Biovistas

International Journal of Biological Research

ISSN: 2321-0524
Volume: 8 Issue: 1

Analysis Of Salt Stress Induced Morphological, Phytochemical Changes Of Some Fabaceae Members And Their Quantification By Spectrophotometric Method

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Abstract

All plants need their own specific ambient environment for their normal growth, and development. The growth and yield of any plant primarily depends on the type of soil, aeration, nutrition, water-logging capacity, salinity, microbial flora, soil acidity, temperature, seed viability, pathogens etc. When any one of these is altered or not up to the desired need plants acquire a disturbance called stress within them and their normal physiology and metabolism is affected. Salinity plays a vital role as cell signalling, stomatal opening, cellular transportation, translocation, ascent of sap which are all dependent on osmotic concentration and these activities are controlled by ion exchange mechanism. A minute difference in these concentrations can lead to sharp adverse effects on plants due to exosmosis and endosmosis. Due to change in the salt concentration of the soil the plant experiences "Salt stress", because of this a sudden shock is experienced within the plants, as a result many heat shock proteins and other molecular chaperons are produced and they come into action to safeguard the plant from death by preventing the degradation of all essential enzymes. Apart from these secondary metabolites such as phenols are also produced during stress period. In certain cases, these salts might also play a beneficiary role depending on the type and quantity of salt in soil. Due to this stress the normal metabolism is also affected such as photosynthesis, respiration etc. Hence quantitative study on Carbohydrates, which are the product of normal metabolism, Proteins which are produced in high concentration initially and later degraded/denatured if stress continues and Phenolics which is a major secondary metabolite produced during stress can reveal the effect and the extent of stress on plants. Hence the main objective of our work is to see how stress can affect normal metabolism in plants and to quantify the phytochemicals produced by them at different stages of stress period.

Keywords: Salt stress, Phytochemicals, Fabaceae members, Spectrophotometric method.

Introduction

Biological stress may be defined as any change in environmental conditions that may have a negative impact on the normal growth and development of plants**.** Stress could be of two types:

Biotic (imposed by other organisms)

Abiotic (arising from excess or deficit in the physical or chemical environment)

Biotic Stress: Plants exhibit stress due to various living organisms such as herbivores, insects,fungal, viral and bacterial pathogens. Although plants do not have an immune system as compared to animals, but the plants have developed intricate defence strategies including production of secondary metabolites to ward off insects and disease.Hypersensitive response and secondary acquired resistance are only small part of diverse mechanisms adopted by plants to resists infection. Plants respond to the attack of insects or microorganisms by

i) Formation of pathogenic-related proteins: These are products of defense related genes that are activated by microbial infection and include hydrolytic enzymes such as proteinase which inhibit activities of proteolytic enzymes secreted by pathogen, lytic enzymes such as glucanase etc.

ii) Biosynthesis of phytoalexins: The microbial infection activates genes that encode enzymes for the synthesis of phytoalexins. Phytoalexins are a chemical diverse group of secondary metabolites (chiefly isoflavonoid and sesquiterpenes) with strong antimicrobial activity.

iii) Change in composition and physical properties of host cell wall: Lignin,suberin, callose and some hydroxyl-

Received : 27.10.2020
Revised : 07.11.2020
Accepted : 10.11.2020
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proline rich glycoproteins are synthesized and accumulated in host cell walls as the response of pathogen invasion. Latter it strengthensby blocking the spread of the invading pathogen.

iv) Programmed Cell Death (PCD): As the hypersensitive response culminates apoptosis around the infection site which deprives nutrient supply to the pathogen and limits its spread in host plant and necrotic lesions are formed to prevent spread of disease.

v) Secondary acquired resistance: Host plants develops resistance against pathogen from few hours to several days as onset of initial infection occurs and this phenomena is stated as "a single encounter with the pathogen increases resistance to entire plant to future attacks by pathogens" is called secondary acquired resistance(SAR)[1].

Abiotic stress: Environmental condition that cause damage are water logging, drought, high or low temperatures, excessive soil salinity, inadequate mineral nutrients in the soil, extreme high or low light etc. Resistance or sensitivity to the given stress depends on the species, genotype, and the developmental age of the plant. Stresses trigger a wide range of plants responses by altered cellular metabolism and gene expression which leads to changes in growth rate and crop yields. The duration, severity and rate at which a stress is imposed influences how plant responds to that particular stress. One type of stress could trigger other type of stress to the plants. Biological species show a range of tolerance to environmental factors. The plants have capacity adaptations enabling them to withstand which do not permit normal growth and development in an un-adapted plant whereas, plants with resistant adaptation enable them to withstand environmental stresses which would otherwise result in the death of un-adapted plants. The plants flourish best in the optimum range. The imbalance of abiotic factors in the environment causes primary and secondary effects in plants. Primary effects such as reduced water potential, cellular degradation which directly alter the physical and biochemical properties of cell eventually lead to secondary effects. Secondary effects are such as reduced metabolic activity, ion toxicity and the production of reactive oxygen species which initiate and accelerate the disruption of cell integrity which may result in cell death[2].

