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Effect Of Moderate Salinity (Nacl) On Seed Germination And Growth Parametersin [*Trigonella foenumgraecum*L.]

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Chapter-1

INTRODUCTION

*Trigonellafoenumgraecum*L.commonlyknownasfenugreek,isavaluableleguminouscrop renowned for its multifaceted uses in culinary, medicinal, and industrial applications. As a significant crop in various parts of the world, particularly in India and middle east, fenugreek plays a crucial role in agricultural economics and dietary practices. However like many crops *Trigonella foenum* faces numerous challenges due to environmental stresses among which salinity stress stand out as a major constraint affecting its growth, productivity and quality. (S.K. Singh et al. 2020)

Salinity stress caused by high concentration of salts in the soil is a pervasive issue worldwide impacting agricultural productivity and sustainability. saline soil contain excessive levels of soluble salts primarily sodium chloride (NaCl), which can disrupt plant physiological processes. For glycophytes, plants like fenugreek that are not adapted to saline conditions, exposuretohighsaltlevelscanleadtoosmoticstress,iontoxicityadnutrientimbalance.these conditions severely affect plant growth, development and yield.

The impact of salinity stress on *Trigonella foenum graceum* is multifaceted. High salt conditions can impede seed germination, reduce seedling vigor, and limit plant growth by affecting photosynthesis, nutrient uptake and protein synthesis. Salinity also can induce oxidativestress,leadingtoaccumulationofreactiveoxygenspecies(RoS)thatdamagecellular components. given the economic importance of fenugreek and its increasing demand, understanding the effects of salinity stress on this crop and exploring potential mitigation strategies and imperative.

Thefollowingexperimentationaimstoprovideanin-depthanalysisofeffectsofsalinitystress on *Trigonella foenum graecum*, focusing on physiological and biochemical interventions.

Enhancing the salinity tolerance of fenugreek not only promises to improve crop yields and quality but also contributes to sustainable agriculture and food security in regions affected by soil salinity.

In response to salinity stress fenugreek activates various biochemical pathways aimed at mitigating the adverse effects. one key response is the accumulation of osmoprotectants, such as proline and glycine betaine, which help maintain cellular osmotic balance and protect macromolecules from damage. additionally, the antioxidant defense system is upregulated to counteract oxidative stress enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) play crucial roles in scavenging RoS and minimizing oxidative damage (Singh et al. 2018)

Salinity stress also effects photosynthesis in fenugreek. high salt concentrations can damage thephotosyntheticapparatus,reducingchlorophyllcontentandimpairingtheelectrontransport chain. This leads to decreased photosynthetic efficiency and carbon assimilation, further contributing to growth inhibition.

Soilsalinityisconsideredasadetrimentalfactorforcropsworldwide.33%ofirrigatedsoilare affected by salinity (Qadir et al., 2014; Hopmans et al., 2021) in case of primary salinization, salinity stress get occur by weathering of rocks on the planet (Parihar et al.,2015)

Secondary salinity stress in crop plants occurs by anthropogenic influences, alteration of hydrological balance of irrigation water most of compounds causing salinity stress in almost of all crop plants includes sodium chloride (NaCl), calcium sulphate, calcium bicarbonate, magnesium chloride and magnesium bicarbonate (Mata-Fernandez et al., 2014)

Based on tolerance to stress for salinity plants are of two types -halophytes and glycophytes (MunnsandGilliham,2015)halophytestoleratingsalinityupto>=(9.8mmhos/cm)i.ecotton, sugarbeetetc. glycophytes tolerating salinity of low level (4mm hos/cm and below) (Shabala,2013)

Salinity stress poses a significant challenge to the cultivation of *Trigonella foenum graecum* through a comprehensive understanding of the physiological and biochemical responses of fenugreek to salinity stress, we can develop effective strategies to mitigate these impacts. by exploring genetic variations, breeding for tolerance, and implementing appropriate agronomicalpractices,itispossibletoenhancetheresilienceoffenugreektosalineconditions. such ensures food security in regions affected by soil salinity.

Reproductionisacriticaltimeinplantlife.Basicseedisayoungsporophyteseedsarealsoan excellent model system for studies of development biology. They are complex structures that areboundedbyaseedcoat,embryoandendospermeachoftheseregionsconsistofsubregion tissues and cell types that undergoes further development [Belmonter et.al.2013]

Development of seedling starts with the germination of seed and radicle emergence. Seed comperises of radicle, hypocotyl and cotyledons.

Salinityaffectsone–thirdoflandalloverworldleadingtodecreaseincropproductivitybyits negative effect on seed growth and germination. Basic process affected by high salt concentrationincludesosmosisionictoxicityandimbalancenutritionchannels.Alteringthose mechanism lead metabolic and physiological changes and possess a negative impact on seed germinationHighsalinityconcentrationinnumberofplantsleadsdecreaseinseedgermination percentage.

Fenugreek(*Trigonellafoenumgraecum*L.)isaperiodiclegumethat'sconsiderablycultivated in utmost regions of the world for its medicinal value and other important uses and other importantusesnitrogenobsessioninthesoil,feedstockofthefoodandchemicalassiduityand beast feed (Petropoulos, 2002; Sadeghzade et al., 2009).

Trigonella foenum is native to an area ranging from Iran to northern India and widely grown in China, India, Egypt, Ethiopia, Morocco, Ukraine and Turkey etc (Petropoulos, 2002). It's generallyusedasaseasoninginfoodmedicationsforitsnutritionalandrestorativeparcelsand it has also been used in folk drug for centuries for centuries for a wide range of conditions including diabetes (Eidi et al., 2007).

Theworld'soldestmedicinal factoryisfenugreek, anditbelongstothelegumefamily.Itwas firstly grown as a pasturage crop; still, fenugreek is substantially grown as a spice crop. Fenugreek has medicinal importance because of production of secondary metabolites such as flavonoid,terpenoids,pyridine-typealkaloids(trigonellineandcholine)andprotein(arginine, histidine, and lysine)

Plants make large variability in salt tolerance and are differenciated as salt sensitive, moderately salt sensitive, moderately salt tolerant, or salt tolerant (Munns and Tester, 2008). Salt-tolerant species genetically acclimated to high-saltness stress are nominated halophytes, while those less acclimated to salt stress are nominated glycophytes (van Zelm et al., 2020). Utmostmajorcrops,similarasrice(*Oryzasativa*),wheat*(Triticumaestivum*),andmaize(*Zea mays*), are glycophytic species that tolerate only low saltness or mild saltness conditions. Severalrecentreviewshaveaddressedthesalinitytolerancemechanismsofhalophytes,which employ specialized strategies to repeal saline surroundings. This review thus focuses on advances in understanding mechanisms of salinity tolerance in glycophytes, including the model factory Arabidopsis (*Arabidopsis thaliana*), and the four most-studied crops: rice, wheat, maize, and soybean (*Glycine max*).

Environmental stresses including salinity stress is a limiting plant growth and productivity circumstance. To acclimatize to salt stress, plants have developed various machenisms to integrateexogenoussalinitystresssignalswithendogenous experimentalcuestooptimizethe balance of growth and stress responses. Accumulating substantiation indicates that phytohormones, besides controlling plant growth and development under normal conditions, alsointervenedifferentenvironmentalstresses,includingsalinitystress,andthereforeregulate plant growth adaptation. we also punctuate how, plant hormones mediate salinity signals to regulateplantgrowthadaptation.Wealsogettoknowthathow,plantsbuildadefencesystem in response to salinity stress by developing the conflation, signaling, and metabolism of different hormones with multiple cross addresses.

About the plant: Fenugreek (*Trigonella foenum graecum*), a short–living periodic medicinal plant that belongs to Fabaceae family, it is used more or less in the various parts of the world as condiment, food, spice, and traditional drug.

Itisnativetothemediterraneanregion,southEurope,andwesternAsia.Itisnowconsiderably cultivated in countries like India, Egypt and Morocco.

The plant generally attains a height of 30-60cm outside and points trifoliate leaves composed of three obovate to oblong leaflets.

The plant produces small, axillary, flowers that are more or less white or pale yellow. These flowersdevelopintoslender,curvedpodsmeasuringupto15cminlength,eachhaving10-20 cuboid, yellowish-brown seeds

Uses:

Fenugreekhasbeenemployedforcenturiesincolorfulculinarytraditions.Itsseedsandleaves areintegraltonumerousdishesinIndiancountry,frequentlyusedincuries,pickles,andspice compositions.Theseedretainandsweetstrongaromaevocativeofmaplesaccharinityandare occasionally employed in the production of maple flavoring. Beyond its culinary operations, fenugreek has a history of use in traditional drug systems, where It has been used for its purported health benefits.

CHAPTER-2

LITERATUREREVIEW

EFFECTOF NaClONSEEDLINGS

Salinity affects one –third of land each over world leading to drop in crop productivity by its altering effect on seed growth and germination. introductory process affected by high salinity condition includes osmosis ionic toxin and imbalance nutrition channels. Altering these machenism lead metabolic and physiological alteration and retains a negative impact on seed germination High salt condition in number of plants leads drop in seed germination chances

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Increasedsaltconditions-Leadsaffectingphotosyntheticpigmentsandchlorophyllconfation, also primary and secondary metabolites fluxes. Proline content increases with high saltness such as in *H.vulgarecultivors* and *O.basilicum* genotypes. salt stress changes biochemical parameters,includingantioxidantsenzymes,andphenolic composites.prolinecontentplaysa pivotal part acting as an osmoprotectant and antioxidant.

Trigonella foenum graecum antioxidant defense system, includes enzymes like superoxide dismutase, catalase, and peroxidase, helps alleviate oxidative damage caused by salt stress. (Basel et al.2021)

Injury causing osmotic stress and ionic toxicity lead to the generation of secondary stress, known as "oxidative stress," a type of stress condition in which the equilibrium between the product of reactive oxygen species (ROS) and the demolishing exertion of antioxidants is altered (Gill and Tuteja 2010)

Oxidative stress in plants is a reason of uncoupled pathways in the metabolism of plants transferringhigh-energystateelectronstomolecularoxygentoformROS(Gilland Tuteja 2010).

