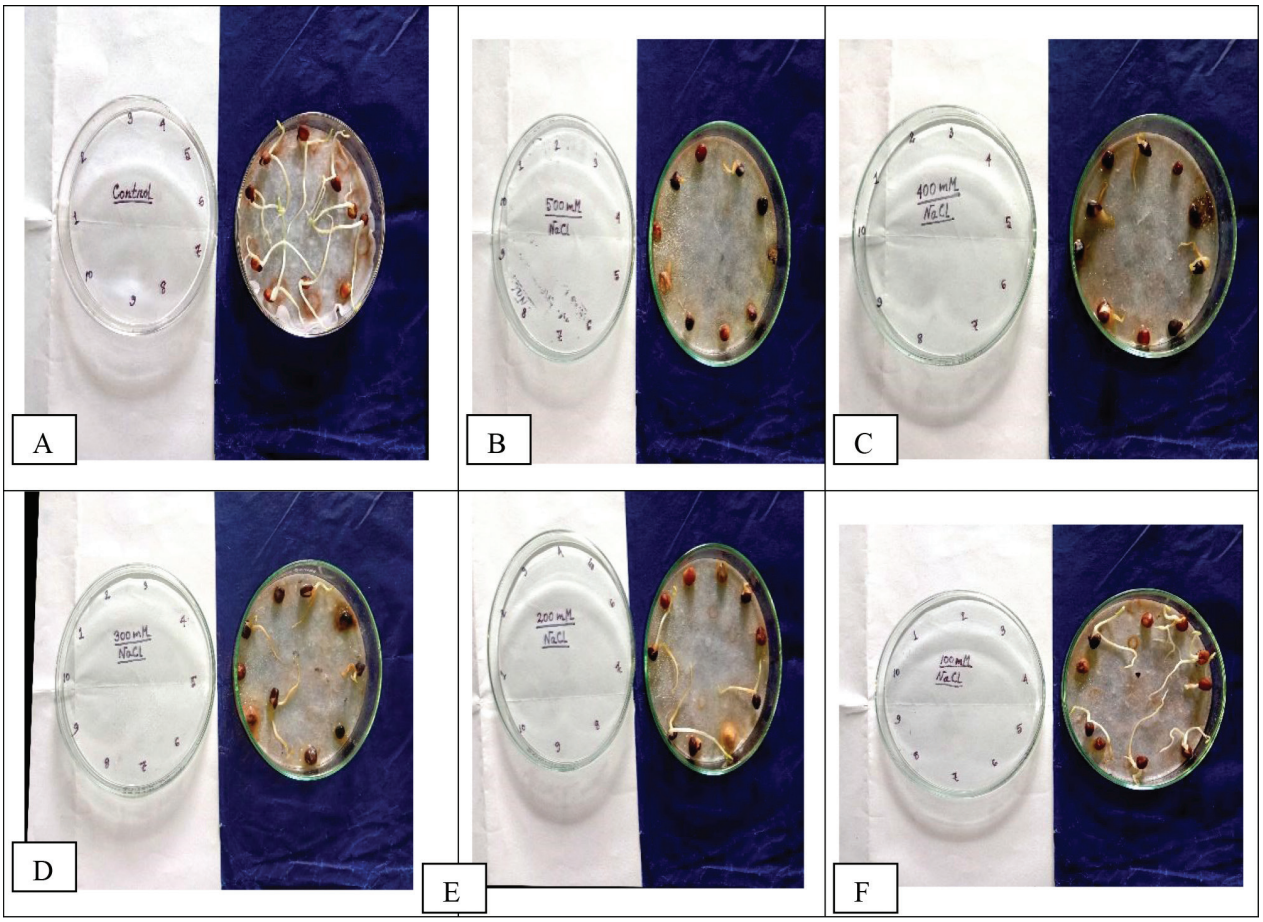


Figure 1. A–F. Plates showing the direct effect of increasing molarity of NaCl priming on germination and root shoot length growth in *Lathyrus sativus* L., (variety Mahatora) after 96 hrs. **A.** Control seeds after 96 hrs of germination; **B.** 500 mM salt primed seeds after 96 hrs of germination; **C.** 400 mM salt primed seeds after 96 hrs of germination; **D.** 300 mM salt primed seeds 96 hrs of germination; **E.** 200 mM salt primed seeds after 96 hrs of germination; **F.** 100 mM salt primed seeds after 96 hrs of germination.

Effect of increasing molarity of NaCl priming on germination vs root length inhibition of *Lathyrus sativus* seeds after 168 hrs (7 days) of germination

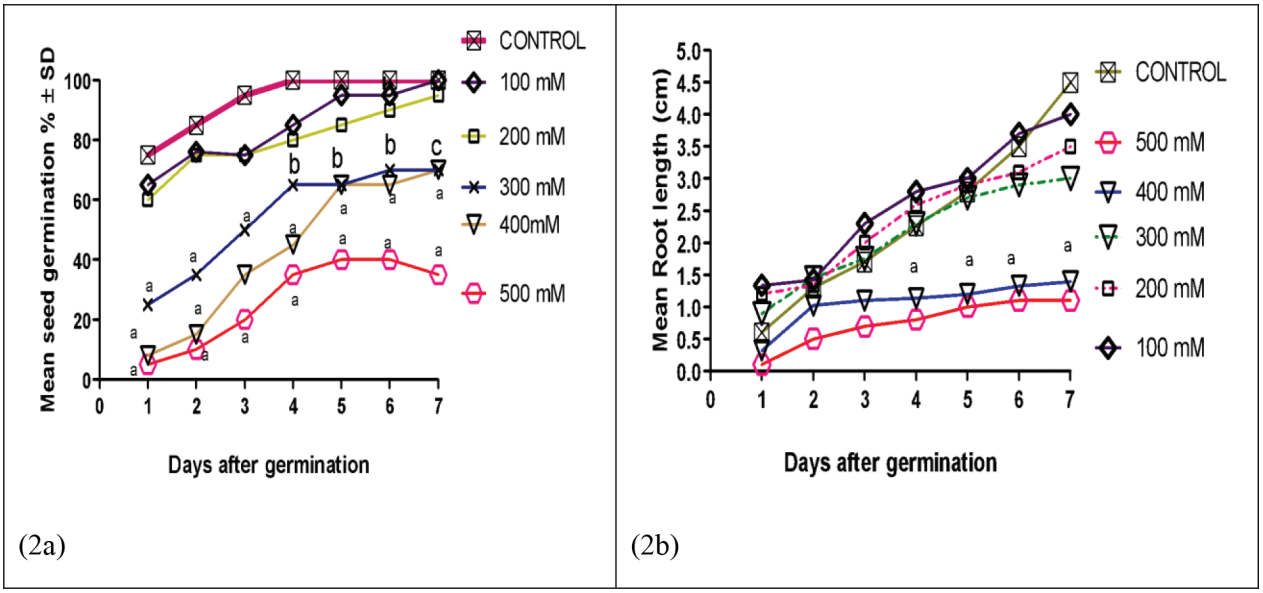


Figure 2. Line graphs (2a, b) showing the effect of increasing NaCl concentrations on seed germination percentage (2a) and root length growth inhibition (2b) in *Lathyrus sativus* L., after 7 days in comparison to control groups.