Salt stress: The major Abiotic stress which limits crop production is salt stress and it is caused by excessive amount of salt in the soil. Salinity occurs naturally or manually by mankind induced process which results in the accumulation of dissolved salts to an extent in the soil water that inhibits plant growth. Soil becomes saline due to various conditions; heavy rains or irrigation followed by rapid evaporation leaving behind plenty of salt deposits in the agricultural fields, inland masses of saline water of lakes, inland deserts etc. Salt stress is always associated with water stress. It makes halophytes xerophytic in nature with thick cuticle, sunken stomata, thick bark etc. called as salt accumulators whereas, glycophytes are very sensitive to salt injury such plants suffer from physiological dryness, reduced rate of photosynthesis, altered metabolism,damage to membranes, toxicity and even may lead to death in extreme condition. In salt stress plants, the roots show synthesis of a special type of protein termed osmotin which helps plants to adapt to the environment during stress while halophytes have been found to synthesize the amino acid proline and other amino acids, galactosyl glycerol etc. Due to the presence of salt in the soil, it reduces the ability of the plant to take up water resulting in reduction of its growth rate called osmotic or water deficit. If excessive amount of salt entersinto the transpiration stream, there will be injury to cells in the transpiring leaves of the plant and thisfurther leads to reduction in growth, this is called salt specific or ion excess effect of salt. Osmotic stress reduces leaf growth, and to a lesser extentof root growth, whichreduces stomatal conductance and followed by photosynthesis. Senescence of old leaves, and transport of salt present in the plant into the young leaves over a long period, results into death by accumulation very high concentration of sodium and chloride. Roots protect plants by preventing them from excessive uptake of salts, and byfiltering out most salt in the soil while taking up water[1]. The other various environmental stresses are briefly discussed below:

Water stress: It is caused due to shortage of water in the soil or its unavailability due to lower water potential of saline water or due to excess water which causes floods. Drought induced water stress develops in the xerophytes; salt-induced water develops in the glycophytes.

Drought stress: Poor precipitation over a prolonged period of time or in a situation where any area receives annual rainfall less than average rainfall.

Temperature stress: High temperature and cold stress (chilling and freezing).

Cold stress: Chilling temperature depress the normal growth of the plant. Typically, tropical and sub-tropical species are susceptible to chilling injury ex. bean, tomato etc. When plants are exposed to 10 to 15-degree Celsius, chilling injury occurs. Freezing injury occurs at temperatures below the freezing point of water. Resistance to freezing temperature involves super cooling and slow dehydration. Deep super cooling is seen in species such as oak, plum etc[3]

High temperature stress: High temperature is dangerous for plants. Plants cannot withstand temperature beyond 45degree Celsius because the enzymes get denatured at 58degree Celsius. Plants bring down the temperature by the process of transpiration besides leaves of plants adopt several adaptations to minimize transpiration such as thick cuticle, wavy, hairy surfaces and sunken stomata etc.[2]

Radiation stress: When the intensity of visible light is very high quantity, it is injurious to the plants as at very high intensity, the atmospheric oxygen oxidizes the entire cell apparatus into Co2 known as solarization or photo oxidation. Infra-red light causes heat injury.[2].

Oxygen stress: It occurs in plants due to oxygen deficiency especially the underground stem and roots. This condition happens if the soil is either rich in clay with tiny pores or if the soil is water-logged.[2].

Acidity stress: Normal pH range suitable for normal growth of plants is between 3-9.If the pH decreases or increases beyond the range of tolerance the plant exhibit serious injury. It happens during heavy rains, leaching of calcium present in the soil makes soil acidic.[2].

Gas stress: Air pollutants are one of the most injurious stress causing strains on the plants. Toxic gases like So2, Ozone and PAN.[2,3].

Plant description: Four economically important plants from the Fabaceae family are selected for the salt stress induced study. Their brief descriptions are mentioned below.

***Cicerarietinum* (Chickpea):** It is a fast-growing plant, reaches upto 1m having tap root system. Stems are hairy, straight or bent. Leaves are sessile, ovate. Chickpea flowers are papilionaceous and solitary, with different colours. It has a pubescent podand is inflated with 2 or 3 seeds[4,5,6]. Cultivated chickpeas are divided into two main groups, the Desi and the Kabuli groups. Desi seeds are small, darker coloured and smooth or wrinkled and the Kabuli seeds are larger and cream-coloured[5].Chickpea is nitrogen fixing leguminous plant which restores soil fertility when grown before cereal or oilseed crops. It is used as disease cycle breaker and helps to reduce pesticide and herbicide usage[4]. The leaves yield an indigo-like dye and have many uses in traditional medicine. Various by- products of chickpea cultivation and processing are used for animal feeding [5,7].

***Lablab purpureus* (Indian bean):** *Lablab* is a leguminous plant. *Lablab* is an annual or short-lived perennial forage legume. It is a summer growing plant. It is a twining, climbing, trailing or upright herbaceous plant which can grow upto a height of 3-6m. It has a pubescent trailing stem with deep taproot system. It has alternate phyllotaxy and trifoliate leaves having rhomboidal shape. The upper surface of leaves is smooth while the underside has short hairs. It has raceme inflorescence. The colour of the flowers varies from white to blue or purple and each flower is about 1.5cm long, typically papilionaceous in shape. *Lablab* fruit is linear with smooth and beaked pods that contain between 2 and 8 seeds which are ovoid, laterally compressed with a conspicuous linear hilumof various colours, usually white to dark brown, and some are black [8,9,10,11]. *Lablab* is a fast-growing plant that can provide fodder in less than 3 months after sowing[12]. Its immature seeds and pods, young leaves are edible and cooked as vegetables. It can also be used as fodder[9,10].The association of *Lablab* with cereal forages such as maize and sorghum has been beneficial in several trials. *Lablab* makes excellent hay, if the leaf is adequately preserved [8,13].*Lablab* can produce good quality silage, alone or mixed with forage sorghum or millet[8,13]. *Lablab* is an N-fixing legume that can be incorporated into cereal cropping systems. They prevent soil infertility by N_2 fixation and break weed and diseases cycles. Fixed N_2 is then available for the next crop in the rotation[13]. The canopy of *Lablab* prevents soil dehydration from sun and wind while the lower leaves are shed and provide mulch to the soil [13].

