CHEMICAL PRIMING FOR IMPROVING SALINITY TOLERANCE IN RICE

By

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(2018-11-065)

THESIS

Submitted in partial fulfillment of the

requirement for the degree of

Master of Science in Agriculture

(AGRONOMY)

Faculty of Agriculture

Kerala Agricultural University, Thrissur

DEPARTMENT OF AGRONOMY

COLLEGE OF AGRICULTURE
VELLANIKKARA, THRISSUR – 680656
KERALA, INDIA
2020

DECLARATION

I, hereby declare that the thesis entitled "***Chemical priming for improving salinity tolerance in rice***" is a bonafide record of research done by me during the course of research and that it has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis entitled **"Chemical priming for improving salinity tolerance in rice"** is a record of research work done independently by **Ms. Liz J Kappen (2018-11-065)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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ACKNOWLEDGEMENT

"Fear not, for I am with you; be not dismayed, for I am your God; I will strengthen you, I will help you, I will uphold you with my righteous right hand"-Isaiah 41:10

I would like to express the deepest appreciation to my committee chair, ***Dr. Deepa Thomas*** *Assistant Professor and Head i/c, Aromatic and Medicinal Plants Research Station, Odakkali, who has the attitude and the substance of a genius. Without her guidance and persistent help this work would not have been possible.*

I express my heartiest gratitude to ***Dr. P. Prameela****, Professor and Head Department of Agronomy, College of Agriculture, Vellanikkara, who continually and convincingly conveyed a spirit of adventure in regard to research and scholarship, and an excitement in regard to teaching. Her dynamism and dedication are worth imitating and for her a student is worth above time. The effort and time you have taken for me would always be remembered.*

No word of thanks can sum up the gratitude that I owe to ***Dr. Meera V. Menon****, Professor Department of Agronomy, College of Agriculture, Vellanikkara and member of my advisory committee for her meticulous help, expert advice, forbearance, critical evaluation, constant encouragement and support throughout my course of study.*

I sincerely thank ***Dr. T. Girija*** *Professor and Head, Department of Plant Physiology and member of my advisory committee for her unwavering encouragement, timely support and critical examination of the manuscript that has helped me a lot for the improvement and preparation of the thesis.*

I owe my deepest gratitude to ***Dr. C. George Thomas*** *former Associate Dean, College of Agriculture, Vellanikkara for the heartfelt help and back-up which gave me enough mental strength for the successful completion of my thesis work.*

I am extremely thankful to ***Dr. Veena Vighneswaran****, Associate Professor (Plant breeding and Genetics) for guiding me in my research work.*

I would like to express my eternal appreciation towards my parents, my lovely siblings, aunty and uncle who have always been there for me no matter where I am, for all their unconditional support and patience. Thank you for being ever so understanding and supportive.

I would like to thank my dear friends and colleagues, especially fellow students of Masters of Agronomy, ***Minu Maiya Issac, Bassila, Moyi, Mounisha and Suhas*** *it has been great to know all of you during my time here at KAU campus. I also thank my dear seniors* ***Dr. Pujari Sobha Rani, Anjali chechi, Reddy sir and Ajmal ikka*** *for being a constant help all throughout my thesis preparation. It would be a sin if I forget to mention* ***Shreela chechi and Samala chechi****, who willingly helped me throughout my lab studies. Words fall short for the help and support of* ***Arathy chechi and Irene chechi****, staff Rice research station, Vytilla. A big thanks to my dear friend* ***Gadha V.P*** *for comforting me during my distress and helping me although out the two years of my M.Sc.*

A word of apology to those I have not mentioned in person and a note of thanks to everyone who helped for the successful completion of this endeavor.

Liz J Kappen

With great pride I dedicate this work to my parents, siblings and teachers of Saint Peters Senior Secondary School, Kadayirippu

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INTRODUCTION

1. INTRODUCTION

Abiotic stress conditions such as cold, salinity, drought and heat stress cause extensive loss to agricultural production worldwide. Research on abiotic stress tolerance in plants is gaining importance due to urgent concern on climate change and resultant food security issues all over the world.

According to FAO, Land and Plant Nutrition Management Service (2008), over six per cent of the world's land is affected by either salinity or sodicity, covering over 400 million hectares worldwide. One among the most encountered problems of rice cultivation in the coastal areas of Kerala is also that of salinity. Salt injury is more pronounced at seedling stage of most crops. This is because salt, especially of chlorides of sodium and magnesium, interfere with the uptake of water and other nutrients vital to plant growth. Speed of germination has a direct bearing on crop productivity. Initial dry matter accumulation is vital to overcome salt stress.

Plants have evolved a battery of mechanisms to adapt themselves to adverse environments. A thorough understanding of molecular and biochemical mechanisms behind this is a pre-requisite for efficiently designing strategies for improving plant tolerance to abiotic stress. Also, there is a need to utilize various crop improvement methods as well as agro-techniques to manage and mitigate abiotic stress for enhancing crop productivity.

Though different methodologies for overcoming abiotic stress like conventional breeding and molecular breeding are common, they are often limited by time or biosafety concerns.

Use of chemical priming is a simple and cost effective, method to manage abiotic stress. Use of chemicals, both synthetic and natural, prior to stress events can impart enhanced tolerance to stress such as salinity, drought etc. The application of chemical agents externally can initiate a mild stress leading to certain biological changes resulting in increased stress tolerance at later stages of the crop. Some of the priming chemicals like sodium nitoprusside, calcium chloride, and beta amino butyric acid have shown promise in laboratory conditions in many crops. When seeds are primed using such chemicals the seeds get a pre-exposure to abiotic stress and this activates its antioxidant system which protects it from future stress. Chemical priming results in the up regulation of antioxidants, which play a major role in salinity tolerance by their protective role in plants. Seed priming methods are easy to adopt and are cost effective and less risky. Rice is the staple food of most Indians and is a crop badly affected by salinity. Though certain rice varieties like *Pokkali* have shown inherent capacity to tolerate salinity, this is not the case with most widely preferred rice varieties. Conferring tolerance to salinity by chemical priming may be promising. Effective utilization of chemical priming in rice can help to expand the area under cultivation and increase productivity in the changing climatic situation.

Hence the study was formulated with the main objective of investigating the effect of different priming chemicals in improving salinity tolerance in popular rice varieties.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Plants experience both biotic and abiotic stress. Biotic stress is caused by pests and pathogens while abiotic stress includes flood, drought, salinity, low temperature, high temperature, heat, nutrient deficiency, metal toxicity etc. One among the main abiotic stress encountered by plants cultivated in coastal regions around the world is salinity. Studies on various aspects of salinity including effects of salinity in plants, how plants tolerate salinity and different methods to improve crop yield under saline conditions are reviewed in this chapter.

2.1 Defining salinity

Salinity can be considered to be arising due to increased levels of soluble salts. The three categories of salinity in soil are saline soils, alkali or sodic soils and saline-alkali soil. Saline soils are found in arid regions, estuaries and coastal regions of the world. When the electrical conductivity (EC) of the saturated extract of the soil in the root zone exceeds 4 dSm^{-1} at 25^0C and the exchangeable sodium is less than 15 per cent, the soil can be considered saline (FAO,1996). The pH of these soil is usually less than 8.5 (Munns, 2005; Jamil *et al*., 2011). Sodic soils have high concentrations of free carbonates and bicarbonates and excess sodium. Sodium occupies the exchangeable sites of clay. The specific features of sodic soils are that they are deficient in nitrogen, phosphorus and zinc. The pH ranges above 8.5 and Exchangeable Sodium Percentage (ESP) is greater than 15. The excess sodium deflocculates the top clay. They are sticky when wet and hard when dry. The hydraulic conductivity of these soils is also low.

Saline-alkali soils are formed as a result of salinization and alkalization. They have ESP more than 15 and pH more than 8.5. Depending upon the presence of sodium ions the soil may be flocculated or deflocculated (Das, 1996).

Various environmental stresses like wind, extreme temperatures, soil salinity, drought and flood affect crop production. One of the most devastating among them is soil salinity (Yamaguchi and Blumwald, 2005; Shahbaz and Ashraf, 2013). Rice cultivation in the coastal belts of Kerala faces the problem of salinity.

2.2. Effects of salinity in plants

The effects of salinity on plants occur as two stages, one as osmotic or water deficit and second as ion excess effect also known as salt specific effect. Osmotic effect occurs when the plant roots are surrounded by salt, entry of water is limited due to changes in water potential. The osmotic effects of salinity continue to last till salt exposure time, resulting in reduced cell expansion, cell division and stomatal closure (Flowers, 2004; Munns, 2002). Gupta and Huang (2014) reported that osmotic stress in the initial stages of crop growth caused disruption of membranes, nutrient imbalance, ROS over production, differences in antioxidant production and decreased photosynthesis and decreased stomatal aperture.

Ion excess effect occurs when the salt enters the transpiration stream causing cell injuries, thereby reducing plant growth. Cramer and Nowak (1992) reported that under long term exposure to salinity, plants experienced ionic stress, which resulted in premature leaf senescence, and therefore reduced photosynthetic area to support normal growth. According to Munns and Tester (2008), as the salinity continued, inhibited growth over a period of time and senescence of older leaves could be observed, and this phase was known as ionic phase.

Overcoming salt stress requires overcoming both the primary and secondary effects of extreme salt conditions. Maintaining whole plant and shoot water status, as well as exclusion of Na^+ ions and maintaining potassium levels in developing parts and rapid leaf growth, contributed to salt tolerance in rice varieties (Yeo *et al*., 1990). Ahmed *et al*., 2011; Wani *et al*. 2016 reported that smaller leaf area, fewer photosynthetic pigments, less gas exchange and lower quantum efficiency of photosystem II as a result of salinity, contributed to reduced length and biomass in shoot and roots. Gucci and Tattini, 1997; Parida and Das, 2005 reported that leaf area decreases, leaf succulence and thickness increases and internodal length also gets reduced. Different plants followed varied ways to get rid of the toxic effect of salt.

2.3 Different mechanisms in plants to overcome saline stress

Plants follow mainly two methods to overcome salinity namely, avoidance and tolerance

2.3 Avoidance

2.3.1 Increased seedling vigour

Plant vigour in rice is one of the key determinants in salinity tolerance. Yeo and Flowers (1986) reported that the concentration of transported Na^+ was lower in fast growing genotypes. Most rice varieties tolerant to salinity were tall varieties or the traditional varieties. Lee *et al*. (2003) reported that indica had higher salinity tolerance than japonica at seedling stage. They showed increased vigour during the initial growth phases. The increased plant vigour resulted in dilution effect, contributing significantly in overcoming salinity stress. Dilution of Na^+ ions per unit area of plant, due to increased plant growth is called dilution effect. In rice this dilution effect comes into play during early stages of plant growth which often coincides with peak saline levels in soil. Kumar *et al*. (2013) and Platten *et al.* (2013) also reported that plant vigour is a mechanism to avoid toxic effect of salinity.