Effect of different salt concentrations on seed germination *Lathyrus sativus* within a span of 7 days

In this assay in comparison to the control it was observed that 500 and 400 mM salt perexposure significantly reduced (40% and 65% respectively at 7 days' intervals). In comparison to control setups, 300 and 200 mM prexposed seeds could augment the salt stress and come up with 70 and 80% germination percentages at 7 days of observation. In 100 mM salt-primed seeds reached up to 100% germination efficiency at 5th day as compared to control seeds which attained 100% germination after 48% hours only. From the results, it might be deciphered that 500 and 400 mM salt exposure is growth inhibitory imparting negative effects on seed germination. Interestingly 200 and 300 mM salt concentrations are tolerable concentrations for the germinating seeds although seed germination percentage got delayed till 5th day which might be the alterations in the cellular metabolic states and subsequent metabolic adjustments of the germinating root tip cells owing to abiotic stress formed within.

Effect of different salt concentrations on mean root length *Lathyrus sativus* within a span of 7 days

This particular observation, in comparison to control it was observed that 500 and 400 mM salt priming significantly reduced the mean root length (less than 2 cm in length) of germinating seeds of *Lathyrus* even after 7 days' intervals of observation. In comparison to control setups of 300 and 200, mM salt-primed seeds could augment the salt stress and come up with less than 4 cm roots after 7 days of observation. In 100 mM pre-exposed seeds reached up to 100% germination efficiency on 5th day as compared to control seeds attaining over 5 cm of length nicely. I that 500 and 400 mM salt exposures are truly growth inhibitory imparting negative effects on root growth. Interestingly 200 and 300 mM salt concentrations are tolerable concentrations for the germinating seeds although the root length growth was hampered to attain up to 4 cm in length till the 7th day which might be the alterations in the cellular hormonal levels and subsequent metabolic adjustments of the germinating root tip cells owing to abiotic stress formed within.

Effect of increasing NaCl concentrations on Mitotic index of *Lathyrus sativus*

Bar diagrams representing the toxic effect of gradually increasing salt concentrations on normal Mitotic index and induction of abnormal Mitotic index on *Lathyrus sativus* L., root tip cells after 72 hrs of germination. Different alphabets within a column represent significant differences at $p < 0.05$ after paired "t" test in comparison to respective normal MIs.

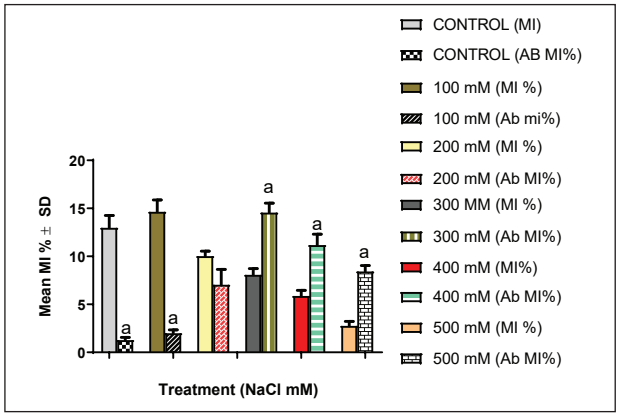


Figure 3. Histograms representing the toxic effect of gradually increasing salt concentrations on normal Mitotic index (MI) and induction of abnormal Mitotic index (Ab MI) in *Lathyrus sativus* L., root tip cells after 72 hrs of germination. Different alphabets within in a column represent significant difference at $P < 0.001$ after paired "t" test in comparison to respective normal MIs.

The comparative normal and abnormal MI%s of different treatments revealed an inverse dose-response relationship. With increasing salt priming there was a gradual decline in normal MI%s and a gradual rise in the abnormal MI%s. But at 400 and 500 mM treated root tip cells both normal and abnormal MI%s were declining. At 500 mM pretreated germinating root tips very few diving cells and abnormal cells could be accounted which were less than 10% in existence and most of the giant strap cells were with multi-fragmentations with dismantled nuclear architectures having hyaline cytoplasm showing nuclear blobs shifted to corners representative of the apoptotic cellular population.

Effect of increasing concentrations of NaCl concentrations on chromosomal division of *Lathyrus sativus*

Increasing salinity has a pronounced effect on chromosomal morphology (induction of chromosomal aberrations) and normal cell division (MI) of *Lathyrus sativus* L., (Fig. 4A–O). At 100 mM salt exposure in comparison to control there is significantly reduced abnormal MI%. Normal MI % was predominant showing very less chromosomal aberrations and abnormal MI% showed only few metaphasic and anaphasic clumping and stickiness. From 200 mM onwards there is a proportional change in normal MI% and there is a relatively high proportion of chromosomal aberrations (statistically insignificant, after paired t-test) showing early separation and centric fission at clumped metaphase. At 300 and 400 mM salt exposed root tips there was a significant reduction in the percentages of normally dividing cells showing up reduced normal MI% and significantly increased abnormally dividing cells i.e., high frequency of Abnormal MI% showing C-mitosis, somatic diads, polyploidy (exceptionally high in frequency), binucleate cells, Karyorrhexis and anaphasic bridges. Giant strap cells with diminishing nucleus was also observed. At 500 mM salt-primed root tip cells there was