ROS include singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and revolutionary hydroxyl radical (OH). ROS undo colorful cellular functions by damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation (Foyer and Noctor 2005)

Plants have basically two antioxidative systems to fight the damages of ROS: enzymatic and nonenzymatic scavenging methods. Enzymatic scavenging method include superoxide dismutase (SOD), catalase (CAT), peroxidase (PX), ascorbate peroxidase (APX), and glutathione reductase (GR). Antioxidant molecules, similar as ascorbic acid, glutathione, α-tocopherol,andcarotenoids,alsoplayimportantplacesinthejunkingofpoisonousby-products of O_2(Gill and Tuteja 2010)

Abiotic stress, in general, and saltness, in particular, make a series of morphological, physiological, biochemical and molecular changes, which unfavourably affect plant growth, developmentandproductivity(ShankerandVenkateswarlu,2011;MunnsandGilliham,2015)

Although *Trigonella foenium* is relatively tolerant to saltness (Kumawat et al., 2017), in numerous countries this species is grown in thirsty and semi-arid regions in soils with high salinity condition, which negatively affect plant growth and productivity (Al-Saady et al., 2012)

Saltness in soil doesn't only change leaf and root hormone attention (Pérez-Alfocea et al., 2010), but also alters root to shoot hormonal signaling (Albacete et al., 2009). Abscisic acid (ABA)playsaroleinthecontrolofleafstomataltransduction,growthofplantandadaptation to deficiency of water and salinity condition, while cytokinins (CKs) play a functional part in theregulationofleafletgrowth,leafsenescence,shootandrootbalanceandnutritivesignaling (Albacete et al.,

2008; Albacete et al., 2009)

Plants respond to high saltness stress through eliciting a range of physiological, biochemical, and molecular mechanisms, which can be distinguished into three major classes (i) 'ion exclusion' to eliminate $Na^+$and Cl^-ions from roots when their accumulation becomes poisonous (ii) 'tolerance of tissue' allowing 'compartmentalized of poisonous ions at cellular andintracellularposition'(Royetal.2014)and(iii)'tolerancetoosmosis'(Paridaetal.2005; Munns and Tester 2008; Roy et al. 2014)

In recent times, work has done to explore the inheritable variation for salt stress tolerance capacityindifferentleguminouscrops.Chickpeagenotypesmadeawiderangeofvariationin their response to salt stress (Lauter and Munns 1986)

In pea, differentiation for traits like as plant height, plant growth rate (PGR), roots and shoot biomasswasknownfollowingfromwebbingof780peaaccessionsundersaltstress(Leonforte et al.2013).Also, another study covering 30 pea genotypes were known for variation for germination (%) chance, root and shoot weight and inorganic osmolytes (Shahid et al. 2012). salinity tolerance of the genotypes *Samarina Zard*, Climax, 9800-5 was set up to be due to lower accumulation of poisonous Na^+in leaf and a advanced Na^+/K^+rate, and abundant antioxidantenzymaticconditionsandosmolyteamountundersaltstress(Shahidetal. 2012a, b)

Animportantpointofthesalinitystressresponseisthattheosmoticsignalcausesabscisic acid (ABA) to get aggregating by converting the expression of ABA biosynthesis genes (e.g., NINE- CIS-EPOXYCAROTENOID, DIOXYGENASE's (NCEDs), ABA-DEFICIENT(ABA),andALDEHYDEOXIDASE-3(AO3)),whichmakesnumerous/many adaptive responses in plants (van Zelm et al., 2020; Du et al., 2023)

Rapid and Na^+-specific halo-tropism (growth of roots down from Na^+) under salinity stress conditions indicates the presence of root-grounded Na^+detectors (Sun et al., 2008;Galvan- Ampudia et al., 2013)

Salinity stress makes tissue-specific modulations in Ca^{2+}circulation and amplitude, and numerousCa^{2+}signalsbeinginvolvedintheconformationofsalt-convincedCa^{2+}signalshave been linked to plants (Knight et al., 1997; Ma et al., 2019; Steinhorst et al., 2022)

A good review about the effect of (melatonin) in the plant was presented in response to saltness.Describedthechangesofexogenousmelatoninthemodulationoftheexpressionof genesmakinginmelatoninmetabolism,theincreaseofthetranscriptionlevelsofmanystress- responsive genes, and recap transcription factors involved in the ROS scavenging and of the genes responsible for the conservation of ion homeostasis. Melatonin regulates hormone metabolismbyup-regulationofgibberellicacid(GA)biosynthesisalsoincludingabscisicacid (ABA) catabolism genes formation. (Zhan et al.)

Transporters helpplants take up salt from the soil, that made ion toxicity and disturb mineral uptakeofplantandionhomeostasis.Saltstressleadstovigorousaccumulationofions($Na^+$and Cl^-) and stops $K^+$and Ca^{2+}uptake and eventually results in ionic imbalance (Isayenkov and Maathuis,2019).Saltstressincreasesreactiveoxygenspecies(ROS)contentinplantandmake oxidative stress. The poisonous impact of ROS is lipid peroxidation, membrane damage, as well as DNA and protein deterioration (El Ghazali, 2020)

Combining different 'omics' approaches such as genomics, transcriptomics, proteomics, and metabolomicsintheplantisthenewandwidertooltodealwithsalttoleranceand adaptation. 'Omics' studies of control and salt stressed plant had authorized the conspicuous evidence of changes in traits, genes, proteins, and metabolome that play a crucial role in making a plant tolerance to salinity stress (Nongpiur et al., 2016; Ismail and Horie, 2017; Jha et al., 2019)

Depending on the type and amount of salt, cells of plant have also different mechanisms for guarding

themselves from harsh effects of salt stress. Plants have made their defence against saltstressatnumerouslevelsbymakingchangesinmolecular,biochemical andphysiological pathways. Some of these processes include ion hemostatis, enzymatic regulation and non- enzymatic regulation of antioxidants, compatible solute attraction and osmotic protection, hormonal regulation made change in expression of stress resistance genes, and regulation of nitric oxide production (Hanin et al.2016)

In recent times, important attention has been made toward the use ofnano-particles(NPs), as one of the most advanced methods, to make improvement in growth and plant performance undergoing salinity stress (Ahamad and Akhtar2021, Das and Das.2019, Duhan et al 2016)

Nano-materialsaremadeofcompoundswithverysmallsize(atascaleof100nmorless),and thesecompoundsmadeactionontheproperties ofmaterialsatthemacrolevel.Nano-materials have a fairly largersurface area when compared to the same mass of material produced in a largerform.materialscanbemademorechemicallyreactivewithNP'sandaffecttheirstrength or electrical properties. NPs have high surface to volume ratio that increases their reaction capacity and possible biochemical activity (Das and Das.2019, Dubchak et al.2010)

Lipids are essential factors of cell membranes responsible for structural conservation and cell functioning.ROSaregeneratedfromseverallifeprocessesinplantsandanexcessofROScan damagecell,tissuesandorgansofplant.Saltstressexposuremadeadisturbance,anoverflow, orindeed adislocation of electrontransport chains (ETC)in mitochondriaand chloroplasts in higherplants,makinginROSaccumulation.ThemajorplaceinvolvedintheproductionofO2isthephotosystemI(PSI).whenlightispresent,O2whichiscontinuouslyhandedbythewater autolysis reaction involved (Reaction 1: $2H_2O \rightarrow 4\ e^- + O_2 + 4\ H^+$) can be decreased to O_2(Reaction 2: $2O_2 + 2\ e^- \rightarrow 2\ O_2$). The extra amount of decreased ferredoxin (Fdred) and the limitedNADPpresenceaccelerationtheautoxidationofFdredtoFdoxandtheformationof O_2(Reaction3:$Fd_{red} + O_2 \rightarrow Fd_{ox} + O_2$).Also,the$Fd_{red}$canreactwith$O_2$toform H_2O_2(Reaction 4: $Fd_{red} + O_2 + 2\ H^+ \rightarrow Fd_{ox} + H_2O_2$). Lipids are main targets of ROS attack andthefreeradicalsoxidationofpoly-unsaturatedfattyacidsiscalledLP(Gutterigeetal1995)

AccordingtotheUnitedStatesDepartmentofAgriculture(USDA)Laboratory,thesoilissaid tobesalinewhenelectricconductivity(EC)ofthesoilsuspensionismorethan4dSm^{-1}which is (equal to 40 mM NaCl) (Munns and Tester, 2008)

Salinity stress, accompanied by osmotic imbalance and ionic accumulation can lead to a deficiency of $K^+$and Ca^{2+}, attenuation of photosynthetic rate, impaired metabolism, and ultimately plant's death (Mudgal et al., 2010)

Saltness have double effects on plants: osmotic as well as ionic. Under the condition salt concentration crosses its normal level, water potential(ψ) decreases in the soil making the reduced absorption capacity ofwaterby roots ofplants. This effect is called an osmoticeffect or water deficiency effect in plants. (Parihar et al., 2015; Rahnama et al., 2010)

Water insufficiency is characterized by a decreased water potential(ψ), loss of turgor, fading, stomatalclosure,andreductionincellgrowth.Alsoontheotherhand,avigorousassimilation of toxic ions, like as $Na^+$and Cl– in cells has a poisonous effect on the plant. At increased concentrations, even non-toxic salts for example (Na_2SO_3) also become toxic, leading to change in the normal physiological processes. Due to salts, the K^+/ Na^+ratio changes in the plant cells, leading to high Na^+ concentrations and cellular homeostasis (James et al., 2011)

Soilsalinityiscalledasanincreasedconcentrationofsolutesaltsinsoils,leadingmorethan4 dS/m electric conductivity. Salinity on other hand affects plant growth and development throughitseffectonphysiologicalandbiochemicalpathways(Nabatietal.2011),oppressively constraining major cereal crop production, with yield losses of 60% in wheat (*Triticum aestivum*L.)(El-Hendawyetal.2017)and50%in rice(*Oryzasativa*L.)(VanGenuchtenand Gupta1993),anddecreaseof51.43%dryweightand53.18%leafarea,inmaize(*Zeamays*L.) (Hussein et al. 2007)

The major genes included in salt rejection are salt overlay sensitive (SOS) homolog genes (*OsSOS1*,*OsCIPK24*,and*OsCBL4*)inrice(Martínez-Atienzaetal.2007),(*TaSOS1*and*TdSOS1)*inwheat(Xuetal.2008;Fekietal.2011)and(*ZmNHX7)*as a Na^+/H^+ antiporter in maize (Bosnic et al. 2018).