2.3.2 Initial entry of salts from roots

Roots with weak barrier areas are potential entry sites for Na^+ bypass movement from soil (Yeo and Flowers, 1986). Salts such as Na^+ and K^+ in soil enter the plants through different transporters present in root cells. Garciadebleas *et al*. (2003) reported the existence of such transporters in root cells. They also suggested that the movement of the ions was based on osmotic potential. The rate at which sodium is taken up by rice roots is affected by the genetic variation of different rice varieties. Wide variability in ion uptake among different rice varieties provides scope for genetic improvement of different rice varieties. According to Krishnamurthy *et al*. (2009) sodium transport to the shoot in rice is via apoplastic pathway, while Das *et al*. (2015) reported that in rice the transport of salts is controlled by selective uptake by root cells along with water through symplastic and apoplastic pathways.

2.3.3 Plant level transport and compartmentalization

The sodium that enters the plant system gets accumulated in the older leaves, leaf sheath and culms. Yeo and Flowers (1982) reported that in rice, expulsion of Na^+ from developing young leaves and sequestering in older leaf, would allow young leaves to actively participate in plant growth, while mature leaves accumulate sodium. In this way the number of leaves lost due to Na^+ accumulation is equalized to the new leaves formed. Munns, (2002) reported that the rate of leaf death is very important for the very survival of a plant. Old leaves accumulated higher concentration of sodium ions, chloride and nitrate than young leaves (Wang *et al*., 2012).

In saline tolerant *Pokkali,* absorbed sodium ions were found to get compartmentalized in lower parts of stem (Shylaraj *et al*., 1994). Ochiai and Matoh (2002) Restricting excess sodium transport to shoot is one of the key factors to alleviate saline injury in plants. Tolerance of a plant to salinity can be measured by its sodium accumulation in older plant parts; however, this cannot be the sole criteria to measure salt tolerance of a plant. The selective uptake of sodium into reproductive organs and flag leaf is also observed in certain plants. Healthy flag leaf and reproductive organs is a measure of tolerance.

2.3.4 Intra cellular compartmentalization

Though under normal conditions plant compartmentalization of salts is not so visible, the levels of accumulation increase under stress condition. Accumulation of sodium in cell cytoplasm is an example of intra cellular compartmentalization. Hajibagheri *et al.* (1987) found that the cytoplasmic sodium reached only 40 mol m^{-3} when the soil salinity was 100 mol m^{-3}, while at normal levels it varied between 40 to 70 mol m^{-3}.

2.4 Stress response in plants

Plants acclimatize to changing environment initially by sensing and activating signaling pathways and later signal transduction pathways are activated. According to Shulaev *et al*. (2008) the signal transduction molecules could be newly synthesized compounds or compounds released from their conjugated form(s), such as salicylic acid or they could be by-product of membrane degradation which include reactive oxygen species (ROS), phenolic compounds or antioxidants. Amino acids such as proline and ectoine also serve as osmolytes and osmoprotectant to protect plants under extreme salt, drought and desiccation stress. Increased accumulation of proline occurred in salt tolerant plants in comparison with sensitive ones (Ashraf and Foolad, 2007; Habib *et al*., 2012).

According to Pastori and Foyer, (2002) stress response in plants usually occurred in the sequence of - stress stimulus, signals, transducers, transcription regulators, target genes and stress responses like morphological, biochemical and physiological changes. They continued that plants used common pathways and components for any kind of stress-response relationship and this phenomenon is called cross-tolerance, which allowed plants to manage any kind of stress after experiencing any one specific stress. Stress tolerance are often regulated by transcription factors and the activation or de-regulation of these transcriptional factors often resulted in reduced growth and development in plants, the major mechanism by which plants survive stress. Such metabolic arrest could lead to reduced yield and so regulating the activity of transcriptional factors, at the same time coping stress is important.

2.5 Role of proline in plants

Proline is an amino acid produced by plants under normal environment and under stress. Kishor *et al.* (2015) reported that proline has an important role to play in cell growth and differentiation. Proline is the most common endogenous osmolyte accumulated as a result of various abiotic stress in plants (Szabados and Savoure, 2010; Slama *et al*., 2015). Though proline got synthesized by two pathways namely glutamate pathway and orinithine pathway, glutamate pathway contributed the major share in proline accumulation under stress condition in plants. Glutamate synthesis pathway was mainly found in grasses while ornithine pathway was seen in legumes. Glutamate is the precursor of proline (Rhodes *et al.*, 1986; Szekely *et al.*, 2008; Sharma and Verslues, 2010). Proline acts as a proteogenic amino acid, also it plays an important role in biotic stress tolerance in plants (Kishor *et al.*, 1995; Sharma and Verslues, 2010). Proline has the ability to act as chaperons and help maintain protein integrity and help other enzymes to function properly. Proline get synthesized at low levels in all tissues under normal condition and under stress condition they get transported to roots. Later it gets compartmentalized in mitochondria and get degraded into glutamate and finally released as energy (Ober and Sharp,1994).

The role of ABA in stress tolerance is well known but the relation between ABA and proline is quite unknown. Ober and Sharp (1994) reported that when ABA levels were kept low in root zone of maize, the proline accumulation also decreased in maize. Proline deposition is important for osmotic adjustment. They help the root to elongate and take up water under stress condition. This may be suggestive of the relationship between proline and ABA.

Salt –inducible proline transporter was isolated from barley and they were found to express in root cap region under saline stress (Ueda *et al.*, 2001). This study was indicative of the role of proline during salt stress. The role of proline at different growth stages of the plant is also important. Proline was found to get accumulated in large amount during the transition from vegetative to flower initiation under normal conditions and transportation of proline to reproductive organs suggested its role in flower development (Chiang and Dandekar, 1995; Schwacke *et al.*, 1999; Mattioli *et al.*, 2009). Kishor *et al.* (1995) reported that P5CS in tobacco got over expressed under stress condition and resulted in more proline accumulation. Accumulated proline enhanced flowering in tobacco. Proline also influenced flowering time (Kishor *et al.*, 2015).

Proline acted as an excellent osmolyte, metal chelator, a molecule with antioxidant action and as a signaling molecule (Shamsul *et al.*, 2012). The antioxidant properties of proline help plants in ROS scavenging. Apart from the production of proline in plant parts, the exogenous application of proline was also found to be effective.

2.6 Chlorophyll content, carotenoids, flavonoids in plants under salinity stress

Decrease in chlorophyll is considered as a typical symptom of oxidative stress (Smirnoff, 2004). Chlorophyll degradation and membrane lipid per oxidation due to increased reactive oxygen species reduced the membrane fluidity and selectivity (Verma and Mishra, 2005). Under salinity stress both chlorophyll A and chlorophyll B was found reduced in *Vigna subterranean* (Turan *et al*., 2007). Carotenoids are involved in photosynthesis, they also regulate oxidative stress defence mechanisms (Gill and Tuteja, 2010). Flavonoids help in lipid peroxidation inhibition (Di Ferdinando *et al*., 2012). The amount of chlorophyll, carotenoids and flavanoids in plants under salinity stress is an important aspect in understanding the stress response of plants.

2.7 Salinity tolerance in rice

Salt tolerance in rice is known to be a quantitative trait, indicating the involvement of multiple genes (Chinnusamy *et al.*, 2005). The immediate response of rice to saline stress is partial closure of stomata. Rice is sensitive to salinity mainly during early seedling stage (Lutts *et al*., 1995) and reproductive stage (Todaka *et al*., 2012).

The salinity of 4 dSm^{-1} is moderate level of salinity, while a salinity above 8 dSm^{-1} is considered high (IRRI knowledge portal). The salinity tolerance mechanism in rice can be divided into two: ion exclusion and osmotic tolerance (Munns and Tester, 2008). Ion exclusion take place by retrieval of Na^+ ions from xylem and moving them out from plant to soil. Osmotic tolerance on the other hand is considered as the ability of the plant to tolerate salinity related water deficit and to maintain leaf expansion and stomatal conductance (Rajendran *et al.,* 2009). According to Roy *et al*. (2014), rice follows another salt tolerating mechanism called tissue tolerance. Tissue tolerance involves the sequestering of Na^+ in vacuole, synthesis of compatible solutes such as proline and production of enzymes which can detoxify ROS.

When salinity is too high the plant gets totally killed and at moderate levels, they show various morphological and biochemical symptoms. The major morphological symptoms shown by rice under saline stress are white leaf tip which immediately follow tip burn, stunted growth, low harvest index, reduced tillering, spikelet sterility, reduced 1000 grain weight, low florets per panicle, altered flowering duration, rolling of leaf and reduced root growth (IRRI knowledge portal).

The major biochemical changes observed are increased uptake of sodium and preferential deposition of sodium in older leaves, increased chlorine ion uptake and reduced potassium uptake, lower uptake of phosphorus and zinc uptake. So, a saline sensitive plant would have a higher Na^+/K^+ ratio compared to a tolerant plant. Under salt stress the dry weight of both root and shoot was found to be low. There is an increased production of reactive oxygen species (ROS) in response to salinity stress. Non- toxic organic compatible solutes and polyamine levels also tend to increase (IRRI knowledge portal).

Certain traditional rice varieties of Kerala show remarkable ability in withstanding salinity, acidity and flooding. These varieties like *Pokkali*, *Ezhome* are taken up by farmers since a long time. *Pokkali* varieties are mostly tall and are less yielding compared to mostly cultivated varieties.

2.8 Salinity tolerance of *Pokkali*

The salinity tolerance in *Pokkali* varieties is due to the presence of *Saltol* genes. According to Thomson *et al*. (2010), saltol is mainly controlling shoot Na^+/K^+ homeostasis. Saltol is reported to be a polygene present in chromosome 1 (Waziri *et al*., 2016). Saltol helps the plant maintain low Na^+ absorption, high K^+ absorption, and low Na^+/K^+ ratio (Gregorio *et al*., 2002). Bonilla *et al*. (2002) reported that saltol QTL was mapped between RM 23 and RM 140.The shoot $Na^{+\,/}K^+$ ratio is maintained low in *Pokkali*, as a result of which the harmful effect of Na^+ is kept at check. Osmotically the long-distance signals restrict growing shoots and this occurs before the actual accumulation of Na^+ ions. Leaf expansion and stomatal conductance occur normally due to osmotic tolerance. Ion exclusion occurs when Na^+ ions are removed from the xylem and they get moved into the soil. This allows ions such as sodium and chloride from accumulating in leaves as it moves towards roots. Sodium ion gets sequestered in vacuole, compatible solutes get synthesized and enzymes responsible for the detoxification of reactive oxygen species start to get released. This mechanism is known as tissue tolerance mechanism (Roy *et al*., 2014).

2.9 *Pokkali* cultivation practices

Cultivation practices starts from the month of May. Bunds are erected followed by taking mounds of 1 m^2 base and 50 cm height (Kerala Agricultural University, 2016). As the south west monsoon begins, salts and toxic chemicals are washed away. The seeds after being tightly wrapped in baskets of coconut or teak leaf are soaked for 12 to 15 hours for germination (Thomas, 2002; Paimpilli, 2007). Once the soil salinity reduces due to rain, germinated *Pokkali* seeds are sown over the mounds. As the seedlings reach a height after one month, the mounds are levelled off; with not much disturbance to its roots, using a spade. Manures and fertilizers are not applied as *Pokkali* farming is completely organic. *Pokkali* matures within 120 days and are harvested using sickles by farmers in country boats. Panicles are cut leaving behind the straw which later decay in the water, the decaying straw serves as food for shrimps (Gayatri and Raveendra, 2009). Crop cultivation is followed by shrimp cultivation as the soil salinity increases from January to May.