***Trigonella foenum-graecum* (Fenugreek or Methi):** The English name Fenugreek (*Trigonella foenum-graecum* L.) is derived from French *fenugrec* and Latin *faenugraecum*, meaning "Greek hay" ("Online etymology dictionary", 2017). It is an annual herb belonging to the family Fabaceae, with leaves consisting of three small obovate to oblong leaflets. It has a tap root with bacterial associations forming root nodules. Its stems are erect, upto 50 cm high, sometimes branched. The flowers are papilionaceous, borne in leaf axils, white, lemon-yellow or purplish blue in colour [14]. The fruits occurs like pods of 2-10cm long, thin and pointed, and contain 10-20 seeds. The seeds are 6-8mm long, oblong or square, green-olive or brownish in colour, with a very strong and spicy odour[14,15]. Fenugreek has a long medicinal history of usage[16] with very few side effects[17]. Recent studies have shown that these plants have a promising result in prevention and treatment of a wide variety of diseases[18] like diabetes[19], hypertension [20], atherosclerosis[21], cardiovascular disease[22]. It is also known to increase breast milk supply in nursing mothers[23]. It reduces menstrual and menopausal problems, improves digestion. Fenugreek contains Carbohydrates such as Fructose, Sucrose, Xylose; Amino acids such as Cysteine, Proline, Tryptophan, Phenylalanine and Lysine; Minerals such as Calcium, Phosphorous, Potassium, Sodium, Copper; Secondary metabolites such as Glycosides, Flavonoids, Phytosterols, Phenolic Compounds, Alkaloids and some amount of fats, fibers and mucilage[24].Fenugreek is a medicinal plant used worldwide since ancient times. The spice was documented since 15th century. Many studies in last decade highlighted the biological activities and therapeutic properties of this species due to the presence of bioactive secondary metabolites and especially diosgenin, it's a steroidal saponin, has been investigated for its medicinal uses and found that the fenugreek employ as a raw material for steroidal hormones production[25].

***Vignaradiata* (Mung bean or Green gram):** The mung bean is a leguminous plant. The mung bean plant is an annual, erect or semi-erect, reaching a height of 0.15-1.25[26,27]. The plant is hairyand haswell-developedtap root system. Wild types are prostrate ,while cultivated ones are erect[29]. The leaves are elliptical or ovate in shape arranged alternate and trifoliolate manner. The flowers are papilionaceous, pale yellow or greenish in colour. The pods are long that contain 7 to 20 small seeds which are various in shape[27,28]. In India, two other types of mung beans are found, one with black seeds and other with brown seeds[27]. Mung bean is one of the major edible legume across Asia including India. It is mainly grown for its edible seeds and is cultivated across Asia. The

mung bean is used as a cover crop before or after cereal crops which acts as a green manure and they are N_2fixing legume that can provide large amounts of biomass and N_2 to the soil[29]. It is used to prepare various delicacies like soups, porridge, snacks, bread, noodles and ice cream[27].

Phytochemicals studied: Three major Phytochemicals or Bio chemicals are studied in salt stress induced plants. Their brief descriptions are given below:

Carbohydrates in plants: Carbohydrates are organic molecules made of Carbon, Hydrogen and Oxygen. Carbohydrates are made up of 2 basic compounds, aldehydes and ketones. Generally, carbohydrates are sugars or starches which are a major food source and a key form of energy for most organisms. They are also called as saccharides or carbs.
Carbohydrates are of various types such as monosaccharides, disaccharides and polysacharides.
Monosaccharides: The carbohydrate monomer is known as monosaccharides. Two monosaccharides of considerable importance in cells are ribose and deoxyribose.
Disaccharides:A disaccharide is formed when 2 monosaccharides become linked together in a glycosidic bond through the removal of the water from the hydroxyl groups of the monosaccharides. Sucrose, composed of glucose and fructose is the most widely distributed disaccharide in the plant world and one of the major products of photosynthesis.
Polysaccharides: The addition of many monosaccharides together with the elimination of water molecules, produces long chain of carbohydrates known as polysaccharides. Two of the most widely distributed polysaccharides in plants are starch and cellulose.
The carbohydrates are important to plants in many ways.Polysaccharides like starch serve as storage of energy in plants. The most abundant carbohydrate, cellulose serves as a structural component of the cell wall of plants and many forms of algae. The carbohydrates form the supporting tissues of the pant, enabling them to achieve erect growth. It also provides immediate energy to the plant cells. Some carbohydrates are in the form of starch which is stored in the seeds, fruits, roots etc., for the later use. (Merriam Webster medical dictionary)

Proteins in plants: Proteins are large macromolecules having one or more long chain of amino acid residues. Proteins are formed from amino acid monomers through the splitting of a water molecule from two adjacent amino acid. Proteins can be classified as simple proteins and conjugated proteins.
Simple proteins**:** They are those compounds that, on hydrolysis, yield only amino acids.
Conjugated proteins**:** conjugated proteins are associated with a non-amino acid component. This additional component is usually referred to as a prosthetic group.
Proteins are found in large quantities in storage organs, such as endosperm or cotyledons. Simple proteins, such as albulins, globulins, glutelins, and prolamins are examples of storage proteins. During germination the storage proteins are hydrolyzed by enzymes to small peptides and amino acids that nourish the embryonic axis in early growth and development. Some proteins appear to function primarily as structural units or protective structures. Other proteins such as glycoproteins and glycolipids are found at membrane surface, where they take part in membrane organization and function. The best know roles of proteins are as enzymes in catabolic and anabolic processes.All enzymes are proteins and are subject to the numerous reactions that proteins undergo. Their activity may be altered by changes in temperature, hydrogen ion concentration, heavy metals and so forth[31].