There are mainly three salt tolerance mechanisms proposed by(Munns and Tester2010): ion exclusion–thenetexcretionofpoisonousionsfromtheshoot;tissuetolerance–thesepration oftoxicionsintospecificplanttissues,cellsandsubcellularorganellesofplant;andshootion- independent tolerance – the care of growth and water uptake nondependent of the extent of Na^+accumulationintheshoot.Transpiration(T)alongwithtranspirationuseefficiency(TUE) (Haris et al 2010) leaf area, seed germination, antioxidants production, growth of early seedling, and harvest index (HI) are some of other physiological parts likely involved to salt tolerance, like the maintenance of plant water relation (Gholizadeh et al 2014).

Salt stress today has emerged as a major global agricultural issue, leading to change ~20% of thetotalagriculturalareaintouncultivableregionsonearth,mainlythirsty(arid)andsemiarid geographicallands,atanincreasedannualrateof ~1%−2%(Mohantyetal2021).Bytheyear 2050,saltpollutantswillacquireanimpactonnearly50%offarmingareas(Butcharetal.2016)

A study made by Banakar and their team members resulted a decrease in the production of *Trigonellafoenum*plantsalongwithhighersoilsalinity,i.e.,by10%(3.38dsm^{-1}),25%(6.28 ds m^{-1}), and 50% (11.67 ds m^{-1}) (Banakar et al 2022)

In a study carried out in northern India concluded that, fenugreek is more susceptible to high salt condition than *Coriandrum sativum* and *Foeniculum vulgare* plants (Yadav et al. 2013)

Abscisic acid (ABA) plays a part in the regulation of leaf stomatal functioning, plant growth andadaptationtowaterdeficitandsaltstress,whilecytokinins(CKs)playan greatpartinthe maintenance of leaf growth, leaf senescence, shoot and root balance and nutritional signaling (Albacete et al., 2008; Albacete et al., 2009).

It has been seen in today's time that gibberellins (GAs) interact with mycorrhizal association to improve growth in salt stressed tomato plants (Khalloufi et al., 2017).Salinity disturbs the mineral relations of plants by decreasing N and other mineral nutrients availability through competition with Na^+ and Cl^- (Murtaza et al., 2013).

Endophytes from salinity prone environments naturally adapt to salinity conditions and can help plants to more effectively cope with the saline environments during their development, thus promoting the management of saline land areas.

Endophytes make symbiotic or pathogenic relationships with plants in most of cases. They include beneficial effects such as increased plant nutrients uptake, like nitrogen, phosphorus, andions.Andregulationofplantgrowthanddevelopmentbytheregulationofplanthormones including (cytokinin and ethylene).

In case of wheat examined with an endophyte, (*Pantoea agglomerans)* calledYN1, seen that from healthy wheat stems shown noticeable increases in morphological aspects like plant heightandrootlength;chlorophyll,carotenoid,andprolinecontentsandanoticeabledownfall in malondialdehyde content under 150 mM NaCl stress, which indicates the capability of (YN1)inincreasingplantsalttoleranceoradaptability.(Manjunathaet al.)concludedthatthe endophytic fungi increases salt tolerance capacity in wheat at the seedling stage.

NO (Nitric oxide), as a major signaling part, plays an effective part in plant development and in resistance gaining to plants from various environmental conditions, such as salinity conditions.Ininfluenceofsalinity,plantspromoteNOconflationsubstantiallybyadditionthe exertion of a NOS-suchlike enzyme or NR and by inhibiting the exertion of GSNOR.

Salinity decreases the plant height, fresh weight, total dry weight, photosynthetic colour content, and amount of protein in plants. The negative effects of salinity can be soothed by exogenousNOtreatment in wildvarietyofbarley. Exogenousoperation ofSNPincreasedthe freshweightandshoot/rootextensionof(*Nitrariatangutorum)*seedlingsundersalinitystress. Leaf anility and root damage convinced by salinity stress were also soothed. Meanwhile, operationoftheNOeatingcPTIOandmammalianNOSassetL-NAMEsignificantlyworsened stress-convinced damage under high-salinity stress conditions (Zhao et al 2004)

Exogenous melatonin provided significantly reduced saltness-convinced ROS. Following 12 days of salinity stress, H_2O_2 attention increased by 37.5%, while melatonin pre-treatment of cucumber maintained a low H_2O_2 attention throughout the trial. analogous results were also observed in salinity-stressed rapeseed seedlings, and the operation of exogenous melatonin dropped the H_2O_2content by 11.2 %. (Liang et al.)

ExogenousmelatonindecreasesH_2O_2andO_2^-attentionbycrackingantioxidantenzymes.This process has been verified in numerous plant species, similar as rapeseed, radish, cucumber, rice, maize, bermudagrass, soybean, watermelon, kiwifruit, and *Malus hupehensis*. In cucumber,theexertionofmajordefensiveantioxidantenzymes—includingSOD,CAT,POD, and APX—in melatonin pre-treated plants was significantly advanced than control plants. Undersaltstress,exogenousmelatoninoperationalsoeffectivelyincreasedtheconditioningof APX,CAT,SOD, cover, GR,andGPXinmelatonin-treatedseedlingscomparedtotheirnon- treatedcounterparts.also, melatoninteracts withROS by perfecting attentionsofantioxidants (ASA-GSH)

Generally,lowsaltattentioninducesastateofdormancyanddecreasesthegerminationchance. Meanwhile, high salt attention for plant inhibits the seed germination and decreases the germination chance (Shannon and Grieve, 1999, Khan and Weber, 2008)

The germination of utmost crops fails on saline soils. This is frequently a result of high salt concentrationsintheseedplantingzone.Inhotanddrysurroundings,highevapo-transpiration resultsinwaterloss.Thisresultsinsalinityaroundtheplantroots.Thissalinityinterfereswith the plant's capability to take up nutrients (Bernstein and Hayward, 1958)

High saltness situation take 1.5 million hectares of land out of product each time (Pitman and Läuchli, 2002, Munns and Tester, 2008).

Therefore, 50 of cultivable lands will be lost by the middle of the 21st century (Wang et al., 2003)

wearestilldevelopinganunderstandingoftheunderpinninginheritableresponsestostressand the complex relations between stresses and environmental cues, networks of gene expression regulation,physiological responses and eventually plantfitness(Cramer et al2013;Siddiqiet el 2021)

The*E2*knockout mutant $e2^{CR}$not only has a docked flowering time but also appreciatively regulates the recap position of ROS scavenging-related genes, which enhances the salinity tolerance of soybean, forming a foundation for the identification of early growing and salt- tolerant soybean kinds. (Dong et al,2022)

Lateembryogenesisabundant(LEA)proteinsareinvolvedinplantstresstoleranceforbearance and play a pivotal part in adversity resistance (Abdul Aziz et al.2021).

In rice (*Oryza sativa*), five*LEA*genes (*OsLEA1*,*OsLEA2*,*OsLEA3*,*OsLEA4*, and*OsLEA5*) were mainly over-regulated under stress failure and handed better physiological adaptation to drought stress in seedlings (Kamraudin et al.2021)

The biosynthesis and signaling exertion of other phytohormones similar as ethylene are also told by saltness stress. formation ofethyleneconflation in caseof avariety ofstresses such as salt stress is well known. Biosynthesis of ethylene or its direct precursor, ACC (1- aminocyclopropane-1-carboxylate)isconvincedtoaremarkabledegreebysalinity stress. Thus, ethylene accumulates in plantlets freighted with salt shock

SiliconoperationenhancesH$^+$-ATPaseexertioninthetubemembraneandtonoplast.increased H-ATPaseexertion upon Si operation facilitatestheexportofNa$^+$ out ofthecell (Lianget al., 2006b)

StudiesundersalinitystresshaveshownreduceddamageandtranslocationofNa^+totheshoot withSisupplementation.Forcase,increasedK^+/Na^+ratewasobservedwithSisupplementation in alfalfa *(Medicago sativaL.*) and salinity tolerant and salinity-sensitive chickpea (*Cicer arietinum*L.) genotypes (Wang and Han, 2007; Garg and Bhandari, 2016), and translocation of $Na^+$to upstanding parts was reduced in rice (Matoh et al., 1986)

Silicon accelerates the exertion of enzymes similar as peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, and acyltransferase related to lignin biosynthesis. Numerous studies reported Si intermediated improvement in the lignification and suberization in roots (Caietal.,2008;Flecketal.,2011).Indiscrepancy,somestudiesshowedanegativecorrelation between Si and lignin conflation.

Although the involvement of histone variations in abiotic stress priming has been reported in several cases in Arabidopsis, the places of histone marks in modulating transcriptional responses in more plant species, especially economically important crops, remain largely unknown. In soybean, the relationship between histone variations and recap has only been assessed in the environment of a single salinity treatment (Song etal.,2012; Sun etal.,2019)

The expression of transcriptional processes is important in maintenance of a broad range of cellular processes underpinning salinity mostly. Recent studies have proposed that epigenetic features similar as histone marks may be altered as a consequence of priming, which in turn modify the chromatin binding and modulate transcriptional responses during posterior stress exposure (Fu etal.,2021; Sun etal.,2020; Yung etal.,2021; Zheng etal.,2019)

Microtubule networks can rapidly alter their organization in response to salt stress, which is consideredvitalforplantgrowthandadaptation.Forinstance,cytosolicsaltimbalanceresults in met alicrotubule depolymerization and salt sensitivity in *Arabidopsis sos1*mutant (Shoji et al.2016).