2.10 Soils of *Pokkali* tract

Pokkali soils are generally acid sulphate soils formed due to repeated tidal activity. They have high amount of clay, organic matter and nitrogenous compounds like ammonia and nitrous oxide. The soils of *Pokkali* tract are generally bluish grey in colour and intensity of colour increase with depth (Varghese *et al*., 1970). They have high water holding capacity and are of fine texture. The large amount of clay makes them fine textured and they develop cracks in summer and get sticky in wet condition. The rising and falling tide deposit sediment load in land and such sediments are called lacustrine and alluvial deposits.

They are rich in silt, clay and large amount of organic matter. *Pokkali* soils are the result of lacustrine and alluvial deposits.

This soil is suitable for organic cultivation of rice due to its high fertility status. About 94 per cent of this tract contains medium to high organic carbon (Annie *et al*., 2013). *Pokkali* system, which consists of paddy cultivation and prawn farming, has high organic matter during paddy cultivation, and various nitrogenous compounds like ammonia and nitrate get added to the soil due to shrimp farming (Krishnani *et al*., 2011).

Electrical conductivity and pH of the soil largely limit the choice of different rice cultivars taken up by the farmers. The EC value ranges from 12 to 24 dsm^{-1} during summer, and during south west monsoon the value ranges from 6 to 8 dSm^{-1} (Tomy,1981). Kramer (1984) reported that the fertility and productivity of *Pokkali* field is decided by tidal inflows which make it rich in Na^+, K^+, Ca^+ and Mg^{2+}. The toxic elements like Al^{3+}, $Fe^{2+}$and Mn^{2+} get washed away by tidal movements. The net level of salt is also decided by water inflow, water outflow, evaporative losses and transpiration.

2.11 Chemical priming

The pre-exposure of seeds and seedlings to chemicals such as antioxidants, plant growth promoters, organic compatible solutes and inorganic salts is known as priming (Borges *et al*., 2014; Hossain *et al*., 2015; Savvides *et al*., 2016). The use of chemicals such as sodium nitroprusside, melatonin, hydrogen peroxide, polyamines, sodium hydrosulfide etc. were found to be useful in overcoming environmental stress. Polyamines which have hormonal activity could be used as priming agent according to Tanou *et al*. (2014). Afzal *et al*. (2016) recommended the use of micro- nutrients such as Zn, B, Fe, Mn, Mo, Cu and Cl which helped to improve many physiological, biochemical, molecular and yield related parameters. The effectiveness of these chemicals depended on the mode of application. Seed priming is the pre-germination treatment given to seeds which alter several metabolic and physiological activities of the seed (Savvides *et al*., 2016). As chemical priming is undertaken before actual germination of the seed, all the changed metabolic and physiological process help the plant respond faster to stress.

Sheteiwy *et al*. (2017) reported increased germination in rice. Seed priming accelerated respiration, gene transcription, activation of endosperm, and translation which were all related with seed germination (Savvides *et al*., 2016). The developments in cellular and molecular level are communicated better within the cell and surrounding cells. Increased communication within the cell and outside the cell helps in increased growth and germination of plants. Seed DNA repair pathway and antioxidant mechanisms were activated during early seed imbibition (Jisha *et al*., 2013). Savvides *et al*. (2016) reported that by using chemicals in priming ionic balance, scavenging of ROS molecules and thereby physiological homeostasis was achieved. Also, molecular adjustments helped to alleviate the negative effects of abiotic stress. The advantage of chemical priming at pre germination stage is that the chemical needs to be applied only once in the crop's lifetime as the one-time application at the beginning generates memory in plants. So, when plants get exposed to abiotic stress later in life, they are pre-prepared to respond faster and this gives the plant enough time to respond effectively. Several workers (Bruce *et al*., 2007; Chen and Arora, 2013) hypothesized that upon chemical priming "priming memory" is created and this helps in overcoming abiotic stress when the plant actually encounters a stress condition.

Apart from seed priming exogenous application of many priming chemicals have shown promising results. Exogenous application could enhance plant tolerance to multiple abiotic stress without altering the genetic material (Savvides *et al*., 2016). The amount of chemical used for seed priming and exogenous application is very less and so cost involved in application is reduced in this technique compared to molecular breeding works which takes years to give final result.

2.12 Role of priming agents

a) Sodium nitroprusside and calcium chloride

Sodium nitroprusside (SNP) acts as NO donor. Thomas *et al*. (2003) reported that NO, help plant in scavenging free radicals by activating antioxidants like CAT, SOD and POD. In plants, NO triggers the production of metabolites and antioxidants, thereby reduce abiotic stress (Ahmad, 2018). Khan *et al*. (2012) reported that when 24 hours of salt stressed leaves were treated with SNP and calcium chloride, leaves showed improved carbonic anhydrase, nitrate reductase and chlorophyll, leaf RWC and leaf ion concentration as compared to leaves treated with NaCl only. They continued that the damage caused by increased hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substance induced membrane damage and electrolyte leakage was blocked by application of 0.2 mM SNP and 10 mM $CaCl_2$. Both chemicals were found to increase antioxidant enzymes such as SOD, catalase, peroxidise, ascorbate, glutathione reductase, proline, glycine betaine. Habib *et al.* (2016) reported that SNP seed priming increased the leaf phenolic and ascorbic acid content in salt stressed plants. Proline accumulation was enhanced by NO in wheat and cucumber under salt stress (Ruan *et al*., 2004 and Fan *et al.*, 2012). SNP is also known to influence shoot and root growth, the pattern in which root and shoot grow (Liao *et al*., 2011; Zhao *et al*., 2015), floral regulation, seed germination, photosynthesis. Zhang *et al*. (2006); Habib *et al*. (2010) reported that SNP can improve seed germination and seedling growth. Priming with calcium chloride for a day with osmotic potential of -1 MPa reduced the salt stress injury (Yousof, 2013). Afzal *et al*. (2012) reported that seed priming with $CaCl_2$, KCl, H_2O_2 induced salt tolerance in Shaheen basmati (saline tolerant) and Basmati-2000 (sensitive). Priming also improved early crop stand, seedling length and seedling dry weight. The author continued that seed priming with calcium chloride brought about small leaf Na^+ accretion at the expense of K^+ uptake for both rice varieties, but the effect was more pronounced in tolerant genotype. Exogenous application of NO improved biomass production and leaf chlorophyll content by activating antioxidants that scavenge free radicals (Dong *et al.*, 2014; Kausar *et al*., 2013). Use of calcium chloride in priming increases the availability of calcium in plants. Calcium is known to increase root growth and leaf development (Amor and Marcelis, 2003). Almeida *et al*.,2016 reported that use of calcium chloride as foliar spray is more beneficial than CaO and calcium chelate. Youssef *et al*. (2017) reported that foliar spray of calcium chloride increased the calcium content in plant leaf.

b) Beta amino butyric acid

Beta- amino butyric acid (BABA) is a 4 carbon, non-protein amino acid with the formula CH_3-CH_2 -(NH_2)-CH_2-COOH. The amino group in BABA is located at the third carbon ie. beta position. BABA is the only isomer of amino butyric acid capable of inducing plant resistance (Cohen *et al*., 2016). According to Cohen *et al*., (2016) beta-amino butyric acid (BABA) is active against abiotic stress and enhances salt, heat and drought tolerance in several plant species. The author also reported that BABA is highly systemic, readily taken up by roots and leaves, and translocated both acropetally and basipetally. BABA is effective as a foliar spray, soil drench and seed treatment.

Ton *et al*. (2005); Jakab *et al*. (2005) reports salt tolerance in Arabidopsis due to the priming effect of BABA. Mostek *et al*. (2016) reported BABA induced salt tolerance in Barley. Jisha and Puthur (2016) reported salt tolerance in bean and mungbean. They also showed that on priming with beta amino butyric acid the photosynthetic pigment content and mitochondrial activities increase. They continued that BABA seed priming enhanced proline, total protein, total carbohydrate, nitrate reductase activity and activity of peroxidise and superoxide dismutase. Reduction of malondialdehyde was also found in seedlings.

Phytotoxicity is a major concern when using plant chemicals and so checking for plant toxic symptoms is of utmost importance. Beta amino-buytric acid can impose growth stress and phytotoxicity in some plants if used at a higher dose.

Transgenerational inheritance has been reported in BABA. Luna *et al*. (2012) suggested the transgenerational effects of BABA due to epigenetic mechanisms. Cohen *et* *al*. (2016) reported the possibility that BABA induced resistance is controlled by various defense signaling pathways.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The research work “Chemical priming for improving salinity tolerance in rice” was conducted during 2018-2020. The materials and methods used in the study are given below:

3.1 Location

The experiment was conducted at Rice Research Station, Vytilla of Kerala Agricultural University which comes under the *Pokkali* tract of Ernakulam district. This institute, located at 10° N latitude and 76°15¹ E longitude, lies 1.2 m above mean sea level, and undertakes research on various aspects of rice and rice-based farming systems in the salinity prone coastal tracts of Kerala.

3.2 Soil

Pokkali soils are acid sulphate soils with extreme acidity with pH ranging from 3 to 5.5. The presence of sulphuric acid, iron and aluminium sulphates which get accumulated by tidal incursions make this soil highly acidic. The soils of this region are clayey with high organic matter content. The periodic high and low tide influences the nutrient status and salinity of the soil. During November this area records EC levels of 12 dS/m to 24 dS/m. This phase is considered as the saline phase. By June when the South West monsoon lashes over Kerala, the salts are washed away reducing the EC levels to 6 dS/m and below. The period from May to October is considered as the non-saline phase during which rice is cultivated. The physico- chemical characteristics of soil in the experimental site are detailed in Table 1.

Table 1. Physico-chemical properties of *Pokkali* soil

Particulars	**Content**	**Method used**
1.Textural composition		
Coarse sand (%)	4.76	Robinson international pipette method (Piper, 1966)
Fine sand (%)	24.00	
Silt (%)	18.24	
Clay (%)	53.00	
Soil type	Clay loam	
2.Chemical properties		
pH	4	1:2.5 soil water ratio Beckman glass electrode (Jackson, 1958)
EC	0.48	1:2.5 soil water ratio Beckman glass electrode (Jackson, 1958)
Organic carbon (%)	2.44	Walkley and Black method (Jackson,1958)
Available N(kg/ha)	64.30	Alkaline permanganate method (Subbiah and Asija, 1956)
Available P(kg/ha)	9.36	Bray-1 extractant ascorbic acid reductant method (Watnabe and Olsen,1965)
Available K (kg/ha)	368.00	Neutral normal ammonium acetate extractant flame photometry (Jackson,1958)

3.3 Climate and weather

The region experiences a warm humid climate. The crop was raised during 2019. The average annual rainfall received was 250 cm. During 2019, the peak monthly rainfall was observed in October (772.5 mm). The mean evaporation rate during the cropping season was 2.7 mm/day. The maximum temperature ranged from 29 – 31^{0}C and the minimum temperature between 21-26^{0}C. The relative humidity ranged from 74 to 82%.