Phenolics in plants: Plants synthesize various types of organic molecules, which may be divided into two major groups, primary and secondary metabolites. Primary metabolites are metabolic intermediates or products found in all living systems which is essential for growth, biochemical pathways etc. Secondary metabolites are intermediateproducts which are produced by plants which are not essential to growth and life rather required for the interaction of plants with their environment. Plant secondary metabolites can be divided into three major classes:

i. Terpenes
ii. Phenolics
iii. Nitrogen containing compounds

Terpenes: Terpenes contributes a large class of natural products which are built from isoprene units. Terpenes have insecticidal property, used in pharmaceutical industries, used as perfumery raw material[32].
Nitrogen Containing Secondary metabolites: A large variety of plant secondary metabolites have nitrogen as part of their structure. Most nitrogenous secondary metabolites are synthesized from common amino acids. E.g., Alkaloids, Glucosinolates, Glycosides, Non-protein amino acids [34].
Phenolics: The most abundant secondary metabolites of plants characterized by presence of aromatic ring structure bearing one or more hydroxyl groups. Phenolics are involved mainly in defense and pathogens. Phenolics are widespread constituents of plant foods and also help in protecting against ultraviolet radiation. They also serve as defense compounds against herbivores pollinators and fruit dispersal. Some of the important classes of phenolic compounds are Phenylpropanoids, Coumarin, Lignins, Flavonoids and tannins. Some naturally occurring phenolic compounds are as follows: Bisphenol A, Cresol, Capsaicin, Gallic acid, Polyphenol, Ortho phenyl phenol etc. These compounds are very much essential for the growth of plants and are involved in the reproduction process of plants. Because of their antioxidant activities they are used in processed foods as a natural antioxidant. It plays an important role in plant development, mainly in lignin and pigment biosynthesis.

They also provide some structural integrity and scaffolding support to plants. Phenolics serve as important key for the plant in environmental interactions. It will act as protective agents, inhibitors, natural animal toxicants and pesticides against invading organisms, i.e. herbivores, nematodes, phytophagous insects and fungal and bacterial pathogens [33].

Materials And Methods

Collection of soil: Uniformly mixed (50Kg), well aerated soil with good amount of compost was collected from Lalbagh Botanical Garden nursery, Bengaluru. 500mL plastic pots were used for growing of the plants and small holes were made at the bottom for the drainage of excess water. 500 grams of soil was weighed and transferred toeach of them.

Seed collection and plant growth: Seeds of all the four plant varieties were collected from Lalbagh Nursery and soaked in water for 24 hours and kept moist with a cloth to allow the seeds to germinate. After 48 hours, the emergence of plumule was noticed inmost of the plants.The germinated seeds were sown in appropriatelylabeled pots. In each pot about 10 plants was allowed to grow in an equidistant manner.Every day the pots were exposed to sunlight for about 6-8 hours. The pots were watered with 20 to 30mL of tap water either daily or alternatively depending on the soil moisture content. The plants were allowed to grow for about 15-25 days until true leaves were seen. Seed germination picture is shown in Figure.1.

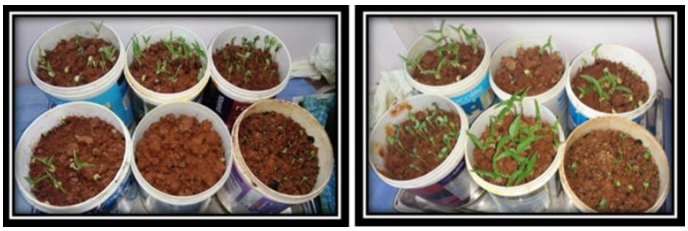

Figure.1: Seeds germinating

Stress treatment: Four sets of three replicates in each were made as follows:

i. SET-01: **Tap water** (Control)
ii. SET-02: **200mM NaCl** solution
iii. SET-03: **300mM NaCl** solution
iv. SET-04: **400mM NaCl** solution

Initially up to 25 days normal water was added to all the sets. Once the plants started to produce true leaves the stress treatment was initiated by adding 30mL of respective salt solution every day. 30mL of water was given to the control set.The plants were keenly observed every day and their morphological changes were recorded and photographed. Fresh plant samples were collected and brought to the laboratories for the biochemical analysis on the 7^{th} day, 14^{th} day and 21^{st} day (if they survived up to that stage)

Phytochemical analysis: Fresh plant samples were collected on 7^{th}, 14^{th} and 21^{st} day and the biochemical parameters, namelyCarbohydrates, Proteins andPhenolics were estimatedusing standard protocols. Standard graph was plotted using the absorbance and unknown values were calculated from the graph.

Estimation of total carbohydrates by phenol sulphuric acid method: 100mg of the sample was weighed into a boiling tubewith 5 ml of 2.5N HCLwas hydrolyzed by keeping it in a boiling water bath for 3 hours and cooled to room temperature.Then it was neutralized with solid sodium carbonate until the effervescence ceased.The volume was made upto 100ml, shaken well and centrifuged at 5200 rpm for 30 minutes. 1 mL of clear supernatant was taken. 1ml of phenol solution was added to each of the test tube.5ml of 96% sulphuric acid was added to each of the test tube and shaken well for 10 minutes and incubated at room temperature.After which the color was read at 490nm using spectrophotometer.The amount of the total carbohydrate present in the sample solution was calculated using the standard graph where glucose was taken as standard**.**

Estimation of proteins in given sample by lowry's method: 1g of fresh plant sample was grinded with 10mL of phosphate buffer and centrifuged to obtain a clear supernatant. 1ml of supernatant was taken into a test tubes and 1mL of 1N NaOH solution was added to all the test tubes and it was incubated at room temperature for about 30minutes. After incubation 10 mL of alkaline reagent was added and incubated for 15 minutes again.1mL of FC reagent (1:1 ratio with water) was added to all the test tubes and incubated for 5 minutes. Similarly, standards was done using BSA (Bovine Serum Albumin). The absorbance was measured at 660 nm using spectrophotometer and standard graph was plotted taking protein concentration (µg/ml) in X-axis versus Absorbance (at 660nm) in Y- axis.The amount of protein present in the given sample was estimated from the standard graph.