In addition, the salinity stress mediated phospholipase D enhances the formation of phosphatidicacid(PA),whicheffectivelycorelatestoMAP65-1in*Arabidopsis*andreinduces its microtubule-stabilizing functioning, forming cortical microtubule reassembly and improving salinity tolerance in plants (Zhang et al.2012).

PGPB can live in the soil (rhizosphere) surrounding the plant roots, epiphytically attached to roots,stems,orleavessurfaces,orasendophytesinsideplanttissues.PGPBareabletopromote the growth of plants by different mechanisms such as nutrient uptake from soil (phosphate, nitrogen,iron,etc.),modulationofplanthormonelevels(auxins,ethylene,abscisicacid,etc.), or enhancement of plant resistance to pathogens by the activation of defence mechanisms referred to as Induced Systemic Resistance (ISR) or the production of antimicrobials. Before the last few years, PGPB have increased as a biotechnological tool with capabilities in agricultureasaneffectivetoolagainstthetraditionalchemicalfertilizersandpesticides.(Saad M.M; Eida A.A; 2020)

Jasmonates (JAs) are lipid-based plant hormones that regulate an array of processes in plants, particularly involved in defence mechanisms and stress tolerance. Delgado et al. refined the role of JAs in plant salinity tolerance, chiefly based on a genome-wide association study, and concluded that MYC2 transcription factor and JASMONATE ZIM-DOMAIN repressors are key components in JA signaling. The authors provides a detailed knowledge of JAs against plant salt stress thought be useful as a mentor in breeding programs. (Delgado c et al 2021)

Calmodulin-like proteins (CMLs) are thought to be involved in salt stress maintenence in Arabidopsis. Zhang et al. isolated a CML gene, *MpCML40*, from (*Pongamia),* and concluded thatitsheterologousformationcouldimprovesalttoleranceinyeastcellsandincreasetherate of seed germination

and the length of roots when exposed to salinity and osmotic stresses in Arabidopsis (Zhang Y et al 2021)

To mount an effective response to cope with salt stress, plants have developed the ability to sensebothethehyper-osmoticcomponentandtheionicNa^+componentofthestress.Thesetwo sensory modalities are evident in that some responses to NaCl remain distinct from responses to purely osmotic stress. At increased salt concentration in the soil suspension produces hyperosmotic stress in roots. To identify, the molecular identities of plant hyper-osmotic sensors and Na^+sensors have remained exclusive. TheArabidopsis(*Arabidopsis thaliana*) histidine kinase receptor protein HK1 has been shown to complement the loss of the yeast osmosensorSln1andoverexpression/loss-of-functionlinesexhibitdroughtandosmoticstress- associated phenotypes (Trans LS et al 2007

Transcription factors play a crucial role in connecting salt-sensing pathways to various tolerance mechanisms in plants. Several transcription factor (TF) gene families show altered expression levels in response to elevated salt conditions.

These include members of the bZIP, WRKY, AP2/ERF, MYB, bHLH, and NAC families, all of which are known to contribute to salinity stress responses (Trans LS et al., 2004).

Only plants with a natural tolerance to salinity are able to survive in environments with high salt levels. In crops, salinity tolerance refers to their capacity to withstand elevated salt concentrationswithoutsignificantgrowthoryieldloss,aslongasthesalinitydoesnotsurpass a specific threshold level known as the electrical conductivity threshold (ECt) (Machado and Serralheiro, 2017).

Various physical and chemical techniques, such as leaching through methods like continuous or intermittent ponding, sprinkling, and soil drying, as well as drainage improvement and the use of soil amendments like gypsum, are commonly applied to improve saline soils. Additionally,biologicalapproachesincludingphytoremediationandconventionalbreedingare also used for soil reclamation (Fita et al., 2015).

However,manyofthesephysicalandchemicalstrategiesarenotenvironmentallysustainable, as they can lead to pollution and ecological damage through their application methods (Arora et al., 2020a; 2020b).

Wheat plants genetically engineered to overexpress TaASR1-D demonstrated improved resistancetobothdroughtandsaltstress,mainlythroughmechanismsinvolvingROSandABA signaling,whichalsoledtoincreasedcropproductivity.Similarly,transgenicmaizeplantswith elevatedlevelsofZmASR1showedbetterdroughttolerance,thoughthishadlittletonoimpact on yield. In rice, higher expression of OsASR1 contributed to better growth and yield under drought by helping maintain stomatal closure (Park Si et al., 2019).

TheABA-independentpathwayalsoplaysavitalroleinhelpingplantstoleratesaltstress.Key components of this pathway include DREB-type transcription factors and genes encoding dehydrin proteins, among others (Zhu, 2002).

Genetically modified plants that produce higher levels of OsASR6 have shown improved resistance to salt stress, whereas plants with reduced OsASR6 expression through RNA interference became more vulnerable. Studies also revealed that OsASR6 can directly bind to OsNCED1, an important enzyme involved in the production of abscisic acid (ABA), both in testconditionsandwithintheplant.Additionally,OsASR6wasshowntoinfluencethebuildup ofreactiveoxygenspecies(ROS)andtheproductionofABAduringsaltstress,indicatingthat ithelpsimprovesalttolerancethroughpathwaysinvolvinghydrogenperoxide(H_2O_2)andABA signaling.

Plants manage salt stress through both ABA-dependent and ABA-independent signaling pathways. In the ABA-dependent pathway, three key components are involved: the PYR/PYL/RCAR receptors (Pyrabactin Resistance-like/Regulatory Component of ABA Receptor), the group A Protein Phosphatase 2Cs (PP2Cs), and the SNF1-Related Protein Kinases 2 (SnRK2s). When abscisic acid (ABA) is present, it binds to the PYR/PYL/RCAR receptors,whichtheninteractwithandinhibittheactivityofPP2Cs.Thisinhibitionallowsfor theactivationofSnRK2kinases—particularlySRK2D/SnRK2.2,SRK2E/OST1/SnRK2.6,and

SRK2I/SnRK2.3—triggering downstream stress responses (Mustilli et al., 2002; Danquah et al., 2014; Mehrotra et al., 2014).

Insalinesoils,thegerminationofmanycropsisoftenunsuccessfulduetothepresenceofhigh salt levels where seeds are planted. In arid and hot regions, intense evaporation leads to moisture loss, which causes salt to accumulate near plant roots. This salt buildup restricts the plant's ability to absorb water effectively (Bernstein and Hayward, 1958).

Quick seed germination and strong initial plant growth are essential for achieving good crop yields, especially under stressful environmental conditions. As research has improved our knowledge of how seeds germinate, new methods have been created to influence these processes for agricultural benefits. Among these, "seed priming" has become one of the most widely adopted techniques (Bewley et al., 2013; Paparella et al., 2015).

Seed priming is a technique where seeds are partially hydrated under controlled conditions to activate early germination processes, but then dried before the radicle (young root) starts to emerge (Giri and Schillinger, 2003).

Recentresearchindicatesthatneurotransmittersplayimportantrolesinvariousplantfunctions. These include promoting root and shoot development, aiding in fruit ripening, aging (senescence), seed and pollen germination, embryo formation, protecting germ tissues, and regulating themovement ofions acrossmembranes (Arnao and Hernández-Ruiz,2018, 2019; Saxena, 2018).

Several studies have shown that various plants contain neurotransmitters such as GABA, acetylcholine,indoleamines,catecholamines,andothers(Bameletal.,2016;Momonoki,1997; Ramakrishna et al., 2009, 2011a, 2011b).

Similar to other environmental stresses, salt stress has a harmful impact on plant growth and reproduction. It disrupts nutrient and hormone balance, leads to ion toxicity, causes oxidative andosmoticstress,andincreasesvulnerabilitytodiseases.Salt stresscanharmplantsinthree mainways.First,excessivesaltinthesoilchangesitsstructureandreducesitsabilitytoretain andtransportwater,resultinginlowerwateravailability.Thiscreateswaterstress,mimicking drought conditions, even if water is present. Second, the presence of toxic ions, especially sodium (Na^+), can damage cell membranes and lead to the breakdown of proteins.

Saltstressisresponsibleforaround70%ofcropyieldlosses.Highsoilsalinitydisruptsvarious physiological,structural,biochemical,andmolecularfunctionsinplants,negativelyimpacting their natural growth and overall survival (Bose et al., 2018).

Restoringsalt-affectedlandforagriculturalusehasbecomeakeyobjectiveforimprovingcrop production globally. High levels of NaCl in saline soils lead to increased sodium uptake and thelossofessentialnutrientslikepotassiumandcalciumfromplantcells.Thisdisruptscellular balance, causes nutrient shortages, increases oxidative damage, slows down growth, and can ultimately lead to cell death (Mukhopadhyay et al., 2021)

Plants are sensitive to salt stress because it disrupts the molecular processes that control their growth and development (Kransensky and Jonak, 2012)

Saltstressinplantsisusuallytestedinexperimentsbyaddingmoreandmoresalt(specifically sodiumchloride,orNaCl)tothesoilorgrowingmedium.Ifalargeamountofsaltisaddedall at once, it creates a sudden "salt shock" that causes the plant to lose water quickly due to osmotic pressure. Over time, the plant also starts to absorb more sodium (Na^+) and chloride (Cl^-) ions, which can be harmful. On the other hand, if salt levels increase slowly—like they mightinnature—theplantfaceslessimmediatewaterloss,andthebuildupofsodiumhappens moregradually.Interestingly,mostofthesalt-tolerancemechanismsdiscoveredandconfirmed in genetically modified plants have been based on these sudden or moderately intense salt shock treatments, not the slow,

natural kind (Shavrukov, 2013).