The EC of soil was low compared to previous years due to unexpected rains and flooding. In normal year usually the EC of 12 dS/m will be recorded by December. However, the maximum salinity recorded was 6 dS m^{-1} in December 2019. All the important meteorological observations are presented in Appendix 1and illustrated in Fig.1

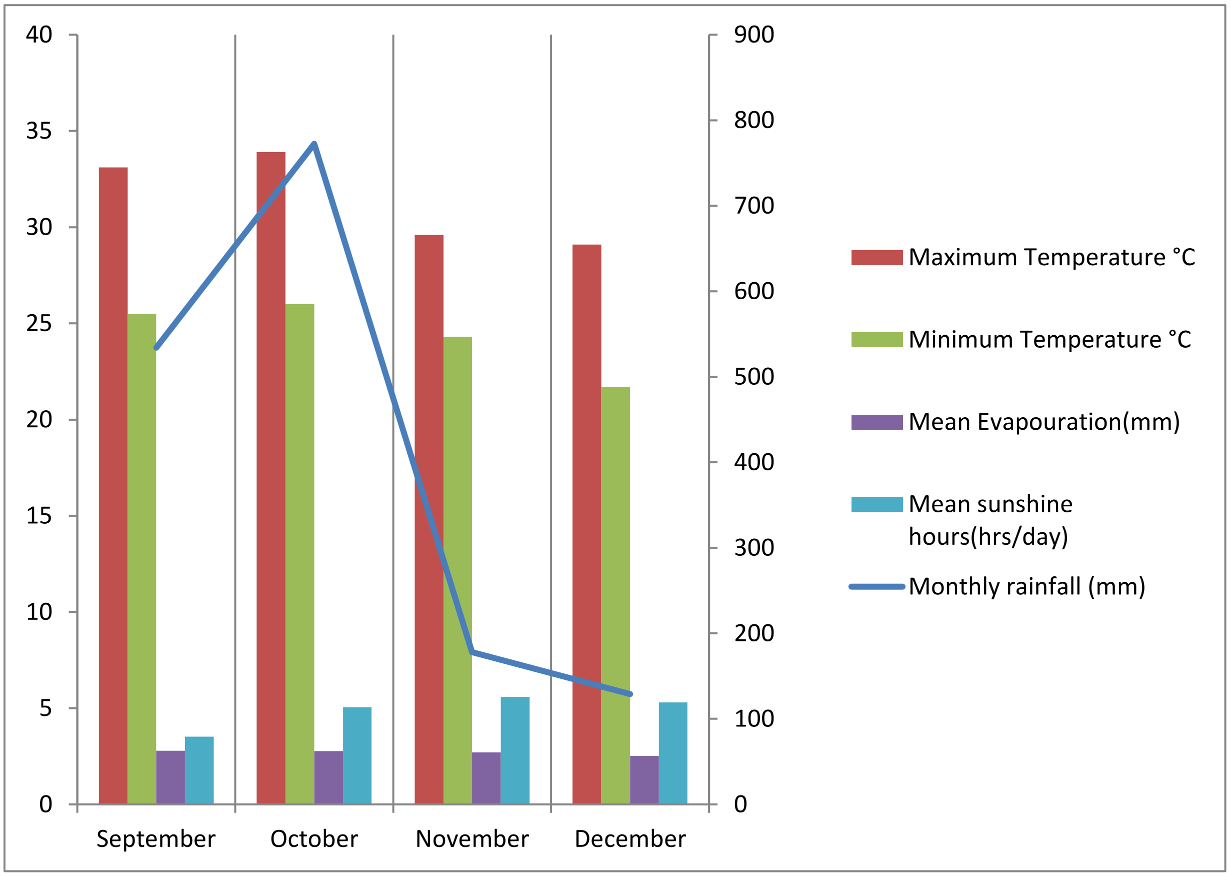


Figure 1. Climatic parameters during the cropping season

3.4 Experiment details

The research programme consisted of three experiments**:**

Experiment 1. Screening rice varieties for salinity tolerance.

Experiment 2. Effect of seed priming on germination of rice varieties under salt stress conditions.

Experiment 3. Effect of foliar spray of priming chemicals on the performance of rice under salt stress condition.

Experiment 1: Screening rice varieties for salinity tolerance

Three popular rice varieties (Jyothi, Uma and Vyttila-10) were screened during *kharif* season for salinity tolerance. Uma and Jyothi are the most popularly used high yielding varieties of Kerala, while Vyttila-10 is an improved medium duration variety tolerant to salinity, released from Rice Research Station, Vyttila.

The seeds were subjected to different levels of salinity (0,3,6,9,12 dS/m) by using different concentrations of NaCl. The concentrations of NaCl which caused retardation in various growth attributes (shoot length, root length and dry weight) were considered as stress imparting salinity levels. The trial was laid out in completely randomised block design (CRD) replicated thrice. The 15 treatments were the combination of three varieties Jyothi, Uma and Vytilla-10 and five levels of salinity including control (T_1V_1,T_2V_1,T_3V_1,T_4V_1,T_5V_1**,** T_1V_2,T_2V_2,T_3V_2,T_4V_2,T_5V_2, T_1V_3,T_2V_3,T_3V_3,T_4V_3,T_5V_3)**.**

The salinity levels tried were 3 dS/m, 6 dS/m, 9 dS/m, 12 dS/m and non -saline control (Gregorio *et al.*,1997).

T_1 - Non-saline,T_2 -3 dS/m T_3 - 6 dS/m, T_4 - 9 dS/m, T_5 - 12dS/m

V_1 - Jyothi, V_2 - Uma, V_3 - Vytilla 10

The quantity of NaCl used for preparing solutions of different salinity levels are given below:

Sl. No.	EC (dS/m)	NaCl(g) per 100 ml distilled water
1.	3	0.2
2.	6	0.4
3.	9	0.6
4.	12	0.8

Screening for salinity tolerance was done at seedling stage. 100 seeds each of Jyothi, Uma and Vytilla -10, were placed in petri dishes with tissue paper that was pre-soaked in respective saline treatments and were incubated for 48 hours at room temperature for germination.

On third day germinated seeds were transferred to floats maintained at varied salinity levels. The setup was prepared with rectangular plastic tubs and trays, plastic net sheets along with fabricated seedling floats (acrylic make) with holes. Two pre-germinated seeds were sown per hole on the fabricated seedling float. Yoshida nutrient solution (Yoshida *et al.*,1976) with respective salinity was added to these floats and the solution was renewed every eight days and the pH was maintained at 5.5 daily by adding either NaOH or 1N HCl. Observations were taken at 10th and 16th days.

Preparation of Yoshida nutrient solution (Yoshida *et al.*,1976)

Two separate solutions containing macronutrients and micronutrients respectively were prepared for growing rice seedlings in floats, the details of which are furnished below.

Composition of macronutrient solution

Element	Chemical	Quantity used (g/ 100 L)
N	NH_4NO_3	11.425
P	$NaH_2PO_4.H_2O$	5.030
K	K_2SO_4	8.925
Ca	$CaCl_2.2H_2O$	14.670
Mg	$MgSO_4.7\ H_2O$	40.500

Composition of micronutrient solution

Preparation of stock solution: Dissolved each reagent separately in distilled water then added 200ml H_2SO_4 and made up the volume to 4L using distilled water.

Element	Chemical	Quantity used for 4L stock solution
Mn	$MnCl_2.4\ H_2O$	6
Mo	$(NH_4)_6Mo_7O_{24}$	0.296
Zn	$ZnSO_4.\ 7\ H_2O$	0.140
B	H_3BO_3	3.736
Cu	$CuSO_4.5\ H_2O$	0.124
Fe	$FeCl_3.6\ H_2O$	30.8
	Citric acid monohydrate	47.6

Preparation of Yoshida solution

To one litre of prepared macronutrient solution, 1.25 ml of micronutrient solution was added with constant stirring and the pH was adjusted to 5.5 by adding concentrated NaOH solution. For five floats 100 L of media was prepared. Floats were prepared by adding 20 L of nutrient media and the EC was adjusted by adding salt to each float.

Observations

Germination percentage~~:~~

Number of seeds germinated in a lot of 100 test seeds was recorded and expressed as percentage

Root length and shoot length

Length of root was measured at 14 DAS and expressed in cm. Length of shoot upto leaf tip was also taken at 14 DAS and expressed in cm.

Phenotypic screening

Standard Evaluation Score (SES) of visual salt injury was done at seedling stage and vegetative stage (Gregorio *et al*., 1997).

Modified standard evaluation scoring method for salinity tolerance in rice

Score	**Visual symptoms/observations**	**Tolerance**
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled	Tolerant
5	Growth severely retarded most leaves rolled,only a few are elongating	Moderately tolerant
7	Complete cessation of growth most leaves dry, some plants drying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Experiment 2. Effect of seed priming on germination of rice varieties under salt stress conditions

The experiment was laid out in two factorial completely randomized block design (CRD) replicated thrice. The experiment was conducted during 02-08-2019 to 18.8.2019.

Treatments

The three rice varieties were Jyothi, Uma and Vytilla-10. The treatment combinations were four priming chemicals sodium nitroprusside(C1), calcium chloride (C2), beta amino-butyric acid (C3), hydropriming (C4) and three salinity levels (based on the results of experiment 1) EC of 6 dS/m(S1), 9 dS/m(S2) and 12 dS/m (S3).

Rice seeds were primed in the respective solution for 12 hours, using1:3 seeds to solution ratio. Seeds were then removed from the solutions and shade dried at room temperature to bring back to its original moisture content. Germination test was conducted by pre-soaking 100 seeds each at different salinity levels and placing in petri dishes lined with tissue paper soaked in respective saline solution. Jyothi and Uma varieties were treated with salinity levels of 6 and 9 dS m^{-1}while Vyttila-10 was treated with salinity levels of 9 and 12 dS m^{-1} based on the results of experiment 1.

TREATMENT COMBINATIONS

V1C1 S1	V1C1 S2
V1C2 S1	V1C2 S2
V1C3 S1	V1C3S2
V1C4S1	V1C4 S2
V2C1 S1	V2C1 S2
V2C2 S1	V2C2 S2
V2C3 S1	V2C3S2
V2C4S1	V2C4 S2
V3C1 S3	V3C1 S2
V3C2 S3	V3C2 S2
V3C3 S3	V3C3S2
V3C4 S3	V3C4 S2

V1-Jyothi, V2-Uma, V3-Vytilla-10

C1- BABA, C2 - $CaCl_2$, C3 - SNP, C4 – hydro priming

S1- 6 dS/m, S2-9 dS/m, S3- 12 dS/m

Floats containing 20 L of Yoshida nutrient solution at respective salinity levels were taken. Three days after sowing primed seedlings were transferred from petri dishes to floats and were maintained upto18 days.

Observations

Germination parameters like germination percentage, shoot length, root length and phenotypic screening were recorded as in the case of experiment 1 up to 21 days.

Experiment 3: Field experiment to study the effect of seed priming

Seedlings obtained from seeds primed by using above mentioned chemicals were transplanted to the main field. Field trial was conducted in micro plots of size 2mx1m to assess the effect of seed priming on the growth and yield of rice in fields with saline water intrusion.