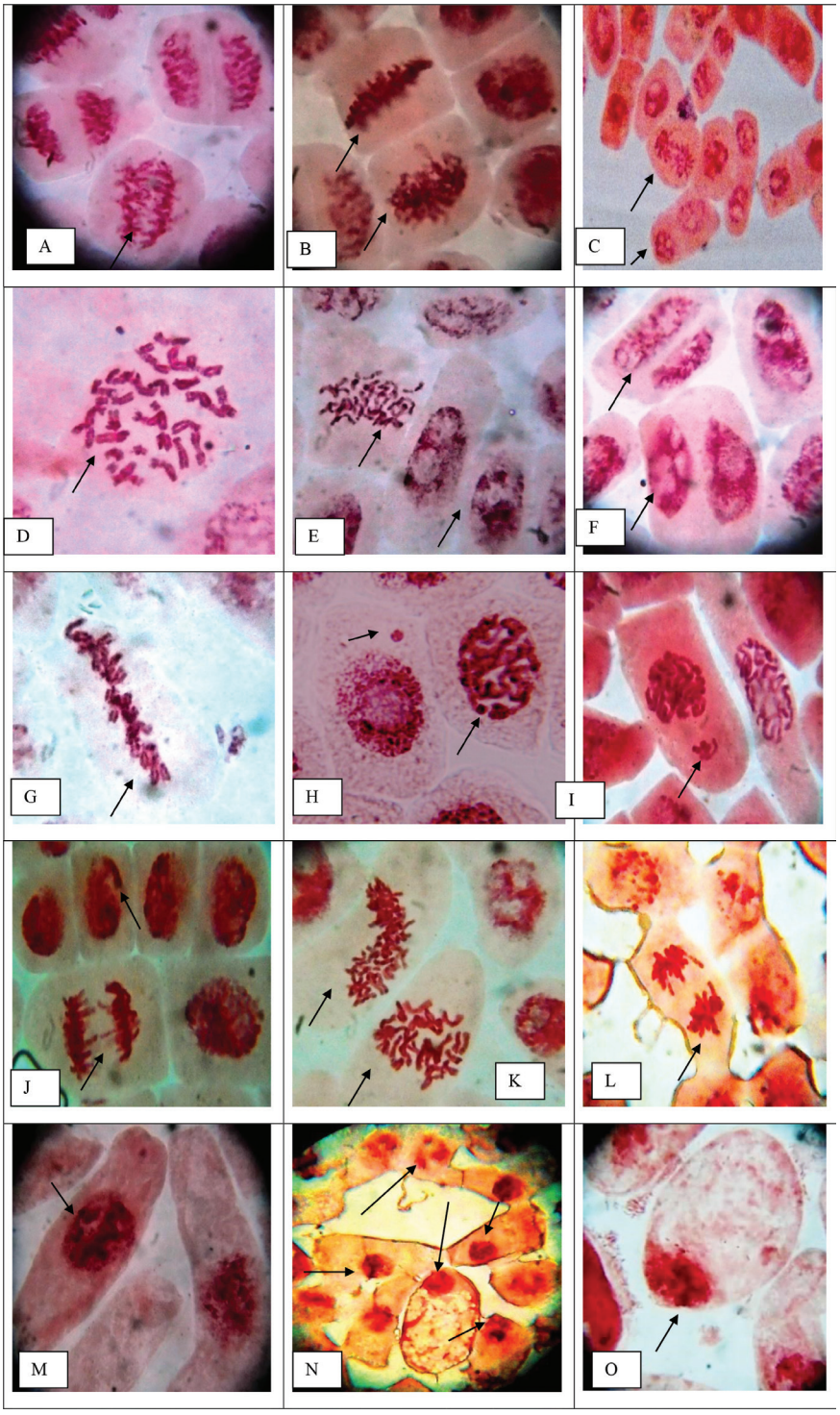


Figure 4. A–O. Meristematic root tip cells of *Lathyrus sativus* L., representing chromosomal alterations after 72 hrs of germination after increasing concentrations of salt priming. Photomicroplates (**A–O**) showing induction of chromosomal abnormalities after varying concentrations of sodium chlorideon germinating root tips of lathyrus sativus step by step induction of cellular death from early stages of cellular toxicity (from 100, 200,300,400 and 500 mM doses respectively). **A.** Anaphasic clumping, metapahic bridges at 100 mM NaCl; **B.** Metaphasic stickiness and ball metaphase at 100 mM NaCl; **C.** Asteroid like anaphase separation and multivacuolated nuclei at 200 mM NaCl; **D.** C-mitosis with isochromosome formation at 200 mMNaCl; **E.** Polyploidy at 200 mMNaCl; **F.** Somatic diads with Multilobed double nucleus at 300 mMNaCl; **G.** Isochromosomes showing pole to pole sticky methaphase at 300 mMNaCl; **H.** Micronuclei formation at 300 mM NaCl; **I.** Early decondensation at prophase with precocious chromatin fragmentations at 400 mMNaCl; **J.** Karyorrhexis and tropokinesis at 400 mM; **K.** Hyperploidy polypoidy at 400 mM NaCl; **L.** Coagulated anaphase, laggard and late separation at 400 mM; **M.** Multifragmented nuclear lobes and nuclear erosions at 500 mM Nacl; **N.** Vacuolated cytoplasm, and dislodged nucleus with karyorrhexis in giant strap cells (500 mM); **O.** Translucent cytoplasm with karyolysis leading to apoptosis (500 mM).

gradual blackening and root tip showing death symptoms and the surviving roots ended up in very less viable cells where almost all the cells were necrotic, highly vacuolated with chromosomal erosions and nuclear dismantling with nuclear blebbing showing apoptotic symptoms. In this highly toxic dose, the normal and abnormal MI % are greatly reduced as more than 50% of cells were enucleated or with apoptotic signatures. This result correlated with the seed germination and root length inhibition assays where the normal growth was greatly halted and almost all the root tips were blackened and dried up owing to the toxicity of high slat stress in the meristematic tissue.

Effect of increasing NaCl concentrations on Nucleolar dynamics of *Lathyrus sativus*