Determination of total phenolics by folin-ciocalteu method:1mL of plant extract was taken in a separate test tube to which 0.5 mL of FC reagent was added to all the test tubes.After an interval of 3 min, 2 ml of 20% Na_2Co_3 was added to all the test tubes.All the test tubes were incubated in warm water for a minute.The test tubes were allowed to cool at room temperature and absorbance was read at 650 nm using spectrophotometer.A standard graph of Phenolicswas plotted taking concentration of x- axis against absorbance at y- axis where catechol was taken as standard.

Results

Plant Sample.01:*Cicer arietinum* (Chick pea)

Morphological observations and discussion

Morphological response towards stress by the plant is shown in Figure.2. Morphologically all the plants showed uniform growth up to 7^{th} day of stress period. As the stress period prolonged drastic morphological changes were observed in the plants. On 14^{th} day, the 200mM and 300mM

plant sample were a bit reduced in size when compared with control plant sample while the 400mM plant sample was very much reduced in sized and it also began to show chlorosis of leaves. On 21st day the 200mM and 300mM plant sample was just half the size of control sample. The 400mM plant sample was very much affected and it showed a dwarf and slender appearance. Morphological response towards stress by the plant is shown in figure.2.

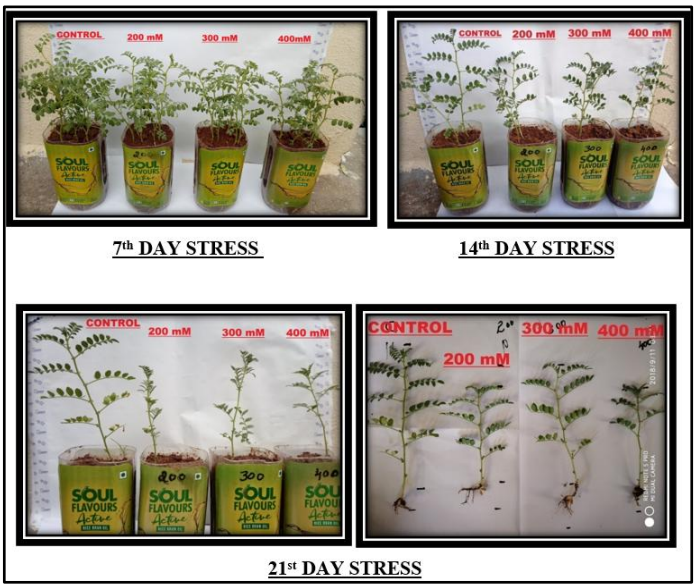

Figure.2: Response of *Cicer arietinum* (Chick pea) towards salt stress

Table.1: Tabular column showing amount of Carbohydrates, Proteins and Phenolics in µg/g of fresh wt. of *Cicer arietinum* (Chick pea)

		Control	200 mM	300 mM	400 mM
Amt. of Carbohydrate in µg/g of fresh wt. of sample	7th day	224	156	160	228
	14th day	184	148	148	188
	21st day	168	200	164	84
Amt. of Protein in µg/g of fresh wt. of sample	7th day	1800	2600	9400	1200
	14th day	2800	4100	3700	6800
	21st day	3000	2500	2800	4600
Amt. of Phenolics in µg/g of fresh wt. of sample	7th day	500	600	480	700
	14th day	1060	680	760	480
	21st day	760	660	400	760

Phytochemical observations and discussion

The phytochemicals produced in various stages of stress period is tabulated in Table..1 and represented graphically in figure.3. From the graph it is seen that there is no much change in the carbohydrate content of the plant samples on the 7th day, this may be due to continuous synthesis of glucose during photosynthesis and its break down during respiration. While on the 14th day as the stress increased, the carbohydrate decreased. This is due to the degradation of vital photosynthetic enzyme and cellular apparatus. When further the plants where treated with stress the carbohydrate content increased in 200mM, 300mM while it completely decreased in 400mM plant sample.

This attributes to the destruction of photosynthetic apparatus resulting in decreased production of carbohydrates. In the case of protein there is a sharp increase in the protein content on the 7th day of estimation. The general pattern observed on the 14th day and 21st day is that, the protein content gradually increased as the concentration of salt increased. The production of various heat shock proteins, molecular chaperons which come into action during the stress period. In the case of phenolics, there was an increase in its content when the salt concentration was increased. This could be due to the synthesis of secondary metabolites which act as defence molecules and protect the plant from the stress shock. On 14th day the phenolic content decreased and the same trend continued on 21st day also except in 400mM concentration sample were there was a sharp increase in the phenolic content.Review of literature suggests that the reducing sugars and soluble protein content initially increases when stress is induced.

An increase in the content of sugars has been attributed to the hydrolysis of starch by enhanced amylase activity . According to another study, the sugar content increases during stress and their concentration is higher in aerial parts than the other parts like root[34]. Some studies reveal that during salt stress, various enzymes like peroxidase, catalase come into action which scavenge the ROS and hence do not cause much disturbance in the cell membrane structure, therefore resulting in a rise in the protein concentration[35].It has also been reported that the total phenolics shows a sharp rise and the plant growth is reduced as major products like lignin and other metabolic products have been converted into polyphenols for defensive purpose. The results obtained in the present study are majorly in agreement with already existing reports.