Three varieties initially used in the experiment were Jyothi, Uma and Vyttila-10. During flash floods of September, the crop was completely submerged for a period of 5 days and as a result both Jyothi and Uma varieties failed to survive. The experiment was continued with variety Vyttila-10.

In 2019, the monsoon showers were very weak during the month of June. As a result, there was a delay in the start of cropping season and the crop was raised from September 16 to December 24, 2019.

Experiment details

Field trial to assess the effect of seed priming in rice fields with saline intrusion was conducted at Rice Research Station, Vyttila. Variety Vyttila-10 and four priming chemicals were used for the study. To study the effect of priming chemicals as foliar spray, the chemicals were sprayed on the standing crop at different intervals. The experiment was laid out in Factorial RBD replicated thrice. The treatments included

Chemicals (seed priming)

P_1-Beta-amino butyric acid -1Mm/L

P_2-Calcium chloride -2 per cent

P_3-Sodium nitroprusside -100 μM

P_4-Hydropriming

Interval for foliar spray (respective priming chemical)

F_1- Weekly intervals (30,37 and 44 DAS)

F_2- Fortnightly intervals (30,45 and 60 DAS)

F_3-Water spray

P1F3R3	P2F3R3	P3F3R3	P4F3R3
P1F3R2	P2F3R2	P3F3R2	P4F3R2
P1F3R1	P2F3R1	P3F3R1	P4F3R1
P1F2R3	P2F2R3	P3F2R3	P4F2R3
P1F2R2	P2F2R2	P3F2R2	P4F2R2
P1F2R1	P2F2R1	P3F2R1	P4F2R1
P1F1R3	P2F1R3	P3F1R3	P4F1R3
P1F1R2	P2F1R2	P3F1R2	P4F1R2
P1F1R1	P2F1R1	P3F1R1	P4F1R1

Figure 2. Layout of Field experiment

Plate 1. Field view after lay out of the experiment

Plate 2. Transplanting

Plate 3. Crop at 40 days after transplanting (variety – Vyttila 10)

Plate 4. Crop at harvest stage

Land preparation

Field preparation commenced during the month of May. Outer bunds were strengthened and sluices for water regulation were repaired. The field was drained during low tide and also with the help of petti and para. Shutters were placed preventing further entry of water. After strengthening of bunds, mounds of $1m^2$ base and 0.5m height were taken at distance of about 90 cm to facilitate the washing down of salts during rains. Lime was applied on top of the mounds. When all salts were washed off by rain the mounds were dismantled and levelled.

Seed priming and transplanting

100 gm each seeds of Vytilla-10 was primed using beta-amino butyric acid (BABA), sodium nitroprusside (SNP), calcium chloride ($CaCl_2$) and water as per the treatment and were shade dried. Seeds were re-soaked in water for 24 hours for germinating the seeds. The germinated seeds were sown in trays. Later 14 days old seedlings were transplanted into main field at a spacing of 20 cm x 15 cm.

Foliar spraying

Foliar spray at the same priming dosage were given using priming chemicals (BABA, SNP, $CaCl_2$, hydro).

Water management

Bunds were strengthened and sluices were set up for regulating water in the field. However, during continuous heavy rains, management of water was not possible and crop experienced complete submergence.

Weed management

Major weeds observed in the field included *Diplachne fusca*, *Eleocharis dulcis Monochoria vaginalis*, and *Ludwigia* spp. These weeds were removed by hand weeding.

Management of pests and diseases

Generally, pest and disease occurrence were less in *Pokkali* as compared to other cropping systems. To protect the crop from non -insect pests like sparrows and purple moorhen the entire field was covered with net.

Harvest

Crop was harvested by cutting the panicles alone, leaving the straw in the field itself. The total duration of the crop was 115 days.

Observations

1.Growth parameters

a) Plant height

Plant height was measured using a meter scale from the base of the plant to the tip of the longest leaf at 20, 40 and 60 days after transplanting and was recorded in cm. This was done for 10 randomly selected plants.

b) No. of tillers per m^2

A quadrat of 0.5m x0.5m (0.25m^2) was used to count the number of tillers. Observations were taken at 20, 40 and 60 days after transplanting.

c) Leaf area index

Leaf area was calculated by measuring the length and width of each leaf of five randomly selected plants calculating the mean area. Leaf area per hill was divided by spacing to get the LAI. Observations were taken at 20, 40 and 60 days after transplanting.

2)Yield and yield parameters

a) No. of productive tillers

A quadrat (1 m^2) was used to find number of productive tillers per m^2.

b) Grains per panicle

The grains per panicle was counted for five panicles in each treatment and average was worked out

c) Filled grains per panicle

Filled grains per panicle was counted for five panicles in each treatment and average was worked out

d) Thousand grain weight

One hundred filled grains were counted and weighed from each treatment and thousand seed weight was estimated and was expressed in grams.

d) Grain yield

Plants in each plot was separately harvested, threshed and dried and weight of grain was recorded and expressed in kg/ha.

3. Biochemical parameters

Chlorophyll content

Third fully opened leaf from each plot was collected at 20, 40, 60 DAT and chlorophyll was assessed using DMSO method (Arnon, 1949 and Yoshida, 1972) and estimated in spectrophotometer and expressed in mg/g.

Proline

Third fully opened leaf from each plot was collected at 20, 40, 60 DAT and proline was assessed using the method of Bates *et al*. (1973). The absorbance was read in spectrophotometer at 520 nm and expressed in µg/g.

3.5 Statistical Analysis

Data were statistically analysed by applying analysis of variance (ANOVA) with the help of statistical programme OPSTAT (Sheoran *et al.*,1998).

RESULTS

4.RESULTS

A study was conducted at RRS, Vytilla during *Kharif*, 2019 season to study the effect of chemical priming in rice for salinity tolerance. The study consisted of three parts. The first part included screening of three rice varieties, Jyothi, Uma and Vytilla-10 for their salinity tolerance at different levels of salinity (non- saline, 3 dS/m, 6 dS/m, 9dS/m and 12 dS/m). The second, study was conducted to assess the effect of priming chemicals, viz. beta amino-butyric acid (BABA), $CaCl_2$ and sodium nitroprusside (SNP) for salinity tolerance improvement in the three rice varieties. The third part was undertaken to verify the results of priming in actual field condition. The data related to growth parameters were tabulated and analyzed statistically and results are presented in this chapter.

PART 1. Screening rice varieties for salinity tolerance

4.1 Response of rice variety Jyothi to salinity levels

The data on germination and growth parameters of different rice varieties recorded at 10, 16, 12 DAS are furnished in Table 2, Table 3 and Table 4, respectively.

In variety Jyothi germination percentage was affected by the level of salinity. At the highest salinity level of 12 dS/m (S5) a significantly lower germination percentage of 78 % was observed while at all other salinity levels germination percentage was on par and the values ranged from 85 to 93%.

Plant height progressively decreased with increase in salinity and height at all the salinity levels differed significantly from each other. The seedling height was highest in control (18.47 cm) whereas it was only 11.70 cm at salinity level of 12 dS/m.

At 16 DAS and 21 DAS, (Table 3 and 4) the seedling heights at 3 dS/m and non-saline medium were on par. In control the plant height increased from 18.47 cm at 10 DAS to 21.37 cm at 21 DAS, while in the case of higher salinity level of 12 dS/m there was only a marginal increase from 11.70 cm at 10 DAS to 12.43 cm at 21 DAS. Statistically significant difference in height was observed between salinity levels also.

Seedling root length was also influenced by salinity and a progressive increase in root length was observed with increase in salinity. The root length in control (9.35cm) and at lower salinity level of 3 dS/m (10.95 cm) were comparable, while the longest roots were observed under the highest salinity level of 12 dS/m (15.50 cm) at 10 DAS. Also, all the salinity levels differed significantly from each other with respect to seedling root length.

By 16 and 21 days after sowing the root length increased in all treatments. The shorter root was observed for control at all stages of observation. In general, all the treatments varied significantly in root length at all the stages of observation.

There was no significant difference in leaf number of the seedlings at different salinity levels at all stages of observation.

4.2 Response of rice variety Uma to salinity level

The data on growth parameters influenced by salinity at 10 DAS, 16 DAS and 21 DAS are furnished in Table 2, Table 3 and Table 4.

In the case of variety Uma, germination was not affected by salinity levels 3,6,9 and12 dS/m. However, the plant height progressively declined with increasing salinity level. Seedling height was highest in control (16.4 cm), while at 12 dS/m plant height was very low (10.267 cm) at 10 DAS. Salinity levels of 3 dS/m, 6 dS/m and 9 dS/m were having comparable plant height at 10 DAS. The seedling height was comparable at salinity levels 3 dS/m, 6 dS/m at all stages of observation. The seedlings shoot length in normal environment progressively increased from 16.4 cm at 10 DAS to 20.23 cm at 21 DAS.

Increase in seedling root length was noticed with increase in salinity level. The root length was lowest for seedlings grown in normal condition (9.35 cm), while highest salinity level of 12 dS/m had the greatest root length (14.2 cm) at 10 DAS. The seedling root length were comparable for non- saline and 3 dS/m at 10 DAS, while at 16 DAS and 21 DAS the root length varied significantly. Root growth was found to be arrested in salinity above 6 dS/m. At 21 DAS and 16 DAS root length was found to increase with increase in salinity upto 9 dS/m and then decrease with further increase in salinity to 12 dS/m.

There was no significant difference in leaf number of the seedlings at different salinity levels at all stages of observation.

Table 2. Growth parameters of rice varieties as influenced by salinity levels at 10 days after sowing

Salinity level	Germination %	Plant height (cm)	Root length (cm)	Number of leaves per seedlings
Variety Jyothi				
S1 (non-saline)	85	18.47	9.35	2.00
S2 (3 dS/m)	89	18.03	10.95	2.00
S3 (6 dS/m)	89	15.40	12.25	2.00
S4 (9 dS/m)	93	14.03	13.95	2.00
S5 (12 dS/m)	78	11.70	15.50	2.00
S.E(m)	2.89	0.04	0.30	0.23
C.D(0.05)	9.23	0.12	1.103	**NS**
Variety Uma				
S1 (non-saline)	93	16.40	9.35	2.00
S2 (3 dS/m)	92	15.37	10.20	2.00
S3 (6 dS/m)	90	15.20	11.40	2.00
S4 (9 dS/m)	88	14.47	12.45	2.00
S5 (12 dS/m)	90	10.27	14.20	2.00
S.E(m)	3.48	0.28	0.13	0.23
C.D(0.05)	NS	0.90	0.48	**NS**
Variety Vytilla-10				
S1 (non-saline)	83	19.30	11.50	2.00
S2 (3 dS/m)	83	18.60	12.10	2.00
S3 (6 dS/m)	80	18.03	13.25	2.00
S4 (9 dS/m)	87	17.27	14.25	2.00
S5 (12 dS/m)	77	16.17	15.35	2.00
S.E(m)	3.28	0.27	0.17	0.23
C.D(0.05)	NS	0.85	0.62	**NS**

Table 3. Growth parameters of rice varieties as influenced by salinity levels at 16 days after sowing