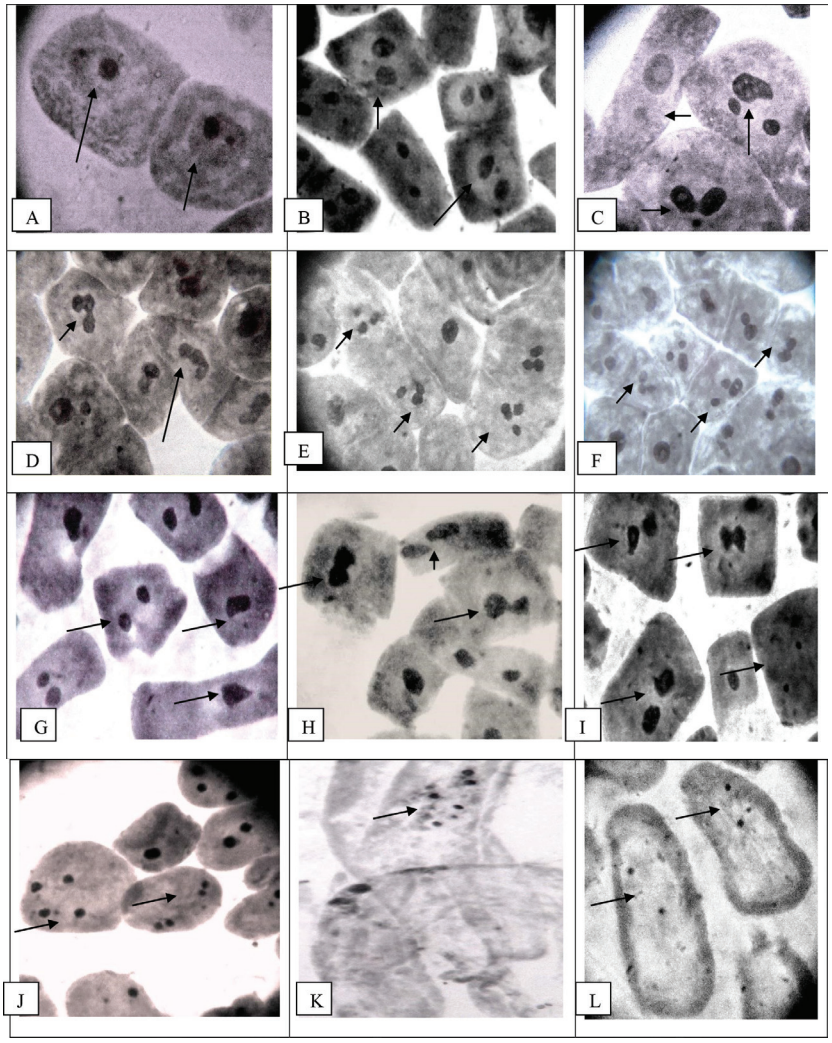


Figure 5. Meristematic root tip cells of *Lathyrus sativus* L., representing nucleolar alterations (frequency and volume) after 72 hrs of germination after increasing concentrations of salt priming. Photomicrophotograhs (**A–L**) showing different shapes and states of nuclear morphometrics after different concentrations of NaCl priming in germinating root tips of *Lathyrus sativus* L., **A.** Control root tip cells showing intact nuclear membranes with double nuclei. **B.** Root cells after 100 mM NaCl pretreatment showing all double nuclei with disappearing nuclear membrane. **C.** Root cells after 200 mM NaCl pretreatment showing no nuclear membranes with big round to oblate and pear-shaped nucleoli showing translucent multiple lesions. **D.** Root cells after 200 mM NaCl pretreatment showing round to oblate micronucleoli showing diplo to streptococci like appearance; **E.** Root cells after 200 mM NaCl pretreatment showing micronucleoli , 4-6 in numbers adhered together. **F.** Root cells after 300 mM NaCl pretreatment showing reduced cellular volumes oblate-shaped micronucleoli with gradual comingling; **G.** Root cells after 400 mM NaCl pretreatment showing altered cellular morphologies with pear to oblong shapes nucleoli; **H.** Root cells after 400 mM NaCl pretreatment showing pear-shaped, eye-shaped and dumbbell-shaped nucleoli, with diminishing nucleolar volume; **I.** Root cells after 400 mM NaCl pretreatment showing dumbbell-shaped nucleoli with nulceolar notches and fragmentations; **J.** Root cells after 400 mM NaCl pretreatment showing multiple micronucleoli formations with translucent cytotoplams; **K.** Root cells after 500 mM NaCl long strap cells with almost no-cytoplasm and multifragmented micronucleoli scattered around; **L.** Root cells after 500 mM NaCl pretreatment showing no only 2-3 micronuceli scatted around corners with ruptured cell walls showing aopototic appearances.

Changing percentages of root tip cells having different number of nucleoli (1–8) after pre-treatment with increasing NaCl concentrations in *Lathyrus sativus*

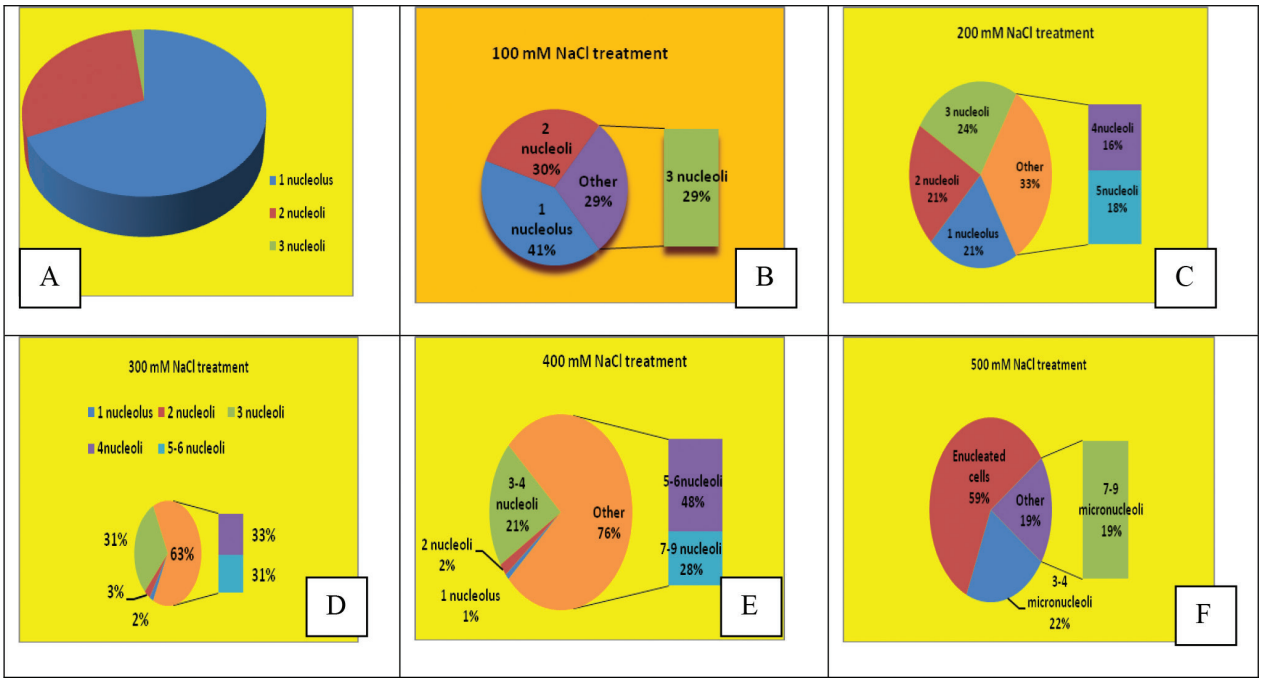


Figure 6. Piecharts representing Meristematic root tip cells of *Lathyrus sativus* L., nucleolar alterations (frequency) after 72 hrs of germination after increasing concentrations of salt priming. Photomicrophotograhs (**A–F**): These relative pie charts are showing different stages, morphological types and numbers of nucleoli in different treatments representing the morphometric and volumetric changes in the nucleus and nucleolus contents in root tip cells of *Lathyrus sativus* L. **A.** Control cells showing the relative percentages of nulcoloar (mono, bi and tri nucleolate) frequency. **B.** In 100 mM NaCl pretreated root tips percentages of tri-nucleolate populations reached nearly 29% followed by **C.** 200 mM NaCl sets where tetranucleate conditions could be accounted in almost 18% of the cells and pentanucleate condition could be seen in 19% of cellular populations. **D.** In 300 mM NaCl treated root tips tetra, penta and hexanucleolate population accounted altogether of 63% of the overall cellular population. **E.** In 400 mM NaCl treated root tip cells 5–6 nucleolated cells were 43% and 7–9 nucleolated cells were upto 20% of the whole population. **F.** At 500 mM NaCl pretreated germinating root cells maximum up 60% of the population were giant strap cells with no nuclear masses remaining with 19% of the cellular population contained 7–9 micronucleolate cells with diminishing nucleolar volumes compared to control and lower treatment groups.

Determination of total soluble protein and POX activity on etiolated roots of *Lathyrus sativus*

From the comparative bar diagram (total soluble protein vs. POX activity) it was found that there had been a differential expression in soluble protein concentrations in comparison to control in all treatment groups (100, 200 and 300 mM NaCl treatments) and in treatment groups having 400 and 500 mM NaCl pretreatments there a reversal of total soluble protein content could be accounted and at 500 mM treated sets the amount of total soluble protein was even less than control. In comparison to total soluble protein vs POX activity, there had been a sharp increase in POX activity in almost all the treatment groups (7.5, 10.3, 11.3, 10.9 mg/mL/min respectively) in comparison to control (6.6 mg/ml/min). But in the 500 mM treated sets there had been a sharp fall in POX activity (3.4 mg/mL/min) which was almost half of the activity of the control group (6.6 mg/mL/min) (Fig. 7). This lessened trend in both total soluble protein content and POX activity could be due to the cellular poisoning of the highest dose augmenting possible halting of both protein synthesis and enzyme activity.