CHAPTER-3MATERIALSANDMETHODS

SEEDGATHERINGANDSOWING:

Trigonella foenum graecum seeds are attained from (HAU, Haryana Agriculture University) Hisar, Haryana. Seeds were HISAR Sonali variety, collected from Department of Vegetable Sciences HAU, Hisar. Seeds are packed and checked all essential parameters germination, moisture content, physical purity, genetic purity and others required for research purpose.

(Fig:1Seedsusedforsowing)

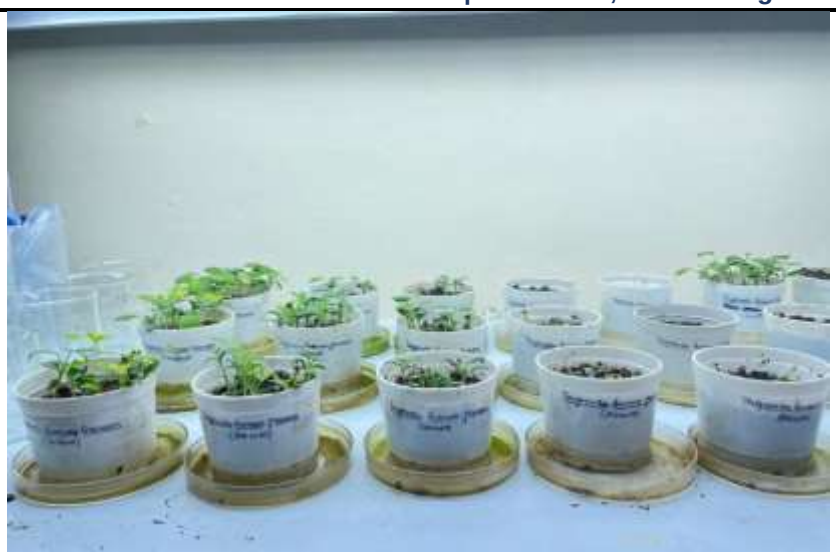
Seeds were sown in autoclaved cocopeat and vermicompost (equally mixed in a container) in sterilized plastic pots. All pots were filled with equal volume of cocopeat and vermicompost mixture ideal for growth.

GROWTHCONDITIONANDENVIRONMENT:

The experiment was conducted in Botany Department Laboratory at Kurukshetra University, Kurukshetra. A proper light condition was given to all the treatments. three replicates of each concentrationincludingcontroland50mMweresownwithpropernumberofseedsineachpot (20perpots).Seedlingswerewell-wateredthroughouttheexperimentwithtapwaterincontrol replicates and 50mM with respective NaCl concentration from stock solution. Stock solution was prepared by adding (2.922g of NaCl in enough water to make it 1 litre) solution.

Seedlingswerealwayskeptatreyunderthemtoavoidmixingofdifferentwaterconcentration.

Propermonitoringisensuredthroughouttheexperimentationtilltheseedlingsattainedproper growth suitable for experimentation.



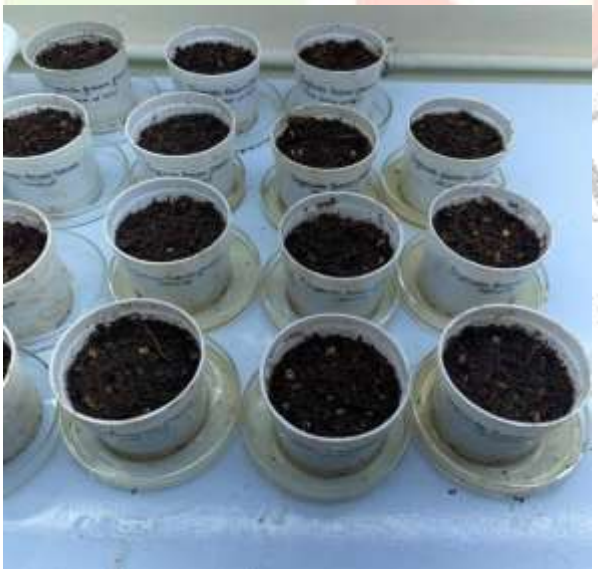
(Fig:2Treatmentsundercontrolled conditions)

GROWTHPARAMETERS:

PercentageOFGermination

The percentage of germination indicates number of seed germinates divided by total number of seed sown multiplied by 100. It indicates the number of seed which produce normal seedlings within specific period of time under control and treated conditions.

$$\text{Percentageofseedgermination} = \frac{\text{Noofseedsgerminated}}{\text{Total noofseedssown}} \times 100$$



(Fig:3Seedsownincocopeatandvermicompost1:1)

Seedling height

Seedling height was measured using a scale from the soil surface to the apex of the plant i.e. main shoot length and expressed in cm (Three replicates were taken).

(Fig:4,SeedlingheightunderControl)

(Fig:5,Seedlingheightunder50mM)

Root length

Ameasuringscalewasusedtodeterminetherootlengthafterplacingthemstraightandcleaned without soil debris. (Three replicates were taken)

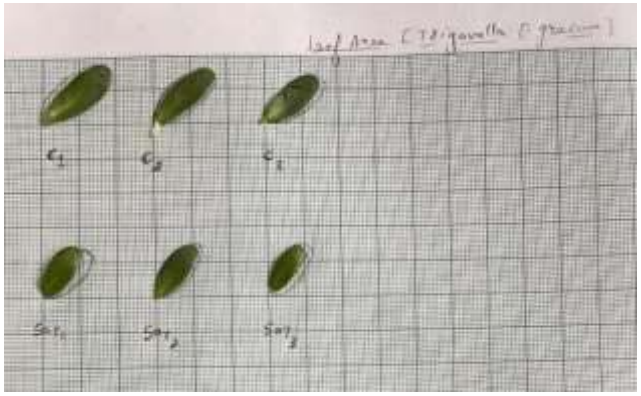
Fresh,dryandturgid weightof seedlings

Seedlingsundereachtreatmentwerecarefullytakenandafterwashingawaythesandstuckto roots with ample amounts of tap water. Plants were cleaned with filter paper to eliminate moisturefromtheirsurfaces.Afterthattheirweightwasmeasuredusingelectronicbalance.In a 60° C oven, all the plants with replicates of three, each control and 50mM were dried until theyattainedaconsistentweightinlabelledpaperenvelopes.Theirdriedweightwascalculated using electronic balance. Similarly turgid weight was calculated after placing the seedlings in water overnight about (8-10hrs), initial and final weight was calculated using electronic balance.

(Fig:6Plantletsusedforweighingfresh,dryandturgidweight)

Leafareaofseedlings

Leafareaisdefinedasthetotalsurfaceareaofleafavailableforphotosynthesis.Itismeasured in $cm^2$with the help of graph paper.



(Fig:7Graphshowingleafareameasuredaftermarkingonbox) Water relation

Relativewatercontent(RWC)

TocompareRWC,Weatherly(1950)formulawasfollowed

$$RWC(\%) = \frac{\text{Freshweight} - \text{Dry weight}}{\text{Turgidweight} - \text{Dryweight}} \times 100$$

ANATOMICALCHARACTERS:

Seedlings were taken carefully out of the cocopeat, washed with tap water in petri dishes to removeadherentsandparticles.Nowshootsaretakenandsectionsarecutwithsterileandfresh blade. Precise sections were taken and seen under the microscope at 4x, 10x and 40x magnification.

BIOCHEMICALESTIMATION:

Chlorophyll estimation

Chlorophyll content of leaves was estimated by the method given by (Arnon 1949). 100mg leaves sample was weighed and washed with distilled water for 2 times. Crush 0.2g of leaves in a pestle-mortar with the help of 10ml 80% acetone along with a pinch of caco3was also added to avoid the photooxidation or destruction of chlorophyll and other pigments. Take the extract after grinding, it was centrifuged at 5000rpm for 20min at room temperature. supernatantwastakenandvolumeraisedto10mlwith80%acetoneandresiduewasdiscarded. Absorbance was taken at 645nm (chlorophyll a), 663nm (chlorophyll b), 510nm and 480nm(carotenoid) in spectrophotometer, against 80% acetone as blank. Chlorophyll content was estimated using the following formula:

Totalchlorophyll=chlorophylla+chlorophyll b

$$\text{Chlorophylla(mg/gfreshweight)} = \frac{12.3(A663) - 0.86(A649) \times V}{a \times 1000 \times W}$$

$$\text{Chlorophyllb(mg/gfreshweight)} = \frac{19.3(A645) - 3.6(A663) \times V}{\alpha \times 1000 \times W}$$

$$\text{Carotenoids} = \frac{7.6(24) - 1.49(24) \times V}{\alpha \times 1000 \times W}$$

Where,

α=light path (cm)

W=freshweightofsample(mg) V= volume of extract =10ml.

Catalaseestimation(CAT)

TheAebi(1984) methodwasusedtodeterminethecatalaseactivity.

Reagentpreparation:50mMH_2O_2:take1.42mlofstocksolution(6%w/v)andmakethevolume of 50ml using distilled water.

Preparation of enzyme extract: take 0.5g of fresh leaves sample in chilled condition in pestle and mortar, grind it with 10ml phosphate buffer to make a fine paste and then centrifuge at 3500 rpm for 10 minutes. Then the supernatant was used as enzyme extract.

Procedure:

- Take1.5mlofphosphatebuffersolutionandadd1.2mlofhydrogenperoxide,thenadd 300µl of enzyme extract in cuvette.
- Thentakeabsorbanceat240nmin spectrophotometer.
- Notedownthereadingsdisplayed

Formula used:

Unitactivity($Umin^{-1}g^{-1}FW$) =changeinabsorbance×totalvolume(ml)

Where, extensioncoefficient=$6.93\times10^{-3}M^{-1}$ $cm^{-1.}$

Experimentation:

The experiments are conducted in Department of Botany, Kurukshetra University, Kurukshetra, Haryana, India.