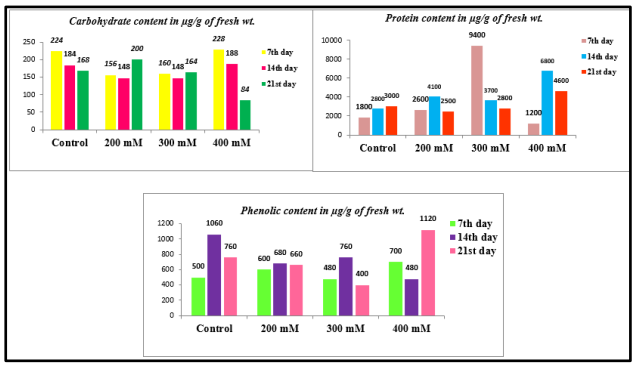

Figure.3: Graphical representation of phytochemical variation during stress conditions.

Plant Sample.02:*Lablab purpureus* (Indian bean)

Morphological observations and discussion:

Morphological response towards stress by the plant is shown in Figure.4. On 7th day of stress period there was no much morphological difference among various plants samples. The control and 200mM plants samples were almost same in size but in 300mM plant sample a greater number of leaves was produced but the leave lamina surface was reduced when compared with control. In 400mM plant sample stunted growth was observed but the leaf size was almost equal to control. On 14th day there was a drastic difference in all the plant samples. Control plant sample had grown luxuriantly while the 200mM plant sample growth had reduced growth and they showed stunted appearance. In the 300mM plant sample, the leaves were much reduced and chlorosis had begun. New leaf production had ceased. In 400 mM sample, abscission of leaves was observed and the plants showed signs of damage. The roots were well developed in control sample while in 200 mM sample they root were slender and weak. In 300 mM sample, the roots system was comparatively better than 200mM. In 400 mM sample, the roots were short and stout giving the appearance of fibrous root.

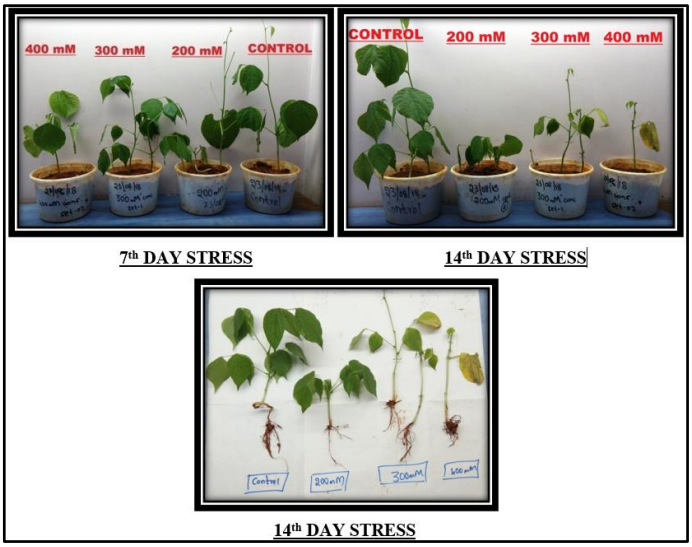

Figure.4: Response of *Lablab purpureus* (Indian bean)towards salt stress

Phytochemical observations and discussion:

The phytochemicals produced in various stages of stress period is tabulated in Table.2 and represented graphically in Figure.5. From the graph, it was noticed that in control the carbohydrate level increased with the stress duration. In the stressed plant sample, there was a small rise in the carbohydrate content as the stress period prolonged. In 200 mM, 300mM and 400 mM plant sample, the protein content was increased when compared to control. As the stress period prolonged, there was no much change in their concentration except in 200mM plant sample. This may be due to the production of various heat shock protein and other molecular chaperons. Similar increase wasnot seen in 300mm and 400mM plant sample probably because the stress level was very high resulting in the proteins being degraded.The phenolic content of the stressed plants was higher when compared to the control. This is because phenolics are a secondary metabolite and plays a major role during stress period. It was observed that as the stress period prolonged, the phenolic content decreased except in 300 mM plant sample. According to literature survey, a report reveals that the phenolics increase only in the shoot system and not in the root system. As salt concentration increases it leads to dehydration which elicits the increase of various secondary metabolites for the protection of the plants[36].In agreement with the results of this study with regard to carbohydrate content, a research paper suggests that the increase in carbohydrate content with increased stress conditions is due to starch degradation, decreased activity of invertase and accumulation of photosynthates[37].

Table.2: Tabular column showing amount of Carbohydrates, Proteins and Phenolics in µg/g of fresh wt. of *Lablab purpureus*(Indian bean)

		Control	200 mM	300 mM	400 mM
Amt. of Carbohydrate in µg/g of fresh wt. of sample	7th day	136	112	94	100
	14th day	312	112	104	130
Amt. of Protein in µg/g of fresh wt. of sample	7th day	1300	1600	1500	1500
	14th day	800	2500	1400	1400
Amt. of Phenolics in µg/g of fresh wt. of sample	7th day	280	300	320	320
	14th day	260	220	340	160