Salinity level	Plant height (cm)	Root length (cm)	Number of leaves
Jyothi			
S1 (non-saline)	19.80	14.10	2.00
S2 (3 dS/m)	19.40	15.50	2.00
S3 (6 dS/m)	16.50	17.10	2.00
S4 (9 dS/m)	15.30	18.95	2.00
S5 (12 dS/m)	12.37	20.50	2.00
S.E(m)	0.21	0.32	0.23
C.D(0.05)	0.68	1.20	**NS**
Uma			
S1 (non-saline)	17.50	11.25	2.00
S2 (3 dS/m)	16.40	13.20	2.00
S3 (6 dS/m)	16.23	14.05	2.00
S4 (9 dS/m)	15.43	14.60	2.00
S5 (12 dS/m)	11.50	14.20	2.00
S.E(m)	0.26	0.170	0.23
C.D(0.05)	0.83	0.63	NS
Vytilla-10			
S1 (non-saline)	22.67	13.95	2.00
S2 (3 dS/m)	19.97	15.30	2.00
S3 (6 dS/m)	18.33	16.00	2.00
S4 (9 dS/m)	18.03	16.85	2.00
S5 (12 dS/m)	16.50	17.75	2.00
S.E(m)	0.43	0.14	0.23
C.D(0.05)	1.37	0.52	NS

Table 4. Growth parameters of rice varieties as influenced by salinity levels at 21 days after sowing

Salinity level	Plant height (cm)	Root length (cm)	Number of leaves
Jyothi			
S1 (non-saline)	21.37	16.05	2.00
S2 (3 dS/m)	20.73	17.05	2.00
S3 (6 dS/m)	17.70	17.10	2.00
S4 (9 dS/m)	15.10	18.95	2.00
S5 (12 dS/m)	12.43	20.50	2.00
S.E(m)	0.47	0.23	0.228
C.D(0.05)	1.49	0.84	NS
Uma			
S1 (non-saline)	20.23	12.05	2
S2 (3 dS/m)	18.40	13.90	2
S3 (6 dS/m)	17.40	14.95	2
S4 (9 dS/m)	16.30	14.60	2
S5 (12 dS/m)	13.23	14.20	2
S.E(m)	0.32	0.10	0.23
C.D(0.05)	1.03	0.35	NS
Vytilla-10			
S1 (non-saline)	26.33	15.05	2.00
S2 (3 dS/m)	24.17	15.25	2.00
S3 (6 dS/m)	21.00	16.30	2.00
S4 (9 dS/m)	19.33	17.05	2.00
S5 (12 dS/m)	19.10	17.75	2.00
S.E(m)	0.53	0.06	0.23
C.D(0.05)	1.70	0.24	NS

4.3 Vytilla -10

The data on growth parameters as influenced by different levels of salinity at 10 DAS, 16 DAS and 21 DAS of Vytilla-10 are presented in Table 2, Table 3 and Table 4, respectively.

Germination of seeds were found to be independent of salinity level of the media even upto 12dS/m**.** Seedling height was influenced by the level of salinity. In plants grown under normal conditions, the plant height increased from 19.3 cm at 10 DAS to 26.33 cm at 21 DAS, while marginal increase in plant height from 16.17 cm at 10 DAS to 19.1 cm at 21 DAS was observed for seedlings grown at 12 dS/m. The height of the seedlings was comparable for non- saline and 3 dS/m at 10 DAS. At 16 DAS and 21 DAS, seedling height for 6 dS/m and 9 dS/m were comparable.

Seedling root length was also influenced by salinity and as in the case of Jyothi and Uma, a progressive increase in root length was observed with increase in salinity. The seedling root length in non-saline and at 3 dS/m were comparable at 10 DAS and 21 DAS, while at 16 DAS the seedling root length of 6 dS/m and 3 dS/m were comparable. Under normal conditions the root length was 15.05 cm at 21 DAS, while the increase was drastic in higher salinity level of 12 dS/m, where root length of 17.75 cm was recorded at 21 DAS. There was no further increase in root length after 16 DAS.

There was no significant difference in leaf number at all stages of observation.

4.4 Visual symptoms in response to salinity

The data on phenotypic scoring of rice varieties are furnished below as Table 4.

In Jyothi the score was found to increase with increase in salinity level from 3 dS/m. No further increase in phenotypic score at different stages was noticed at 3 dS/m. Leaf rolling and white tip symptoms were noticed at 10 DAS even at salinity level of 3 dS/m. Growth retardation in terms of seedling height was noticed for salinity level above 6 dS/m at 10 DAS. At 16 DAS, complete cessation of growth and plant death was noticed for salinity level above 6 dS/m. At 21 DAS, almost all seedlings dried up completely at salinity level above 6dS/m, while plants subjected to salinity level 3 dS/m seedlings remained tolerant showing leaf rolling and white tip symptoms. At salinity above 6 dS/m was found to be critical for the seedling growth.

In Uma, at 21 DAS plants in 3 dS/m salinity level was found affected by salinity. Leaf rolling and white tip symptoms were noticed for salinity 3dS/m at 21 DAS and for salinity 6 dS/m at 10 DAS. At 16 DAS growth retardation was noticed at 6 dS/m and 9 dS/m while growth retardation was noticed for plants raised under 9 dS/m and 12 dS/m at 10 DAS. Complete cessation of plant growth was observed at 16 days in 12 dS/m. At 21 DAS, leaf rolling and white tips were noticed even at the lowest salinity level of 3 dS/m. At salinity level of 12 dS/m all plants dried.

In Vytilla-10 normal growth was observed for plants at all stages in non- saline, 3 dS/m and 6 dS/m. Leaf rolling was observed in salinity level of 9 dS/m at 10 DAS, while at 16 DAS growth retardation was observed for salinity level of 9 dS/m. Growth retardation was noticed at 12 dS/m at 10 DAS and almost all plants were dried at 16 DAS in 12 dS/m. At 21 days cessation of growth was observed at 9 dS/m.

Table.5 Scoring for salinity tolerance of rice varieties at different growth stages

Scoring at different stages				
Variety	**Salinity**	**10 DAS**	**16 DAS**	**21 DAS**
Jyothi	S1 (non-saline)	1	1	1
	S2 (3 dS/m)	3	3	3
	S3 (6 dS/m)	5	7	9
	S4 (9 dS/m)	5	7	9
	S5 (12 dS/m)	5	7	9
Uma	S1 (non-saline)	1	1	1
	S2 (3 dS/m)	1	1	3
	S3 (6 dS/m)	3	5	7
	S4 (9 dS/m)	5	5	9
	S5 (12 dS/m)	5	7	9
Vytilla-10	S1 (non-saline)	1	1	1
	S2 (3 dS/m)	1	1	1
	S3 (6 dS/m)	1	1	1
	S4 (9 dS/m)	3	5	7
	S5 (12 dS/m)	5	9	9

PART 2: Effect of seed priming on germination of rice varieties under salt stress conditions

The effect of the three priming chemicals beta aminobutyric acid (BABA), sodium nitroprusside (SNP) and $CaCl_2$ as well as water in increasing the salt tolerance of three varieties (Jyothi, Uma and Vytilla-10) were analyzed separately and the data are presented below.

4.5 Effect of priming chemicals on rice variety Jyothi

Priming enhanced germination in Jyothi when compared to hydropriming and highest germination % was observed in seeds primed with BABA (95%). This was followed by SNP (90%) and $CaCl_2$(90 %) treated seeds. Seed germination was comparable for both calcium chloride priming and sodium nitroprusside. Germination percentage improved by about 10 percentage with BABA treatment. When the two levels of salinity were analyzed, it was observed that as the salinity increased, priming enhanced seed germination in rice. At salinity level 9 dS/m, 93 % germination was observed. Highest interaction effect between salinity level and priming chemical in influencing germination was observed for calcium chloride priming at 9 dS/m.

Priming chemicals did not influence seedling height at 10 DAS, while at 16 DAS calcium chloride and BABA primed seedlings were found to have more height. The seedling height was comparable in BABA and calcium chloride primed seedlings. The seedling heights were comparable for SNP and water. No interaction effect was observed and salinity did not affect seedling height at 10 DAS and 16 DAS.

At 10 DAS seedling root length was not affected by priming, while at 16 DAS seedling root length was affected by priming treatments. The highest root length was observed for calcium chloride (8.92 cm) and the value was comparable with seedlings primed using water (8 cm) and BABA (7.42 cm). The lowest root length at 16 DAS was for seedlings primed using sodium nitroprusside (6.27 cm). The level of salinity positively influencing seedling root length (7.36 cm) was 9 dS/m at 10 DAS, while at 16 DAS there was no significant difference. Interaction effect was nil at 10 DAS, while the interaction effect was significant at 16 DAS for hydro primed seedlings and it was highest (10.5 cm) in salinity level of 9 dS/m. The seedling root length was comparable for water priming at 9 dS/m (10.5 cm), calcium chloride at 9 dS/m (9.77 cm) and calcium chloride 6 dS/m (8.07 cm). There was no significant difference in number of leaves at any stage of observation.

At 10 DAS, SNP was found to significantly improve dry weight (0.076 g/ seedling). However, at 16 DAS calcium chloride primed seedlings had more dry weight (0.090 g/ seedling). Biomass production was found higher at 9 dS/m salinity level (0.080 g/ seedling) than 6dS/m (0.061 g/seedling) at 10 DAS. At 16 DAS also the highest dry weight was for 9 dS/m (0.077 g/seedling). Calcium chloride at 9 dS/m (0.084 gm) and SNP at 9 dS/m (0.080 gm) had highest and comparable biomass production, while at lower salinity level SNP (0.072 g/seedling) significantly increased dry weight of seedling while the other two were unable to enhance biomass production, but maintained same biomass as that of hydro-primed seeds. At 16 DAS highest dry weight was noticed in calcium chloride primed seedlings (0.091 g/seedling). The values are presented in Table 6, Table 7, Table 8 and Table 9.