Determination of Membrane Permeability/ Electrolyte Leakage after NaCl treatment on etiolated roots of *Lathyrus sativus*

Root electrolyte leakage from *Lathyrus sativus* L. seeds that had not been treated was very low, at less than 10% (Fig. 8). When the seeds germinated after 72 hours of incubation at various NaCl concentrations, the etiolated roots could exhibit a variable response in terms of electrolyte leakage/membrane permeability, and 50% of the electrolyte leakage could fall in the range of 200 to 300 mM treatments. In comparison to the control, there was considerable disruption of membrane leakage that was concentration-dependent in the etiolated roots of *Lathyrus sativus* L.

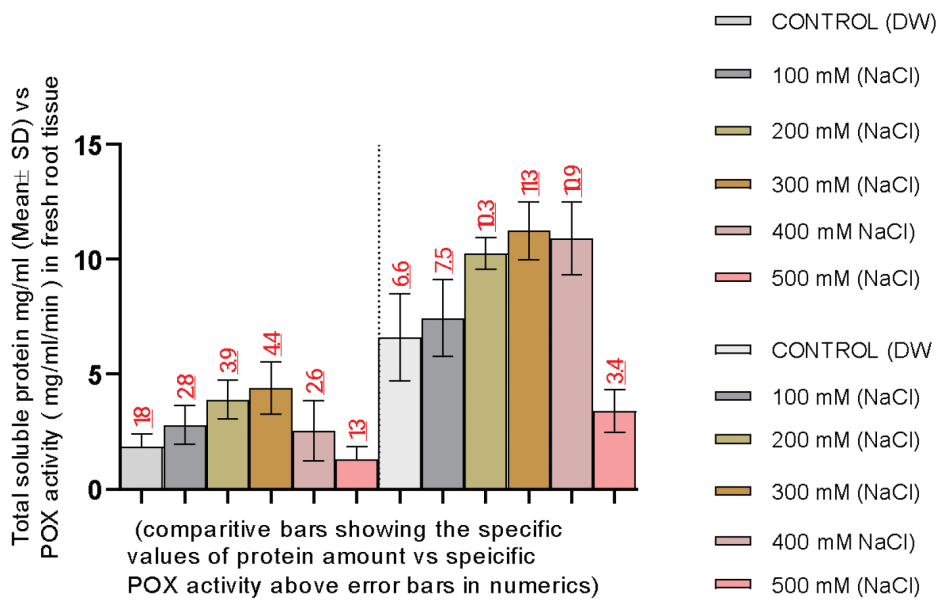
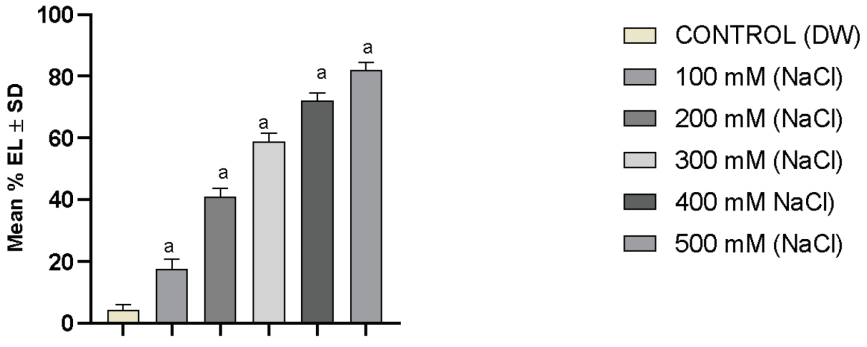


Figure 7. Histogram showing comparative concentrations of total soluble protein vs POX activity in all treatment groups (after 72 hrs of germination) in addition to control groups in *Lathyrus sativus* L., germinating root tip cells.



ANOVA summary	
F	457.3
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R square	0.9948

Figure 8. Histogram showing differences in electrolyte leakage in etiolated roots of *Lathyrus sativus* L., after 72 hrs of germination. Bars with letters within each panel are significantly different at P < 0.0001 according to one-way ANOVA (control vs. treatment) followed by Dunnet's multiple comparison test within treatment groups (100–500 mM NaCl).

Evaluation of alterations in root metabolic/mitochondrial activity i.e., percentage of dehydrogenase activity after NaCl treatment on etiolated roots of *Lathyrus sativus*

There were variable responses in the metabolic profile of the root mitochondrial system (dehydrogenase activity) within positive control (2% H_2O_2), negative control (distilled water) and salt-treated groups. In positive control groups high colour formation (out of formazan complex formation) could be accounted followed by negative control sets (distilled water). In treatment groups there were gradual increase in dehydrogenase activity in 100 to 200 mM NaCl treatment groups in comparison to negative control and inhibition of root metabolic activity at 300 to 500 mM treatment sets, which signified that in the higher (400 and 500 mM treatment) groups there was inhibition of dehydrogenase activity resulting root mitochondrial dysfunction in comparison to Negative control (water) (Fig. 9).

Discussion

In this investigation pronounced increasing NaCl seed priming produced a disruption of normal seed germination and root length inhibition (physiological biomarkers,

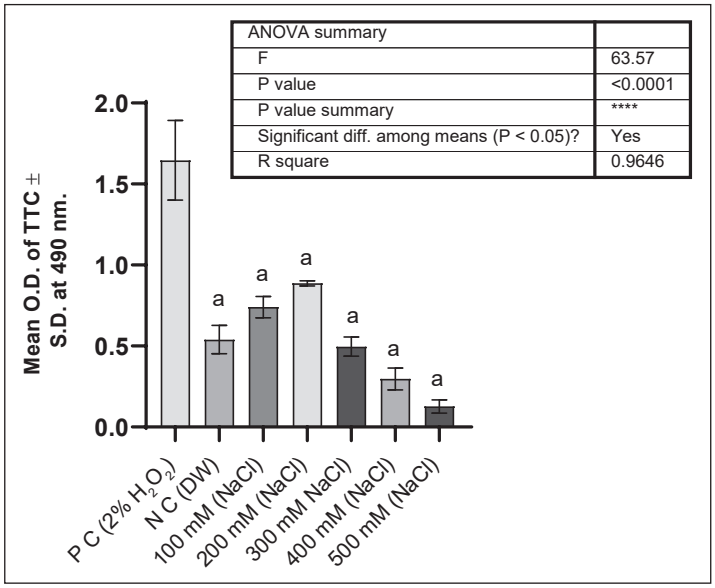


Figure 9. Histogram showing effect of incresing concentrations of NaCl pertreatment in germinating root tips (72 hrs) of *Lathyrus sativus* L., on root mitochondrial (dehydrogenase) activity. The Bars with letter within each panel are significantly different at P < 0.0001 according to one way ANOVA (Positive control vs respective treatment groups) followed by Dunnet's multiple comparison tests within treatment groups.