Equipmentused:

Weighing balance, test tubes, centrifugation tubes, measuring cylinders, beakers, test tube strand,aluminiumfoil,centrifugationmachine,vortexmixture,spectrophotometer,pestleand mortar,icebags,refrigerator,petridishes,micropipette,dryheatovenandothermiscellaneous.

Preparation of phosphate buffer: take a tablet of phosphate buffer, dissolve it in 1000ml of distilled water.

Chemical used: *$CaCO_3$, 80%Acetone(take 80ml of100%acetoneand add 20ml ofdistilled water).

CHAPTER-4RESULTandDISCUSSION

GROWTHPARAMETERS

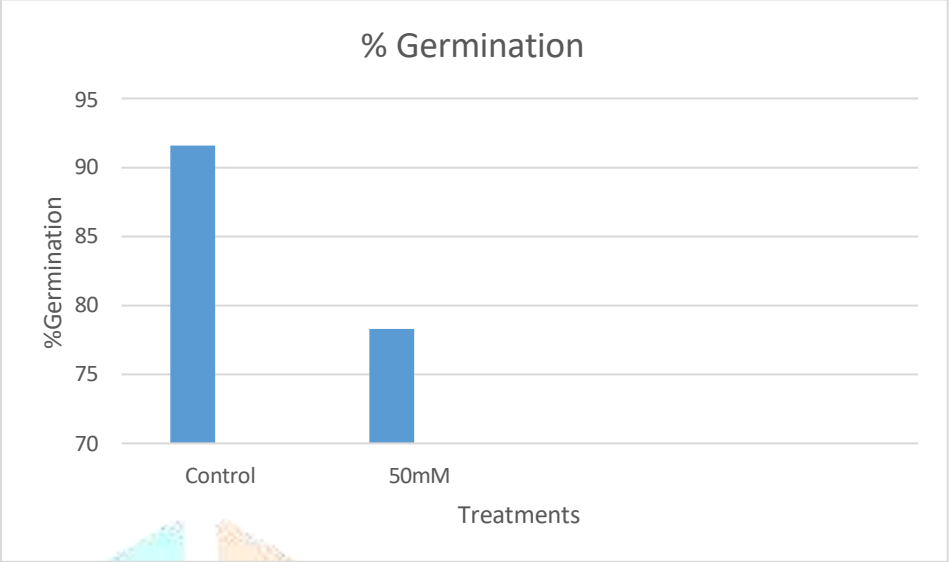
Germinationpercentageof*Trigonellafoenum graecum*:

Treatments	T1	T2	T3	Average(%)	S. D
Control	17	19	19	91.66	1.154701
50mM	15	16	16	78.33	0.57735
Seedsown	20	20	20	100	0

Tableno1:showinggerminationpercentageatlowerconcentrationof(NaCl).

Dueto presenceof salt(NaCl) in thewater germinationpercentageofthe*Trigonellafoenum.*

decreasedbecauseseeds getexhibitingosmoticimbalance duetosalinitystress initiation.

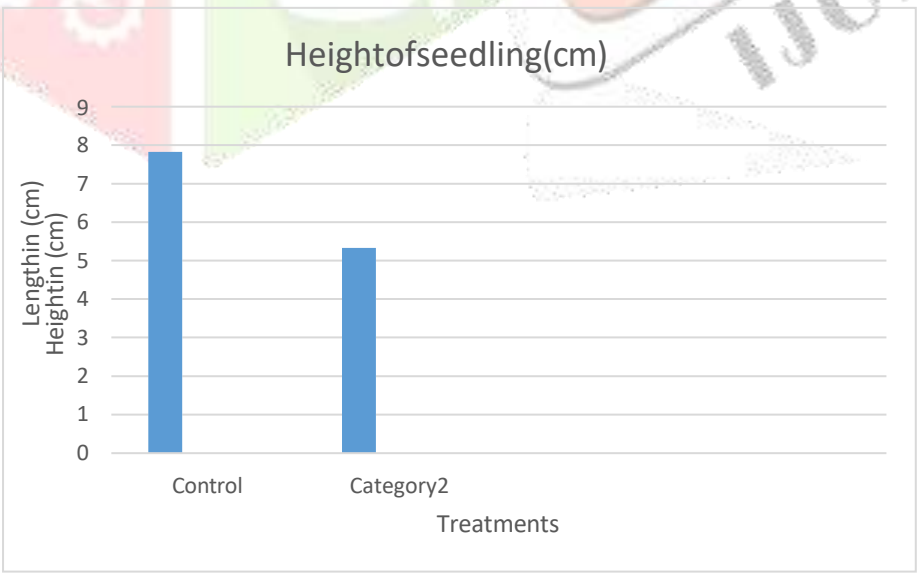


Heightofseedling:

Treatments	T1	T2	T3	Average	S. D
Control	8cm	7.9cm	7.6cm	7.83cm	0.208167
50mM	5.3cm	5.5cm	5.2cm	5.33cm	0.152753

Tableno2: showingaverageheightofgerminated shootin*Trigonella foenum*

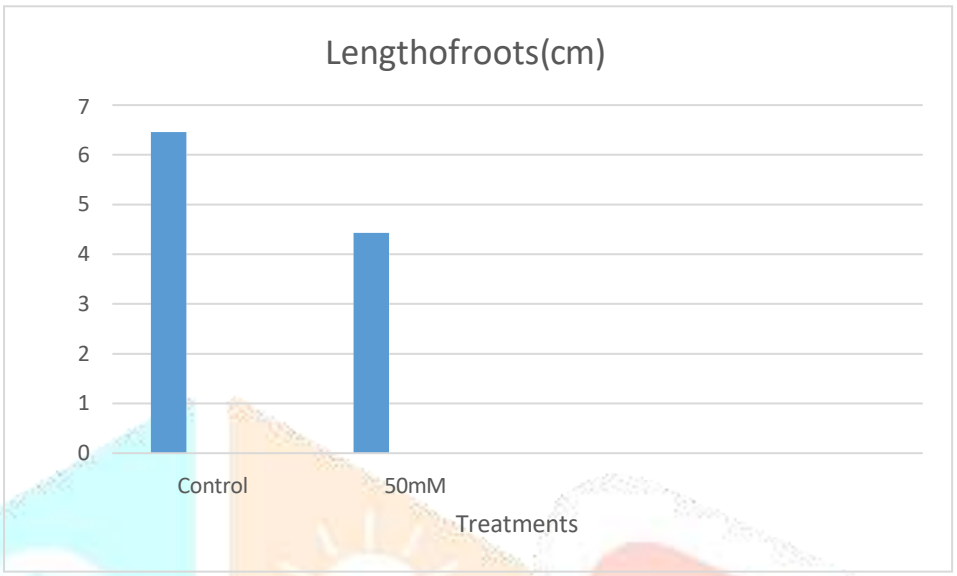
Decreaseinthelengthofshootincaseof50mMconcentrationof(NaCl)isobservedwhenall otherparametersaremaintainedsameinboththecases,thismaybecausedduetothedecreased enzymatic activity and metabolic activity of the plant due to accumulation of salt.



Lengthofroots:

Treatments	T1	T2	T3	Average	S. D
Control	6.3cm	6.5cm	6.6cm	6.46cm	0.152753
50Mm	4.5cm	4.4cm	4.4cm	4.43cm	0.057735

Tableno:3showingaveragelengthofrootsin *Trigonellafoenum*



(Fig:8.1Treatmentreplicas)

(Fig:8.2Treatmentreplicas)

Figure8.1(Treatment1)andfigure8.2(Treatment2)ShowingdifferentconcentrationofNaCl and plantlet growth

Fresh,dryand turgidweightof seedlings:

S. No.	Control	S. D	50mM	S. D
1	0.379g		0.299g	
2	0.349g	0.015044	0.233g	0.035157
3	0.366g		0.245g	
Average weight(g)	0.364g		0.259g	

Tableno:4Freshweight ofseedlings (grams).

S. No.	Control	S. D	50mM	S. D
1	0.031g		0.019g	
2	0.033g	0.004163	0.016g	0.001528
3	0.039g		0.018g	

Average weight(g)	0.034g		0.017g	

Tableno:5Dryweightofseedlings (grams).

S. No.	Control	S. D	50mM	S. D
1	0.441g		0.365g	
2	0.420g	0.011846	0.394g	0.029
3	0.440g		0.336g	
Average weight(g)	0.433g		0.365g	

Table no:6 Turgid weight of seedlings (grams). Seedlings were kept in the water for about 8- 10 hrs.

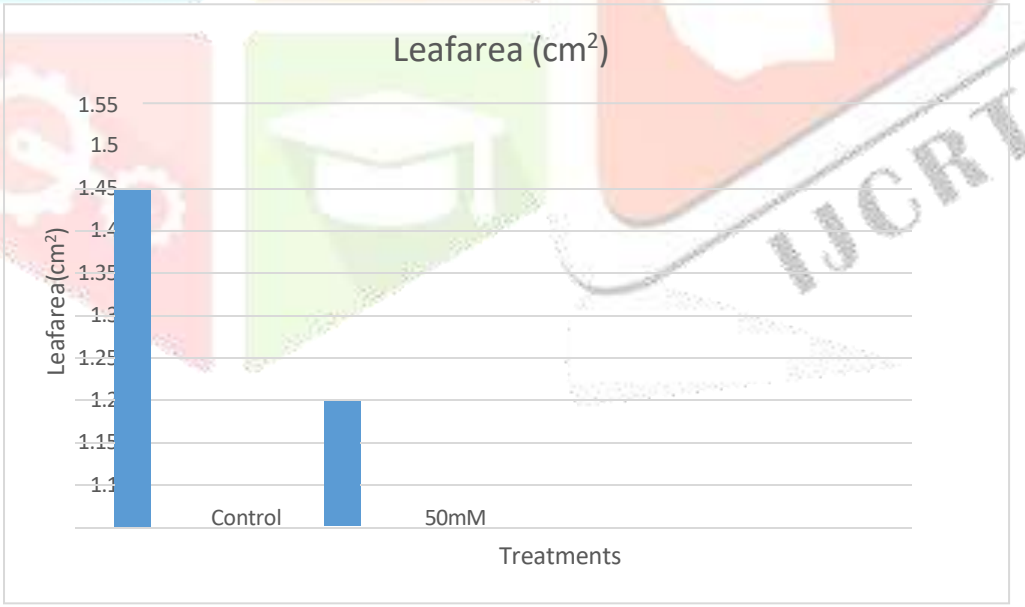
Decreaseinthefresh,dry andturgidweight oftheplantlet

Leafareaofseedlings:

S.No.	Control	50mMconc.
1	1.5cm^2	1.25cm^2

Tableno:7Averageleafareaofseedlingscalculatedusinggraphpaper.