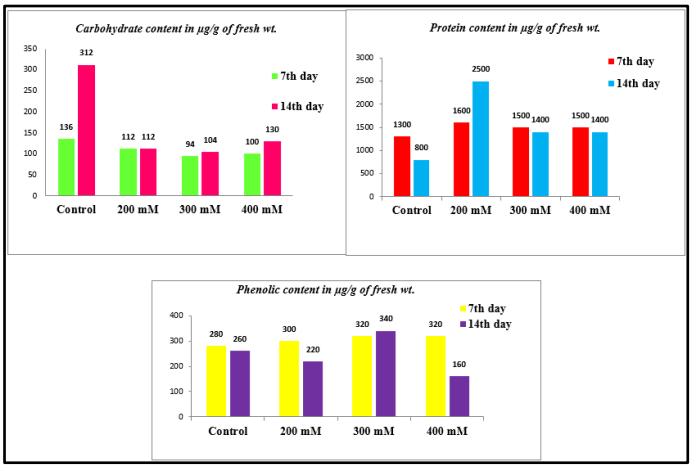

Figure.5: Graphical representation of phytochemical variation during stress conditions

Plant Sample-03: *Trigonella foenum-graecum* (Fenugreek)

Morphological observations and discussion:

Morphological response towards stress by the plant is shown in Figure.6. In Methi, initially all the four set-ups

showed uniform morphology. On 7th day of stress treatment, the 200 mM sample had a dwarf root system. The roots were stout and short. In 300mM plant sample the roots were very thin and sparse. In 400mM plants sample the roots were filamentous, long and delicate. On 14th day of stress period, all set-ups except controlshowed signs of damage such as drying, yellowing and drooping. The root production was severely affected in 200mM and 300mM. While in 400mM there was no much root production and the older roots had become very thin and weak. On 21st day of stress period, the root production was comparatively better when compare to the 14th day and 7th day. This might be due to search of water in the soil highly concentrated with salt. The leaves and the shoot system exhibited shrivelling. Most of the leaves were reduced in size and showed senescence.

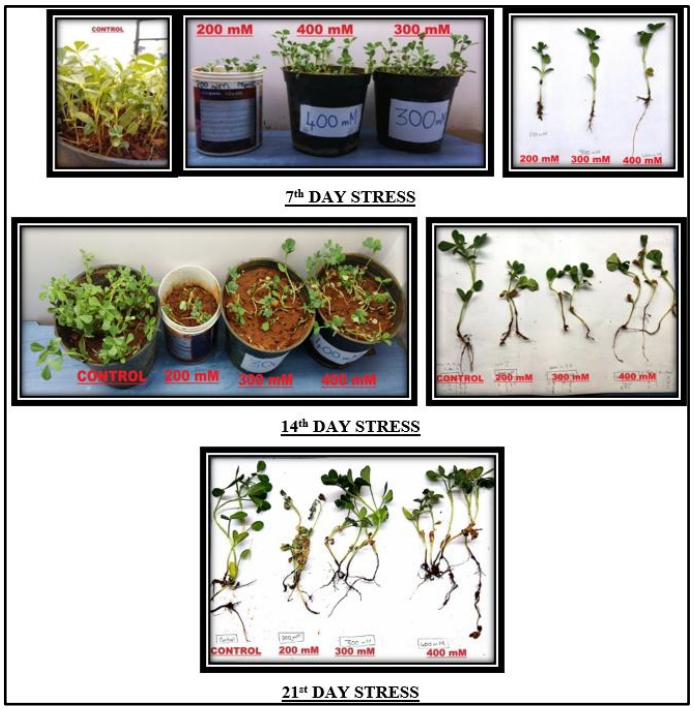

Figure.6: Response of*Trigonella foenum-graecum* (Fenugreek) towards salt stress

Phytochemical observations and discussion:

The phytochemicals produced in various stages of stress period is tabulated in Table.3 and represented graphically in Figure.7. From the graph it was observed that the carbohydrate content increased gradually on the 7th day. On 14th day the carbohydrate content sharply increased. On the 21th day also the same trend was observed except in 300mM, 400mM plant samples.The protein content initially decreased from control to 200mM and later increased. As the stress period prolonged there was a decline in the content of protein in the control plant sample whereas there was a massive increase in the protein content of 200mM, 300mMand 400mM plant samples.Initially the phenolic content increased from control to 200mM plant samples and latter it decreased in 300mM and 400mM plant samples as the stress period was continued there was a drastic increase in the phenolic content of 200mM and 300mM plant samples. While in 300mM plant samples, on 14th day there was increase in the phenolics and it decreased on 21th day. In a similar study conducted on soybean cultivars, it has been reported that the protein concentration decreases due to exposure of NaCl**.**However, in ourstudy there are slight fluctuations in protein concentrations. Also, a similar study on two Turkish varieties of Tobacco is in agreement with the results obtained in our present study.

Table.3: Tabular column showing amount of Carbohydrates, Proteins and Phenolics in µg/g of fresh wt. of *Trigonella foenum-graecum* (Fenugreek)

		Control	200 mM	300 mM	400 mM
Amt. of Carbohydrate in µg/g of fresh wt. of sample	**7th day**	108	140	144	134
	14th day	104	144	168	180
	21st day	116	270	128	108
Amt. of Protein in µg/g of fresh wt. of sample	**7th day**	4000	700	1400	1200
	14th day	1500	3400	1600	2000
	21st day	4600	12,000	4800	2300
Amt. of Phenolics in µg/g of fresh wt. of sample	**7th day**	160	300	220	180
	14th day	400	1000	1020	500
	21st day	880	1300	900	1120

Plant Sample.04: *Vigna radiata* (Green gram/Mung Bean)
Morphological observations and discussion:

Morphological response towards stress by the plant is shown in Figure.8. In green gram, the effect of salt stress was rather pronounced on the morphology of the plants. On 7th day, the control plant sample had grown very well while 200mM plant sample had started showing signs of dryness and drooping. The root was short and thick. The 300mM plant sample was more affected when compared to the 200mM. Despite having a greater number of roots, the roots were all short.Most of the leaves showed senescence. In 400mM plant sample adverse damage was seen and the complete plant started to die. Surprisingly the roots were well developed compared to 200mM and 300mMplants. On 14th day of stress period the control plants had grown well. The 200mM plant root system was affected no new roots were produced. Many leaves started to droop and aging of leaves was seen while in 300mM, 400mM plant sample the complete plant died. Root and leaves production stopped.