Figs 1, 2a, b), phase index changes coupled with a decrease in normal MI% and an increased in abnormal MI% (Fig. 3) coupled with both clastogenic and anegenic changes serving as a cytogenetic biomarker (Fig. 4A–O). Salt priming at 400–500 mM produced total loss in chromosomal complements coupled with cellular apoptosis leading to root cell deaths. NaCl seed priming produced disruption of normal nucleolar frequency and nucleolar volumes (Figs 5A–O, 6A–F) giving a clear indication that higher salt concentrations (from 300–500 mM) could augment severe cytotoxicity. This increasing salinity also creates a menace in germinating root tips metabolic pool in terms of a decrease in total soluble protein concentrations and relative POX activity (Fig. 7) with higher levels of electrolyte leakage from roots (Fig. 8) resulting a possible mitochondrial poisoning thereby; disrupting the mitochondrial respiration cycle, which in turn decreased root metabolic activity (Fig. 9) and possible factor for root cell apoptosis. These results are not only interesting but conclusively can pinpoint that increasing salinity is not only genotoxic but beyond suboptimal concentrations can disrupt several biochemical cycles in germinating cells possibly through membrane damage and mitochondrial deaths.

Many activities, including seed germination, vegetative growth, and fruit setting, are inhibited by soil salinity because it lowers the water potential in plants and interferes with cellular ion homeostasis. Seed germination and vegetative growth are just a few of the activities that are inhibited by increasing salinity, which also would lower the water potential thus disrupting cellular ion homeostasis (Katsuhara and Kawasaki 1996). The cytogenetic response of cells exposed to 50 up to even 600 mM of NaCl was investigated in a brief time period from 0 up to 72 h in cereals (Ogawa et al. 2006; Li et al. 2007; Yumurtaci et al. 2009). The findings demonstrated that during a salt shock, cells' mitotic activity either rapidly declines or is completely halted, leading to cell death (Yumurtaci 2009; Tabu and Demir 2010; Deinlein et al. 2014). Increasing salt concentrations had a pronounced effect on seed germination, radical emergence, and root length growth in *Lathyrus sativus* L., variety Mahatora (Figs 1, 2a, b). In general, the reduction in root emergence and length with increasing salt concentrations confirmed the previous findings in cereals (West et al. 2004; Stavridou et al. 2017). Reduced root elongation is an outcome of altered levels of Abscisic acid, auxin, cytokinin, brassinosteroid, gibberellin, and ethylene, which work altogether to reduce cell cycle activity as a result of the suppression of plant hormone signaling pathways. These substances are thought to be essential for root growth because they promote cell division, cell expansion and elongation, and cell differentiation (Demirkiran et al. 2013; Ryu and Cho 2015; Majda and Robert 2018; Oh et al. 2020). Reactive oxygen species production and calcium signaling pathway inhibition cause oxidative damage to nucleic acid bases that encourage single or double strand breaks in DNA, alter cytosine methylation, and trigger programmed cell death, which are all harmful effects of salt (Duan and Wang 1995; Tuteja and Mahajan 2007; Hossain and Dietz 2016).

Cytological examinations showed a more dramatic reduction in mitotic activity in the roots of *Lathyrus sativus* L. that had received NaCl treatment. The decrease in mitotic activity under salt stress may be explained by stopping mitosis in the interphase or lengthening the G2 phase (El – Ghamery et al. 2003; Yildiz et al. 2009; Chatterjee and Manjumdar 2010). The investigations that are now available show that the disruption of cell divisions starts very early, up to 24 hours after exposure to chloride salts (Grant1978; Katsuhara 1997; Atsushi et al. 2006) and that

it advances concurrently with the duration of exposure to mild and moderate stress, as revealed in the current work. Application of salt resulted in a number of chromosomal aberrations at all stages of mitosis. Depending on the dose, an increase in chromosomal aberrations was seen, particularly after treatment with NaCl. The CAs, such as chromosomal bridges, fractures, and fragments reported here in predominance, may have been brought on by DNA breaks or suppression of DNA synthesis because both chromosome fragments and complete chromosomes cannot be absorbed into the main nucleus during the cell cycle (West et al. 2004; Leme and Marin-Morales 2009).

CA's such as chromosome adherence (Fig. 4A–C), chromosome breaks and loss (Fig. 4E, I, L) and bridges (Fig. 4A, C, J), were the major group of disturbances observed after salt treatment in *Lathyrus* in this present investigation. The highest frequency of CA's was observed in roots exposed to 300 to 400 mM of NaCl. NAs included cells with double nucleus, chromatin fragmentation, nucleus disintegration (nuclear budding, multilobulated nuclei; Fig. 4C, F, I, J), karyorrhexsis (Fig. 4M), karyolysis (Fig. 4O) and changes in number of nucleoli, Fig. 5B–L). Usually in interphase nuclei with one or two nucleoli were predominant (Fig. 6A); however in roots treated with 200–400 mM NaCl as many as 3–8 micronucleoli in squashed root tip cells were also observed (Fig. 5B–L). Changes in nuclear morphology leaded to MN formation. MNs were observed in interphase out of chromatin breakage (Fig. 4H). At 200–300 mM concentration disturbances in mitotic divisions resulting in binucleated cells were also observed (Fig. 4C, F). Increase in the salts concentration up to 400–500 mM significantly blocked cell divisions as evident from root tip growth inhibition (Fig. 1A–F), what explains the low number of observed abnormalities (Fig. 2a). In control roots and roots from pre-exposed to 100 mM of salts MIs were almost normal, however increased in parallel to the salt concentrations after 24 hrs pretreatments which could be prominent after 72 hrs of germination under microscopic squashes.

The impact of salinity on cell demonstrated that a decrease in cell number and a shorter mature cell length were responsible for the growth inhibition of *Arabidopsis* primary roots under salt stress (Ding et al. 1960; West et al. 2004). It was emphasized that salt's ability to suppress the activities of cyclin dependent kinases (CDKs). By complexing with the cyclins, CDKs are regulatory proteins that regulate transcription and control cellular division in response to stressful circumstances (Bamum and O'Connell 2014). In reality, checkpoints control how the cell cycle, which is divided into the G1, S, G2, and M (mitosis) stages, advances. Cell cycle checkpoints regulate the mitotic spindle to control cell size, ensure accurate replication, and maintain the integrity of the chromosomes, preventing cells with damaged or insufficiently replicated DNA from entering mitosis and promoting appropriate segregation at mitosis (Tan 2010). These array of clastogenic and aneugenic abnormalities (c-mitosis, polyploids and somatic diads) observed under salinity conditions are partially explained by disruption in checkpoints, CDKs, and cyclin activity deficiencies with decreased root growth (Oztur et al. 2002; Utani et al. 2010; Qi and Zhang 2019).