Reduction in leaf area of seedlings growing in 50mMconcentration due to ion toxicity andosmotic stress which impacts cell expansion and leaf development.



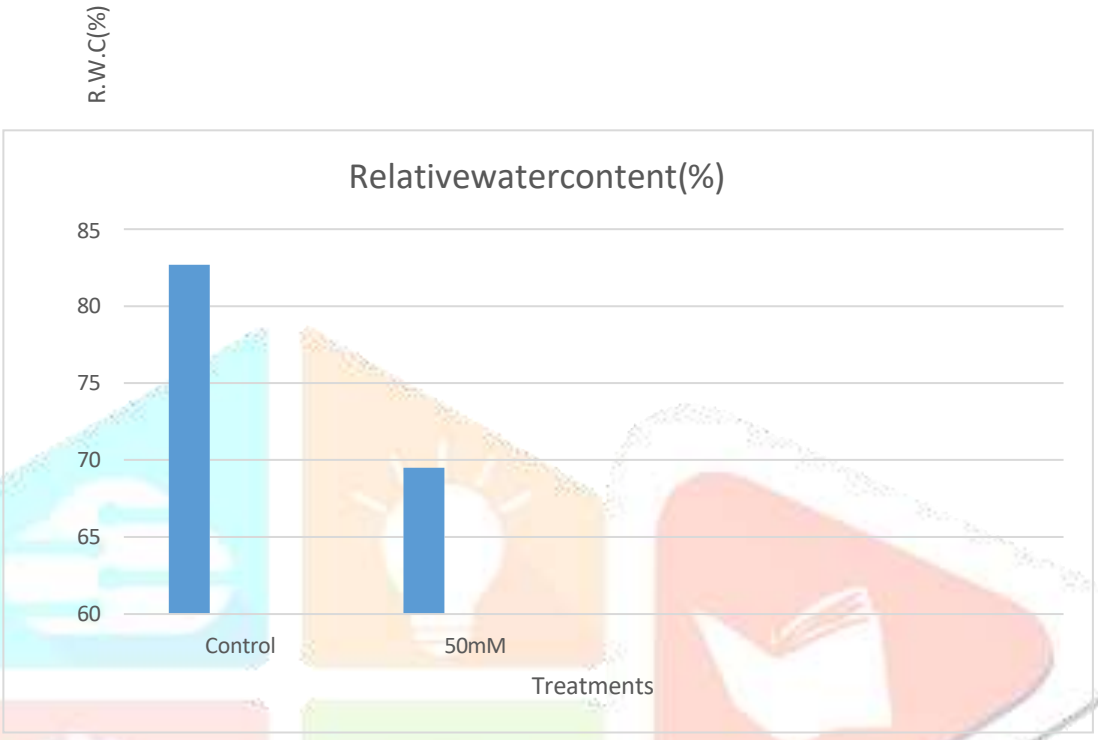
Relativewater content:

UsingformulagivenbyWeatherly(1950) $RWC\% = \frac{Freshweight - Dry\ weight}{Turgidweight - Dryweight} \times 100$

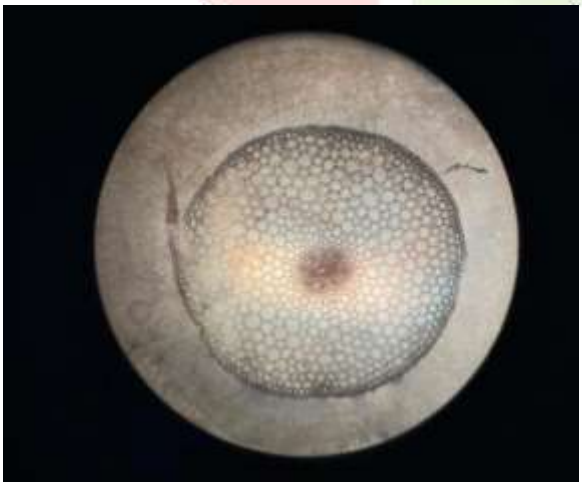
S.No.	Treatments	RWC%
1	Control	82.7%
2	50mM	69.5%

Tableno:8Relativewatercontent ofseedlings

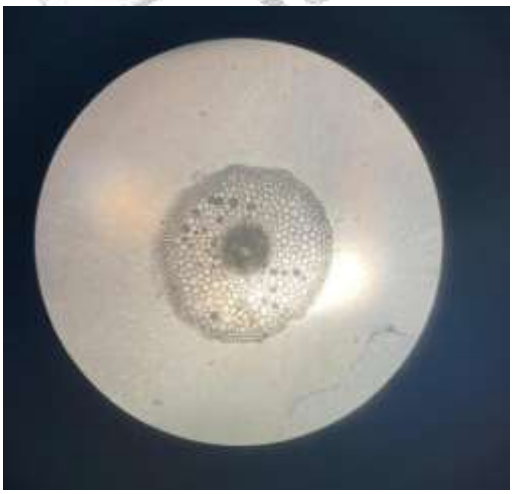
Under50mMconcentrationislowerduetoeithercelldehydration(intercellularwaterdeficit).



ANATOMICALCHARACTERS SHOOT ANATOMY



(Fig:9controlledshootsection)



(Fig:10,50mMconc.Shootsection)

Under control condition shoot exhibits well-defined hexagonal mesophyll cells with tightly packed arrangement and uniform vascular bundles. Whereas noticeable disorganization, cells appear shrunk and more loosely arranged, indicating tissue stress.

Salt stress often causes protoplasmic shrinkage and expansion of apoplastic (cell wall-

membrane)space.Asaresult,incaseof50mMNaClsectionlikelyappearsmaller,more irregular, with expanded intercellular gaps.

Also under controlled condition, vascular tissues (xylem/phloem) are well organized and compact. Salt stress may lead to dehydration-induced narrowing or deformation of xylem vessels and disorganized phloem cells.

Cell wall may appear slightly thicker or more irregular, possibly reflecting protective strengthening against ionic/osmotic stress.

BIOCHEMICALESTIMATION

Chlorophyll estimation

Control	Wavelength(nm)				
Treatments	Chlorophyll	480nm	510nm	645nm	663nm
	C-1	0.475	0.100	0.260	0.663
	C-2	0.492	0.137	0.283	0.668
	C-3	0.419	0.108	0.246	0.648
	Averagevalue	0.462	0.115	0.263	0.659

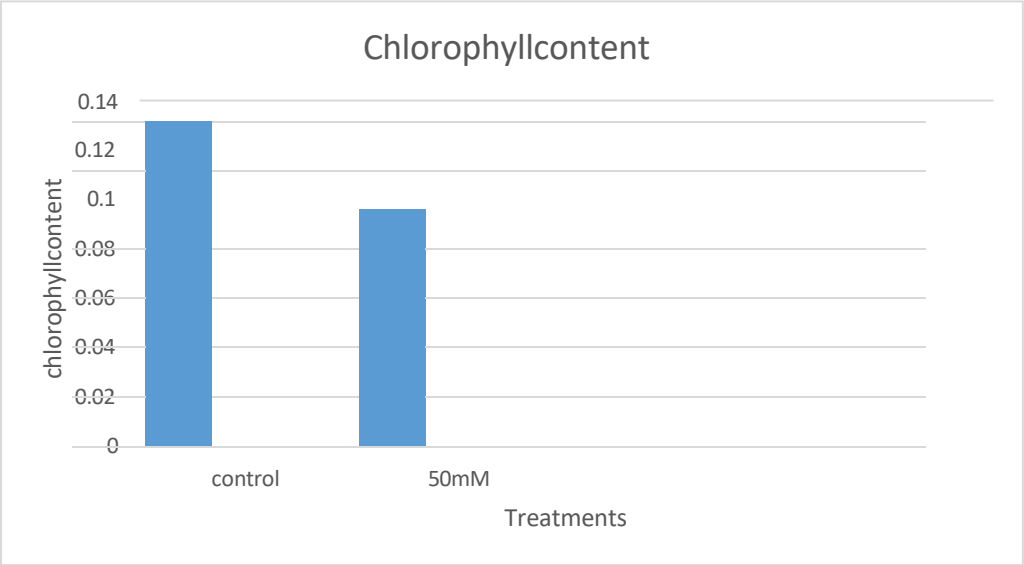
Tableno:9absorbanceatdifferentwavelength

50mM	Wavelength(nm)				
Treatments	Chlorophyll	480nm	510nm	645nm	663nm
	T-1	0.302	0.099	0.193	0.422
	T-2	0.299	0.100	0.240	0.523
	T-3	0.311	0.097	0.209	0.539
	Averagevalue	0.304	0.098	0.202	0.494

Tableno:10absorbance atdifferentwavelength

mg/g(fresh weight)	Control	Chl (a+b)	50mM	Chl (a+b)
Chlorophyll- A	0.029	0.122	0.021	0.090
Chlorophyll- B	0.093		0.069	
Carotenoids	7.332		7.332	

Tableno:11Calculatedresultsofchlorophyll contentfromtable9and 10



Graphrepresentingtotalchlorophyllcontentinboththetreatmentsi.e.controland50mM

Procedureused:

Arnonmethodwasusedto estimatechlorophyll content ofseedlingsgivenin (1949)

Chemicalsused:$CaCO_3$,80%acetone(take80ml100%acetoneandadd20mldistilledwater)

Procedure:Firstly,collectthefreshleavesofsamplethenwashthemwithtapwateranddried it into fold of filter paper.