Table.6 Effect of seed priming on growth parameters in Jyothi at 10 DAS under salt stress conditions

Treatments	**Germination %**	**Growth parameters at 10 DAS**			
		Seedling height (cm)	**Root length(cm)**	**No. of leaves per seedling**	**Dry wt g per seedling**
Variety Jyothi					
BABA	95	8.57	5.07	1	0.065
Calcium chloride	90	8.92	7.17	1	0.071
SNP	90	8.23	5.27	1	0.076
Control /water	85	9.57	6.87	1	0.069
SE (m)	0.29	1.05	1.01	0.14	0.001
C.D(0.05)	1.00	NS	NS	NS	0.003
Salinity levels					
6 dS/m	87	8.68	4.83	1.17	0.061
9 dS/m	93	8.97	7.36	1.08	0.080
S.E (m)	0.20	0.74	0.71	0.10	0.001
C.D(0.05)	0.62	NS	2.16	NS	0.002

Table 7. Interaction effect of priming chemicals and salinity levels on growth parameters of Jyothi at 10 days after sowing

Treatments	Germination %	Seedling height (cm)	Root length (cm)	Number of leaves per seedling	Dry weight (g per seedling)
Salinity level 6 dS/m					
BABA	99	8.77	4.17	1.00	0.057
$CaCl_2$	80	9.67	5.77	1.00	0.058
SNP	90	7.37	4.40	1.00	0.072
Control/water	80	8.90	4.97	1.00	0.058
Salinity level 9 dS/m					
BABA	90	8.37	5.97	1.00	0.073
$CaCl_2$	100	8.17	8.57	1.00	0.084
SNP	91	9.10	6.13	1.00	0.080
Control/water	90	10.23	8.77	1.00	0.081
S.E (m)	0.41	1.48	1.43	0.20	0.001
C.D (0.05)	1.23	NS	NS	NS	0.004

Table 8. Effect of seed priming on growth parameters in Jyothi at 16 DAS under salt stress conditions

Treatments	**Seedling height(cm)**	**Root length(cm)**	**No of leaves**	**Dry w.t (g/ seedling)**
BABA	22.93	7.42	2.00	0.070
$CaCl_2$	23.37	8.92	2.00	0.090
SNP	17.00	6.27	2.00	0.079
Control/water	17.87	8.00	2.00	0.062
S.E (m)	1.74	0.59	0.12	0.001
C.D (0.05)	**5.26**	**1.79**	**NS**	**0.003**
6 dS/m	19.41	7.09	1.83	0.073
9dS/m	21.18	8.21	2.08	0.077
S.E (m)	1.23	0.42	0.08	0.001
C.D (0.05)	**NS**	**NS**	**0.25**	**0.002**

Table 9. Interaction effect of priming chemicals and salinity levels on growth parameters of Jyothi at 16 days after sowing

Treatments	Seedling height (cm)	Root length (cm)	Number of leaves per seedling	Dry weight (g/ seedling)
Salinity level 6 dS/m				
BABA	23.43	7.60	2.00	0.065
$CaCl_2$	20.06	8.07	2.00	0.089
SNP	15.83	7.20	2.00	0.078
Control /water	18.30	5.50	2.00	0.061
Salinity level 9 dS/m				
BABA	22.43	7.23	2.00	0.074
$CaCl_2$	26.67	9.77	2.00	0.091
SNP	18.17	5.33	2.00	0.081
Control /water	17.43	10.50	2.00	0.063
SE (m)	2.46	0.85	0.17	0.001
C.D (0.05)	NS	2.52	NS	0.004

4.6 Effect of priming chemicals on growth parameters in Uma under different levels of salinity

In Uma germination was found highest for $CaCl_2$(95%) and control (95%) and the gemination percentage were comparable. SNP and BABA were having the lowest germination and were comparable. Germination was found to be affected by higher levels of salinity. Seed germination was more at lower salinity level of 6dS/m. Water priming (100%) at salinity level 6 dS/m and calcium chloride (100 %) at salinity level 9 dS/m showed comparable and more interaction effect in improving germination percentage.

At 10 DAS, calcium chloride (11.35 cm) and SNP (11.20 cm) treatments showed highest and comparable seedling height, while at 16 DAS calcium chloride (15.4 cm) priming resulted in more seedling height. At all stages of observation, the lowest increase in seedling height was for water primed seedlings. Though seedling height was more at 6 dS/m (11.65 cm) at 10 DAS, seedling height was highest for salinity level 9 dS/m (13.18 cm) at 16 DAS. Interaction effect on seedling height due to seed priming and salinity levels was nil at 10 DAS, but at 16 DAS possible interaction between priming chemical and salinity on shoot length was observed in calcium at both salinity levels. The values of seedling height on calcium chloride priming, at both levels of salinity (15.60 cm at salinity level 6 dS/m and 15.20 cm at salinity level 9 dS/m) were comparable.

Root growth was more for calcium chloride (10. 52 cm) primed seedlings at 10 DAS, while at 16 DAS BABA primed seedlings (11.32 cm) and calcium chloride primed seedlings (8.87 cm) had the highest and comparable root growth. The lowest root length was observed for water priming at 10 DAS (8.65 cm) and SNP (6.18 cm) at 16 DAS. The root length increased at salinity level 6 dS/m (9.93 cm), while at 16 DAS more root length was observed at salinity level 9 dS/m (10.03 cm). There was significant interaction effect at 10 DAS between priming chemicals and salinity levels in calcium chloride (10.70 cm) and SNP (10.47 cm) at salinity level of 6 dS/m and calcium chloride (10.33 cm) at salinity level 9 dS/m. BABA at salinity level of 6 dS/m, was having highest influence on root length(12.367cm) at 16 DAS. None of the priming chemicals had any influence on number of leaves, salinity level was not found to influence leaf number and there was no possible interaction effect due to treatments at any stages of observation.

$CaCl_2$(0.062 g/seedling) and SNP (0.061 g/seedling) recorded the highest and comparable dry weight at 10 DAS while calcium chloride primed seedlings had the highest dry weight (0.073 cm) at 16 DAS. The lowest dry weight was observed for seedlings primed using BABA at 10 DAS and 16 DAS.

More dry weight (0.060 gm/ seedling at 10 DAS and 0.066 gm/seedling) was recorded in seedlings maintained at 9 dS/m salinity. The dry weight was influenced by interaction effect of SNP and salinity level 9 dS/m (0.066 gm), water primed seedling maintained at 9 dS/m (0.063 g/ seedling), calcium chloride at 6 dS/m (0.063 g/seedling) had comparable values at 10 DAS.

All recorded values were analyzed using ANNOVA and presented as Table 10, Table 11, Table 12 and Table 13.

Table 10. Effect of seed priming on growth parameters in Uma at 10 DAS under salt stress conditions

Treatments	**Germination %**	**Growth parameters 10 DAS**			
		Seedling height(cm)	**Root length(cm)**	**No. of leaves**	**Dry wt. (g)**
Variety Uma					
BABA	90	10.07	9.87	1	0.052
$CaCl_2$	95	11.35	10.52	1	0.062
SNP	90	11.20	9.57	1	0.061
Control /water	95	9.85	8.65	1	0.058
C.D (0.05)	**0.71**	**0.97**	**0.27**	**NS**	**0.003**
Salinity levels					
6 dS/m	95	11.65	9.93	1.25	0.056
9dS/m	90	9.58	9.4	1.08	0.060
C.D (0.05)	**0.50**	**0.69**	**0.19**	**NS**	**0.002**

Table 11. Interaction effect of priming chemicals and salinity levels on growth parameters of Uma at 10 days after sowing

Treatments	**Germination %**	**Seedling height (cm)**	**Root length (cm)**	**Number of leaves per seedling**	**Dry weight (g)**
Salinity level 6 dS/m					
BABA	90	11	9.77	1.00	0.054
$CaCl_2$	100	12.27	10.70	1.00	0.063
SNP	89	12.90	10.47	1.00	0.055
Control /water	100	10.63	8.77	1.00	0.054
Salinity level 9 dS/m					
BABA	90	9.33	9.97	1.00	0.051
$CaCl_2$	90	10.43	10.33	1.00	0.062
SNP	89	9.50	8.67	1.00	0.066
Control /water	90	9.07	8.53	1.00	0.063
C.D(0.05)	**1.00**	**NS**	**0.382**	**NS**	**0.004**

Table 12. Effect of seed priming on growth parameters in Uma at 16 DAS under salt stress conditions

Treatments	**Seedling height (cm)**	**Root length(cm)**	**No of leaves**	**Dry w.t(g)**
BABA	12.60	11.32	2.00	0.056
$CaCl_2$	15.40	8.87	2.00	0.073
SNP	13.18	6.18	2.00	0.065
Control /water	9.58	8.07	2.00	0.061
C.D (0.05)	**0.34**	**2.47**	**NS**	**0.002**
6 dS/m	12.20	7.19	2.00	0.061
9 dS/m	13.18	10.03	2.00	0.066
C.D (0.05)	**0.240**	**1.749**	**NS**	**0.002**

Table 13. Interaction effect of priming chemicals and salinity levels on growth parameters of Uma at 16 days after sowing

Treatments	**Seedling height (cm)**	**Root length (cm)**	**Number of leaves per seedling**	**Dry weight (g)**
Salinity level 6 dS/m				
BABA	13.87	12.37	2.00	0.059
$CaCl_2$	15.60	9.23	2..00	0.071
SNP	11.33	2.97	2.00	0.062
Control /water	8.00	4.20	2.00	0.054
Salinity level 9 dS/m				
BABA	11.33	10.27	2.00	0.053
$CaCl_2$	15.20	8.50	2.00	0.075
SNP	15.03	9.40	2.00	0.068
Control /water	11.17	11.93	2.00	0.068
C.D (0.05)	**0.48**	**3.5**	**NS**	**0.003**

4.7 Vytilla -10

Since Vytilla-10 was found to be tolerant to salinity, the levels of salinity tried for priming were 9 and 12 dS/m

Sodium nitro prusside seed priming enhanced the germination percentage in Vytilla-10 to 95. The lowest germination percentage was observed for water priming (85 %) and BABA (85%) and the percentage of seed germination in both were comparable. Germination was found inhibited at higher salinity level, 12 dS/m (85 %). Germination was higher for salinity level of 9 dS/m (90 %). Highest positive interaction effect was found for SNP at salinity level 9 dS/m (100%) combination.

Seedling height at 10 DAS were comparable in all the treatments except BABA (9.18 cm), whereas at 16 DAS seedlings primed with calcium chloride had the greatest seedling height (16.98 cm). The lowest increase in seedling height was observed for water primed seedlings at 16 DAS. Seedling height was higher at salinity level of 9 dS/m at 10 DAS (10.82 cm) and 16 DAS (17.46 cm).

Though there was no interaction effect at 10 DAS, interaction effect was noticed at 16 DAS for seedlings primed using sodium nitro prusside at salinity level of 9 dS/m (18.70 cm). The lowest interaction effect was observed for water primed seedlings at salinity level 12 dS/m.

The root length at 10 DAS were comparable for all treatments except BABA (4.35 cm), while at 16 DAS the highest root length was observed in seedlings primed using calcium chloride (13.17 cm). The lowest root length at 16 DAS was observed for seedlings primed using water and BABA. At all stages of observation, there was no significant effect on seedling root length due to salinity level. Due to interaction effect longest roots were observed for seedlings primed using calcium chloride (9.37 cm) and sodium nitroprusside (9.20 cm) at 12 dS/m, hydropriming (8.27 cm) and calcium chloride (7.33 cm) at 9 dS/m. Number of leaves were insignificant at 10 DAS and 16 DAS.

At 10 DAS (0.084 g/ seedling) and 16 DAS (0.099 g/seedling), highest dry weight was observed for seedlings in $CaCl_2$ treatment. More dry matter production was seen in higher salinity level 12 dS/m (0.079 gm) at 10 DAS while it was non -significant at 16 DAS. Priming with calcium chloride at salinity level of 12 dS/m increased the dry weight (0.089 g/seedling) at 10 DAS significantly, while at 16 DAS there was no interaction effect. All values related to effect of seed priming influencing growth parameters of Vytilla-10 are presented in Table 14, 15 and 16.