Big and little MNs were the two types (as per nuclear volume) that were present here. Acentric chromosomal fragmentation (3a: E, G, and L) may be the root cause of salt-treated small micornuclei, whereas chromosome loss (3a: I) may be the root cause of large micronuclei (Leme et al. 2008; Herrero et al. 2011). A distinguishing trait of NA is interphasic nucleus morphological alterations (West et al. 2004). Cells with nuclear buds or lobulated nuclei were present in these cells as a result of the alterations. The process of cell death may be triggered by nuclear anomalies, which are an indication of DNA fragmentation, according to various investigations (Katsuhara 1997; Zhu 2002). Cell death induced on by salt stress was investigated in barley roots (Demirkiran et al. 2013). Nuclear DNA cleavage was observed one hour after the addition of 500 mM NaCl, and DNA fragmentation was identified eight hours later. According to this findings, high salt stress swiftly encourages DNA deterioration, which causes cell death. Later studies showed that excessive formation of reactive oxygen species (ROS), which have a detrimental effect on DNA and cellular structures, and an imbalance in ion homeostasis are both responsible for cell death in response to salt stress (Affenzeller et al. 2009; Boulon et al. 2010). In this study, germinating roots primed with NaCl concentrations of 400 and 500 mM showed higher frequencies of cell death (Figs 4M, N, O, 5K–L). These results suggest that DNA is negatively affected by increased sodium ion concentrations have a negative impact on DNA stability and ion homeostasis, which negatively affects *Lathyrus sativus* root meristem cells.

A site of ribosome synthesis, the nucleolus is a subnuclear structure which appears to be the primary structures implicated in the activation of cellular stress responses (Ohbayashi et al. 2018), is the only storehouse of rDNA containing rRNA cistrons (Butorina and Kalaev 2000). Stressful events can affect the morphology and functionality of the nucleolus in both plant and animal cells. According to reports (Boulon et al. 2010) these alterations are connected to variations in the organism's transcriptional activity. Recent report said that (Mazzeo and Marin-Morales 2015) nucleolar activities is the most sensitive indicator of cytotoxicity when compared to other tests like chromosomal aberrations, mitotic index, and micronucleus. The volumetric changes along with the frequency of nucleoli per nucleus counted could be considered as measureable cytogentic biomarkers (shapes like dumbbell, pear, eye-shaped, chain-like adherences, etc.) that are most frequently detected in bioassays for the assessment of the cytogenotoxicity of contaminants. The coordination of processes for the interaction and modification of RNA and proteins in proliferating cells may be affected by such modifications. The nucleoli should therefore receive specific consideration because changes to these structures can act as strong cytological markers, which can be used as a key parameter in investigations of environmental monitoring (Lima et al. 2019). In this present investigation morphological features like nucleolar number, volume and shape etc., had been taken into consideration in the ger-

minating root cells of *Lathyrus sativus* L., for evaluation of salt-induced stresses (Figs 5B–L, 6). With increasing concentrations there was visible alterations in the nucleolar number, shape and volume (Kalinina et al. 2018) which would possibly arising out of adaptation strategies of the germinating root tip cells of this test plant against increasing ion-mediated ROS accumulation. Although in higher plant systems detailed link-up investigations depicting nucleolar alterations vis-a-vis stress signaling pathways are rare, but multiple studies could propound that plant nucleolus has a direct sensing ability to counter stresses such as increasing salinity through differential responses involving different biochemical pathways to quench salt stress (Boulon et al. 2010; Kalinina et al. 2018). Investigations have revealed that stress can trigger dramatic morphological alterations in plant nucleoli and protein content in living cells which are direct outcomes of diverse nucleolar transcriptions under increasing stress (Yildiz and Aki 2019). Just like animals, plant don't have p53 transcription factor for genome stability, but plants have their unique stress-sensing responses and genome-stability maintenance machinery which are localised in plant nucleolus (Yildiz and Aki 2019). At 400–500 mM NaCl pretreated germinating root cells there were the formation of giant strap cells which were showing translucent cytoplasm and muti-fragmented micronucleoli within apoptotic/nectrotic cells. Definitely, these numerous micronucleoli arose out of salinity-driven ROS outburst and ROS-mediated stress cellular stress which would have disrupted the total pool of r-DNA cistrons thereby augmenting disruptive protein synthesis and cellular metabolism. Possibly, here the cells could not perform any transcriptional activity which was reflective in substantial decrease in soluble protein content and POX activity (Fig. 7). At the biochemical levels of stress responses, total soluble protein content and POX could be considered as natural elicitors within the cell cytoplasm (Anuradha and Rao 2001; Yildiz and Aki 2019) that confer viable and variable protective tools against osmolyte imbalance (increasing NaCl stress) for the plant cell to survive. In this investigation NaCl pretreatment at 100, 200 and 300 mM resulted increased levels of soluble protein with POX activity in germinating root tissues of *Lathyrus sativus* L. Interestingly, this increasing salinity suppressed POX activity, which easily could have also triggered an upsurge of ROS propagation by local as well as long-distance signalling cascades. This outburst of ROS in germinating plant roots thus in turn could have stimulated the plant's metabolic system to change in such a fashion that it might be taken into the possible consideration of a potential signature of "biochemical biomarkers" owing to disruptive cellular homeostasis and ROS imbalances. This follows earlier reports (Anuradha and Rao 2001) where increasing salinity root cells were trying to adjust with inherent tolerance to salt. But the decrease in protein content is due to the effects of sodium chloride on protein synthesis (Wang et al. 2003), where above suboptimal levels of tolerance (here 400–500 mM) could affect protein synthesis and provoke its decline (Wang et al. 2003). Protein content in *Vignia unguiculata* (L) Walp., in comparison to control, significantly increased in the stems of plants grown with 100 mM of sodium chloride (Ravelombola et al. 2022). To survive under stress, plants accumulate proteins that protect cells from stress effects (Ravelombola et al. 2022).

In earlier studies, increasing salt concentrations were shown to disrupt membrane leakage caused by membrane lipid peroxidation, which ultimately resulted in the loss of cell electrolytes (Demidchik et al. 2019). Electrolyte leakage is a characteristic of the stress response in whole plant cells. This phenomenon is widely used as a test for the stress-induced damage of plant tissues because it serves as a "biophysical marker" of plant stress tolerance (Demidchik et al. 2019). All primary stress factors, including heavy metals (Hniličková et al. 2019) and oxidative stress due to salinity (Demidchik et al. 2003; Demidchik et al. 2019) can cause electrolyte leakage, which affects a wide range of species, tissues, and cell types. Following the application of a stress factor, the electrolyte leakage is almost immediately noticed and lasts for a few minutes to several hours. Even while electrolyte leakage has a significant physiological impact and a connection to stress tolerance, the processes underlying this phenomenon are still poorly understood. Effects of NaCl stress and membrane permeability on *Lathyrus sativus* L., because it may regulate and adapt the transport and exchange of intracellular chemicals, as the root cell membrane is selective. It is the initial site of stress injury at the cellular level. The plant cell is most immediately harmed by ROS injury from increasing NaCl pre-treatment in germination root tips through disruption of cell membrane structure and function, increase in membrane permeability, decrease in membrane stability, and enhancement of passive leaking of ion cells and macromolecules. As result, enhanced membrane penetrabilitya biophysical indicator of cytotoxicity" is the clearest sign that cell membranes have been damaged. *Lathyrus sativus* L. roots that were germinating after pre-treatment with varying concentrations of NaCl, in this experimental setup showed an almost dose-dependent increase in electrolyte leakage (Fig. 8), which is consistent with the recent findings (Hniličková et al. 2019).