◦Weigh0.2goffreshleavesof sample

◦ Take10mlof80%acetoneandgrindthesampleinprecooledpestleandmortarindark condition

◦AddapinchofCaCO3toprevent photooxidationofchlorophyll content

◦Thenrisethevolumeto10mlusing80%acetoneifrequiredandcentrifugethesampleat3500 rpm for 10 minutes at room temperature

◦Then analyse the content in spectrophotometer at different wavelengths as mentioned in the above table no 9 and 10. Blank used as acetone

◦ThechlorophyllcontentwasthenestimatedusingArnon's equation

TotalcarotenoidalsocalculatedsimilarlyusingMaclachlanandZalik(1963).Justabsorbance was taken at different wavelengths i.e. 480nm and 510nm against acetone as blank.

Catalaseestimation

S. No	Control	Absorbanceat 240nmafter aminute interval				Average	Enzyme activity
1	C-1	0.136	0.132	0.120	0.111	0.025	31.75Umin^{-1}g^{-1}F.W
2	C-2	0.171	0.159	0.153	0.145	0.026	
3	C-3	0.170	0.166	0.160	0.155	0.015	

Tableno:12Absorbanceforcatalaseat240nmforcontrolsampleReplicasandcalculated activity

S. No	50mM	Absorbanceat240nmafter aminute interval				Average	Enzyme activity
1	T-1	0.173	0.154	0.143	0.137	0.036	49.06 Umin-1g-1 F.W
2	T-2	0.227	0.208	0.202	0.191	0.036	
3	T-3	0.213	0.201	0.192	0.183	0.030	

Tableno:13Absorbanceforcatalaseat240nmfor50mMconc.Replicasandcalculated activity

Procedureused:

TheAebi(1984)methodwasusedtodeterminethecatalaseactivity. Reagent preparation:

50mMH2O2-Take1.42mlofstocksolution(6%w/v)andmakethevolumeof50mlofdistilled water

10mMAscorbate-Take0.08gofascorbatewhichisdissolvedin50mlofdistilledwater Preparation of enzyme extract:

Take0.5goffresh leavessamplein chilledpestleand mortar

Then grind them with 10ml of phosphate buffer to make a fine paste and then centrifuge the same at 3500 rpm for 10 minutes.

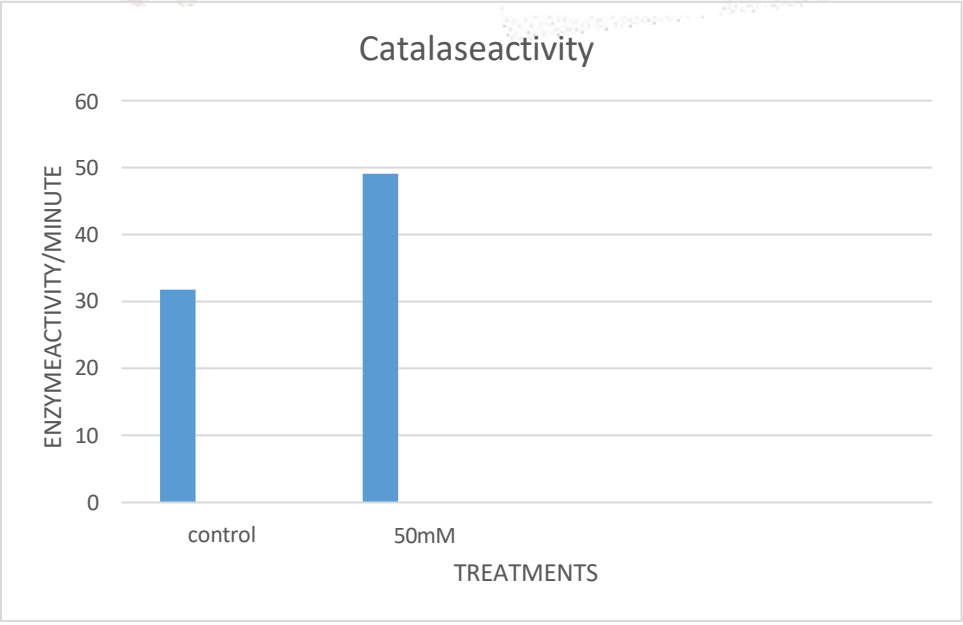
Supernatantwasusedasenzymeextract Procedure:

- Take1.5mlofphosphatebuffersolutionandadd1.2mlofhydrogenperoxide,thenadd 300µl of enzyme extract in cuvette.
- Takeabsorbanceat 240nm in spectrophotometer
- Notedown thereadings.

Formulaused:Unitactivity(Umin^{-1}g^{-1}FW)$=\frac{\text{changeinabsorbance/minute}\times\text{totalvolume(ml)}}{extensioncoefficient\times volumeofsample(ml)}$

Where,Ext.coefficient=6.93×10^{-3}M^{-1}cm^{-1} Total volume =3ml

Volumeofsampleused=0.3ml



Graphrepresentingtheactivityofenzymecatalaseinboththetreatmentsi.e.controland50mM

Chapter-5CONCLUSION

Under moderate salinity levels (50 mM NaCl), (*Trigonella foenum-graecum L.)* (fenugreek) undergoes distinct physiological and biochemical changes when compared to plants grown under non-saline (control) conditions. These changes include alterations in germination rates, noticeable reductions in root and shoot length, and decreased fresh and dry biomass, all contributing to diminished overall plant vigour. These growth reductions are primarily due to osmotic stress and ion toxicity, which impair water absorption, disrupt nutrient uptake, and trigger oxidative stress through the excessive production of reactive oxygen species (ROS).

At the physiological level, salinity stress leads to a decline in chlorophyll content and photosyntheticactivity,thoughitalsostimulatesanincreaseinantioxidantenzymeactivityas a stress response. In contrast, fenugreek plants grown under control conditions (0 mM NaCl) exhibitoptimaldevelopment,characterizedbygreaterbiomassaccumulation,strongerrootand shoot systems, and efficient photosynthesis—thanks to the absence of salt-induced stressors that would otherwise interfere with key metabolic and physiological processes.

Althoughfenugreekshowsacertainlevelofadaptationtomoderatesaltstressthroughvarious compensatory mechanisms, these defences are not completely effective in negating the negativeimpactonplantgrowthandproductivity.Asaresult,strategiessuchastheexogenous application of osmoprotectants, or thedevelopment of salt-tolerant cultivars throughselective breeding, could play a vital role in enhancing the crop's resilience in saline environments.

Salinity remains a major abiotic factor limiting fenugreek productivity, affecting this nutritionallyimportantlegumeatmultiplebiologicallevels.Thecurrentstudyemphasizeshow fenugreek responds to salt stress by initiating a range of physiological and biochemical responses.

ImplicationsandFutureDirections:

This investigation reveals extensive morphological, physiological, and biochemical shifts in fenugreekundersaltstress.Tofurtherimprovecropperformanceundersuchconditions,future studies should focus on the following areas:

- Uncoveringthemolecularpathwaysinvolvedinsalinity tolerance
- Establishingbreedingprograms aimedatdevelopingsalt-resistantvarieties
- Evaluatingagronomictechniquesthatalleviatesaltstresseffectsinfieldconditions

Understanding how different genotypes react to salinity will be crucial for improving growth and productivity in salt-affected regions. The *Hisar Sonali* variety used in this study also exhibited similarstress-induced modifications in both morphological and physiological traits, affirming the broader impact of salinity on fenugreek cultivars.

Fenugreek(*Trigonellafoenum-graecum*)demonstratesarangeofdefensiveresponsestosaline environments, including hormonal modulation, accumulation of osmoprotectants, and activationofantioxidantsystems**.**However,these naturalmechanismsalonearenotsufficient to counteract the effects of high salt concentrations. To enhance resilience under such stress, targeted agronomic practices—such as salicylic acid (SA) priming, the application of plant growth-promotingrhizobacteria(PGPR),andtheuseoforganicfertilizers—haveshowntobe effective.Theseinterventionsnotonlysupportstressmitigationbutalsoimproveplantgrowth and maintain seed quality, especially in arid and semi-arid regions where salinity is a major concern.Acomprehensivestrategythatincorporatesgeneticselection,biochemicaltreatments, andsoil/microbialmanagementoffersasustainablepathwayforcultivatingfenugreekonsalt- affected soils.

Thisstudyintegratesinsightsfromplantphysiology,agronomy,andbiotechnologytopropose

aholisticframeworkforimprovingfenugreek'ssalttolerance**.**Suchanapproachenhancesthe crop's potential as a nutritional and economic resource in saline-prone farming systems, contributing to both food security and environmental sustainability.

While *Trigonella foenum-graecum* inherently exhibits some ability to adapt to salt stress through physiological and biochemical shifts, elevated salinity levels still result in significant negative effects. These include stunted growth, impaired photosynthetic function, and disruption of cellular equilibrium. The contrast between stressed and non-stressed plants clearly indicates that salinity is a critical abiotic constraint limiting fenugreek production in affected regions. Gaining a deeper understanding of these responses is vital for formulating strategies that improve the crop's salt tolerance and ensure reliable yields under stress.

In response to sodium chloride (NaCl) stress, fenugreek plants engage in osmoregulation by accumulating compatible solutes such as proline and soluble sugars. These compounds help sustain cell turgor and stabilize internal structures. Nevertheless, these adaptations are often inadequate under severe salt conditions, leading to reduced plant vigour and yield.

Overall,saltstresssignificantlydisruptsthegrowth,physiologicalperformance,andmetabolic balance of fenugreek compared to unstressed controls**.** Although the plant activates several defence mechanisms, the overall impact of salinity is deleterious, underscoring the species' sensitivity to saline environments and reinforcing the need for integrated stress mitigation strategies.

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