Phytochemical observations and discussion:

The phytochemicals produced in various stages of stress period is tabulated in Table.4 and represented

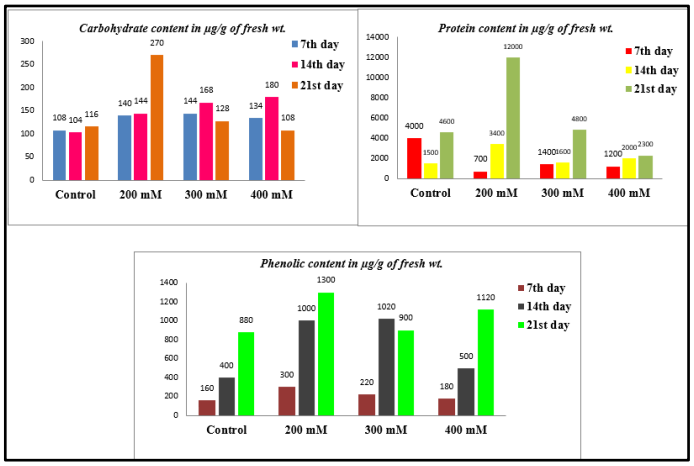

Figure.7: Graphical representation of phytochemical variation during stress conditions.

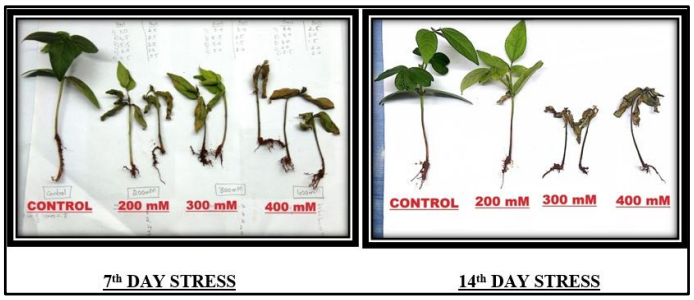

Figure.8: Response of *Vigna radiata* (Green gram)towards salt stress

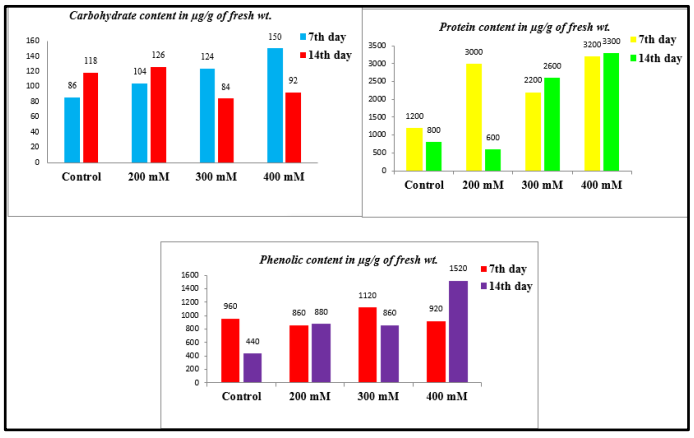

Figure.9: Graphical representation of phytochemical variation during stress conditions.

graphically in Figure.9. The carbohydrate content gradually increased as the salt stress concentration increased. When the stress period was extended,carbohydrate content increased only in control and 200 mM where as in 300mM and 400mM they declined in a faster phase, this might be as a result of high salt concentrations which would have denatured the essential photosynthetic enzymes and proteins.The proteins increased as the salt stress concentration increased. On the 14th day there was a noticeable decline in protein content of the 200mM plant sample while in 300mMand 400mM plant samples, the protein had increased when compared with control. This could be due to the production of defence proteins to protect the plant from damage.The phenolic content initially showed decline from control to 200mM and increased in 300mM and suddenly decreased in 400mM. On the 14th day of stress period there was a sudden rise in the phenolic contents of stressed plants when compared with control plants. There was a very high increase in the phenolic content of the 400mM plant sample. This could possibly be due to the increase insecondary metabolites production for defence in plants.

Table.4: Tabular column showing amount of Carbohydrates, Proteins and Phenolics in µg/g of fresh wt. of*Vigna radiata* (Green gram)

		Control	200 mM	300 mM	400 mM
Amt. of Carbohydrate in µg/g of fresh wt. of sample	**7th day**	86	104	124	150
	14th day	118	126	84	92
Amt. of Proteins in µg/g of fresh wt. of sample	**7th day**	1200	3000	2200	3200
	14th day	800	600	2600	3300
Amt. of Phenolics in µg/g of fresh wt. of sample	**7th day**	960	860	1120	920
	14th day	440	880	860	1520

Acknowledgement

We would like to specially thank the Department of Botany, St. Joseph's College, Bengaluru for giving us the permission, necessary equipment's and chemicals to carry out this project work. We express our deep gratitude to Ms. Poonam R Ahuja our project supervisor for her keen interest, valuable guidance,constant encouragement and painstaking efforts during the course of our research work. We would also like to extend our gratitude to Dr. Neelam Mishra and Dr. Vaishnavi. M for their tireless assistance and guidance during this research work. We thank them for their constructive criticism and useful suggestions apart from the invaluable guidance given to us.

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