Another adverse effect of salinity is the build-up of salts in the root apoplast, which can disrupt cellular water connections and hinder growth as well as lead to wilting and cell death. Later, sodium data were presented to support this concept (Flowers et al. 1991). However, as NaCl salinity also causes the apoplast to accumulate Cl^- ions, which act as an osmoticum (Shahzad et al. 2013), high Cl^- concentrations in the apoplast may harm cells (Geilfus et al. 2018). In an in vitro investigation on barley, Yamashita et al. (1994) discovered that when the roots are stressed with NaCl, PM vesicles of the roots increase their permeability to Cl^- such membrane leakage may result from sodium's displacement of calcium, which on the other hand stabilizes the membrane (De Costa et al. 2007). Furthermore, under NaCl salinity, the thickness of membranes may be impacted; although Cl- ions are anticipated to be exposed to the aqueous phase because sodium is assumed to be connected to the carbonyl and phosphate oxygen of the membranes' lipid head groups. According to different

reports (Cordomi et al. 2008; Klasczyk et al. 2010), this localization of the two ions creates a dipole moment to the lipid head groups that is opposite from what polarized water generally creates under non-stressed situations. Though hypothetical, it's possible that a thickening of the PM under Cl^- salinity could result in decreased metabolic activity, which will inhibit growth. Changes in the cell's surface area to volume ratio have been observed to have an impact on the electrostatic potential of the PM, which has also been reported to be impacted by salt induced changes (Klasczyk et al. 2010).

A higher diffusion of oxygen from the roots is indicated by increased root oxidizability (RO), mostly to combat the harmful substances nearby the site of action. When TTC salt is used to detect RO, electrons from the mitochondrial transport are actually absorbed. In other words, improved RO also signals increased ROS production. The current investigation demonstrated that roots at higher concentrations (500 mM NaCl; Fig. 8) had less root oxidizability as measured by TTC-reduction. The rate of root respiration was found to have noticeably decreased, and the root oxidizability indicates that mitochondrial poisoning is likely what caused the cellular death (apoptosis) (Figs 3, 4M–O, 9). The greater quantity of NaCl modified cellular stress in germination seeds via ROS formation, according to a decline in root oxidizability. According to Ghosh et al. (2023) when ROS damage or rupture the root cell membrane, a significant number of intracellular ions and organic compounds leak out, which causes physiological metabolic disturbance. It is obvious that NaCl stress has an impact on the biochemical and physiological features of *Lathyrus* roots. *Lathyrus* root mitochondrial activity considerably increased from 100 to 300 mM NaCl pretreatments (in comparison to negative contol, 2% H_2O_2; a peroxyl radical genarator), with a distinct downward trend in 400 and 500 mM treatments. Additionally, we can deduce that exposure to 400 and 500 mM concentrations of NaCl caused deeper injury vis-à-vis ROS generation augmenting severe membrane and mitochondrial damage to newly sprouted roots that would have hastened the physiological metabolism disorder resulting root cell death, a direct outcome of disruption in membrane architecture (owing to electrolyte leaking). (Figs 8, 9). Reports already opined that, in reaction to Cl- salinity, the pea (*Pisum sativum*) root's mitochondrial respiration had been specifically inhibited, and a switch from the glycolysis pathway to the pentose phosphate pathway was evident (Hason-Porath and Poljakoff-Mayber 1970). In wheat mitochondria under NaCl stress (Jacoby et al. 2015), it was found that electron transport routes were blocked and respiratory kinetics was disrupted. This stress was also accompanied by an increase in the amount of ROS produced by the mitochondria per unit of oxygen consumed. However, in several plants the beneficial effects of salt stress enhance "epigenome" and brings about epigenomic modifications too (Adhikari and Das 2023) that in long run could enhance salt mediated stress adaptations in climate resilient crops. Till today the bulk of research related to various regimes of salt treatments are only restricted to monocots (Shan et al. 2024) and species of *Allium* and very few dicot crops including woody cash crops have been subjected to salinity induced changes with detailed genotoxic profiling studies (Darwish et al. 2023). Therefore, commercial cash crops need to be brought into the process of elaborate investigative bio-assays in greater numbers, to garner newer insights into this complex process of salinity-induced ROS generation. Similarly, the ameliorative roles of different chemical and phytoconstituents should be taken into more broader applications to enhance the plants' own internal resistance against salinity-induced physiological stress which might alter the drastic genomic loss or gross cytogenetic changes (Omar et al. 2023; Tabur et al. 2023).

This climate resilience crop has a tremendous opportunity to come out as the "future wonder crop" based upon its readiness to get adopted to several ecological and anthropogenic stresses and these findings might be insightful to improve this crop with the help of state-of-the-art biotechnological applications as a "wonder crop" with a rich source of soluble proteins for readymade consumption for domestic and human populations. So optimum salt tolerance levels could be established primarily utilizing cytological bioassays during seed germination. However, it is not conclusive as the establishment of further molecular categorization is needed to detect end-point suboptimal salt tolerance mechanisms in crop plants.

Conclusion

The salt tolerance mechanism in plants is a very complicated and unexplored area of plant breeding in consideration of pulse crops, especially legume crops. This whole experimental study for the first time conclusively proved that salt priming above the suboptimal level (100–200 mM) triggered severe cytogenotoxic responses in germinating root tips of *Lathyrus sativus* L., variety Mahatora from genomic (chromosomal and nucleolar) standpoint. There was a significant increase in root metabolic activity (dehydrogenase enzyme activity in root mitochondria), increased production of soluble protein (as a biochemical tool for osmolyte balance against stress) and increased POX activity as an elicitor against ROS stress owing to salt priming (100–300 mM). These are important findings for the first time in this pulse crop that might highlight its resilience capacity against increasing salinity stress and in vivo ROS outburst during the process of germination. According to this study, rising sodium and chloride ions in the root cells combined with a severe cytological and biochemical stress in the cellular microcosm had a negative impact on the growth of Lathyrus sativus. This work specifically illustrates a wide range of biochemical and cellular toxicity in roots that would effectively catch scientists' interest and motivate them to look into all potential molecular pathways of salt tolerance in other key types of pulse crops in the near future. This report can demonstrate that, apart from conventional model plants i.e., *Vicia faba* and *Allium cepa* L., which are the two most

widely adopted plant-based assay systems worldwide, *Lathyrus sativus* L., root tip cytogenetic biomarkers can stand out rightly to elaborate all promising outcomes, supporting it as an easy-to-handle, alternate, vis-à-vis a cost-effective bioassay model for all plant scientists.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author's contributions

All authors contributed to the study's conception and design. Material preparation, data collection and analysis of results were performed by [Dr. Dipan Adhikari], and [Mr. Rahul Ghosh] and the work was performed manually by Mr. Sagar Dig (P.G., Research Student) in the laboratory. The first draft of the manuscript was written by [Dr. Dipan Adhikari] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript

Availability of Data

All materials are reported in the text, and all the data collected are reported in the manuscript.

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