Table 14. Effect of seed priming on growth parameters in Vytilla-10 at 10 DAS under salt stress conditions

Treatments	**Germination %**	**Growth parameters at 10 DAS**			
		Seedling height (cm)	**Root length (cm)**	**No of leaves per seedling**	**Dry w.t (g/ seedling**
Priming chemicals	**Variety -Vytilla 10**				
BABA	85	9.18	4.35	1.00	0.076
Calcium chloride	86	10.33	8.27	1.00	0.084
SNP	95	10.83	6.93	1.00	0.078
Water (control)	85	10.60	6.13	1.00	0.069
C.D (0.05)	**0.87**	**1.06**	**2.32**	**NS**	**0.001**
Salinity level					
9 dS/m	90	10.82	6.08	1.00	0.074
12 dS/m	85	9.66	6.77	1.00	0.079
C.D (0.05)	**0.62**	**0.75**	**NS**	**NS**	**0.001**

Table 15. Interaction effect of priming chemicals and salinity levels on growth parameters of Vytilla-10 at 10 days after sowing

Treatments	**Germination %**	**Seedling height (cm)**	**Root length (cm)**	**Number of leaves per seedling**	**Dry weight (g)**
Salinity level 9 dS/m					
BABA	80	9.03	4.20	1.00	0.072
$CaCl_2$	91	10.83	7.33	1.00	0.079
SNP	100	11.73	4.50	1.00	0.077
Control /water	91	11.67	8.27	1.00	0.068
Salinity level 12 dS/m					
BABA	90	9.33	4.50	1.00	0.080
$CaCl_2$	80	9.83	9.20	1.00	0.089
SNP	90	9.93	9.37	1.00	0.080
Control /water	80	9.53	4.00	1.00	0.070
C.D (0.05)	**1.23**	**NS**	**3.28**	**NS**	**0.001**

Table 16. Effect of seed priming on growth parameters in Vytiila-10 at 16 DAS under salt stress conditions

Treatments	**Seedling height (cm)**	**Root length(cm)**	**No of leaves per seedling**	**Dry wt.(gm)**
BABA	15.70	7.17	2.00	0.073
$CaCl_2$	16.98	13.17	2.00	0.099
SNP	16.08	11.70	2.00	0.092
Control /water	14.53	6.78	2.00	0.071
C.D (0.05)	**0.37**	**0.77**	**NS**	**0.003**
9 dS/m	17.46	9.37	2.00	0.084
12 dS/m	14.19	10.04	2.00	0.084
C.D (0.05)	**0.26**	**NS**	**NS**	**NS**

Table 17. Interaction effect of priming chemicals and salinity levels on growth parameters of Vytilla-10 at 16 days after sowing

Treatments	**Seedling height (cm)**	**Root length(cm)**	**No of leaves per seedling**	**Dry wt.(gm)**
Salinity level 9 dS/m				
BABA	15.97	7.20	2.00	0.073
$CaCl_2$	18.00	12.53	2.00	0.099
SNP	18.70	10.77	2.00	0.093
Control /water	17.17	6.97	2.00	0.071
Salinity level 12 dS/m				
BABA	15.43	7.13	2.00	0.074
$CaCl_2$	15.97	13.80	2.00	0.099
SNP	13.47	12.63	2.00	0.091
Control /water	11.90	6.60	2.00	0.072
C.D (0.05)	**0.52**	**1.09**	**NS**	**NS**

4.8 Visual symptoms of primed rice varieties under salinity stress

4.8.1 Phenotypic scoring for Jyothi under salinity stress

Greater score implies that the variety is more susceptible

At 10 DAS all three priming chemicals at 6 dS/m and 9 dS/m showed a score of 1 while water priming had a score of 5 at salinity levels 6 dS/m and 9 dS/m. At 16 DAS priming chemicals BABA and calcium chloride at salinity level of 6 dS/m had a score of 3, while SNP priming had a score of 5 and water priming was having a score of 7, while at higher salinity level of 9 dS/m $CaCl_2$ scored 3 while BABA (score 5) and SNP had a score of 7 (susceptible). Water priming was most susceptible (score 9) at 16 DAS. The data on phenotypic scoring at 10 DAS and 16 DAS are presented as Table 18.

4.8.2 Phenotypic scoring of Uma under salt stress

Priming with BABA, $CaCl_2$ and SNP had a score of 1 (in both salinity levels) at 10 DAS while water priming at 6 dS/m had a score of 3 and 9 dS/m had a score of 5. At 16 DAS priming with BABA, $CaCl_2$ and SNP had a score of 3 at salinity levels of 6 dS/m and 9 dS/m. The highest score at 16 DAS was for water (score 7) priming at 9 dS/m. Greater the score the more susceptible is the variety at that salinity level.

Priming in Uma was not found to be effective in increasing growth, but can be said to improve the tolerance level. $CaCl_2$ was found to increase germination % in Uma. Use of $CaCl_2$ as priming agent was found effective in increasing dry matter production at 9 DAS and 14 DAS. SNP was more effective in influencing dry matter production at higher salinity level during the initial stages**.**

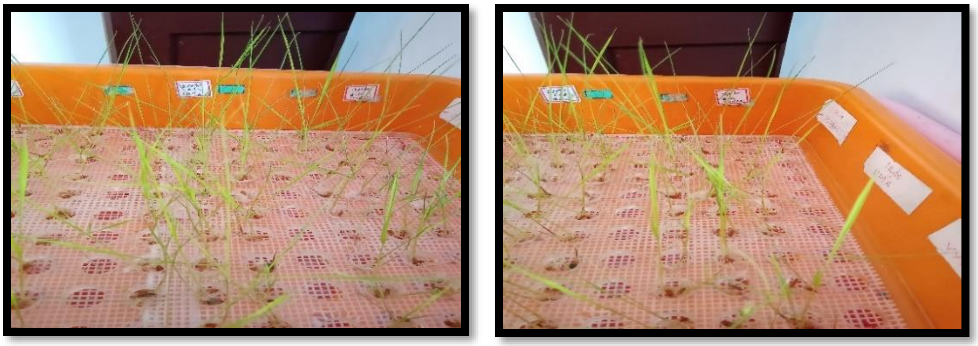
4.8.3 Phenotypic scoring of Vytilla-10 under salt stress

At 10 DAS plants pre- treated with BABA, $CaCl_2$ and SNP were found growing normally without any salt injuries at salinity levels 9 dS/m and 12 dS/m. The injury level for hydro treated plants was more at 12 dS/m (score 5) than at 9 dS/m (score3). At 16 DAS the plants pre -treated with BABA, $CaCl_2$ and SNP were found to be affected by salt injury (score-3). The water treatment at 9 dS/m had a score of 5 compared to the other treatments. At 12 dS/m, salt injury was higher for water primed plants (score-9). Priming with BABA was less effective compared to SNP (score-3) and $CaCl_2$ (score-3) in overcoming salt injury. The data are presented in Table 18.

Table 18. Phenotypic scoring for rice varieties under salinity stress

Treatments	**Jyothi**		**Uma**		**Vytilla-10**	
	Score at					
	10 DAS	**16 DAS**	**10 DAS**	**16 DAS**	**10 DAS**	**16 DAS**
Variety Jyothi						
Salinity level of 6 dS/m					**Salinity level of 9 dS/m**	
BABA	1	3	1	3	1	3
$CaCl_2$	1	3	1	3	1	3
SNP	1	3	1	3	1	3
Control /water	3	5	3	5	3	5
Salinity level of 9 dS/m					**Salinity level of 12 dS/m**	
BABA	1	5	1	5	1	5
$CaCl_2$	1	3	1	3	1	3
SNP	1	3	1	3	1	3
Control /water	3	9	3	9	5	7

Jyothi 6 dS/m at 10 DAS



Jyothi 6 dS/m at 16 DAS

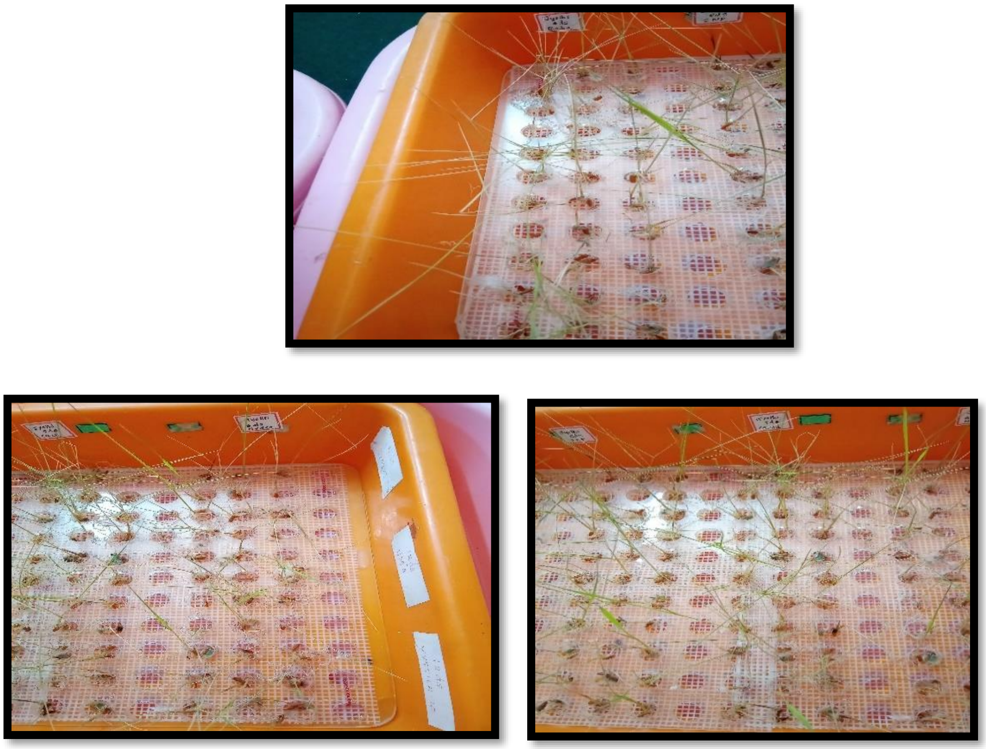
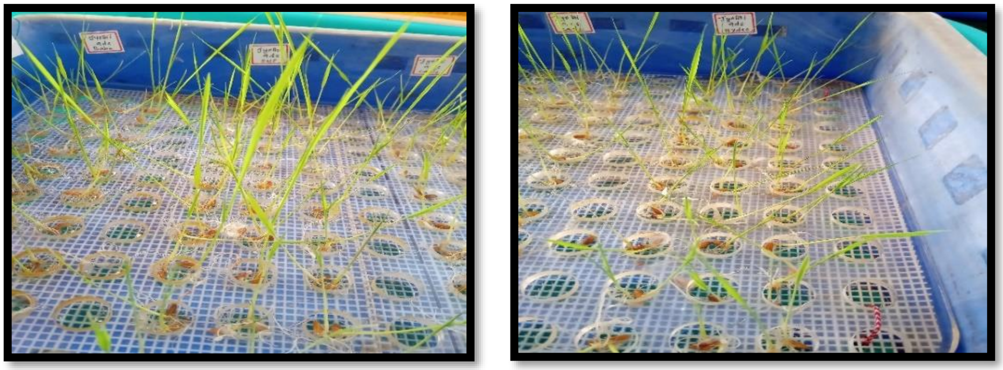


Plate 5. Variety Jyothi at salinity level 6 dS/m, seedlings from primed seeds

Jyothi 9 dS/m at 10 DAS



Jyothi 9 dS/m at 16 DAS

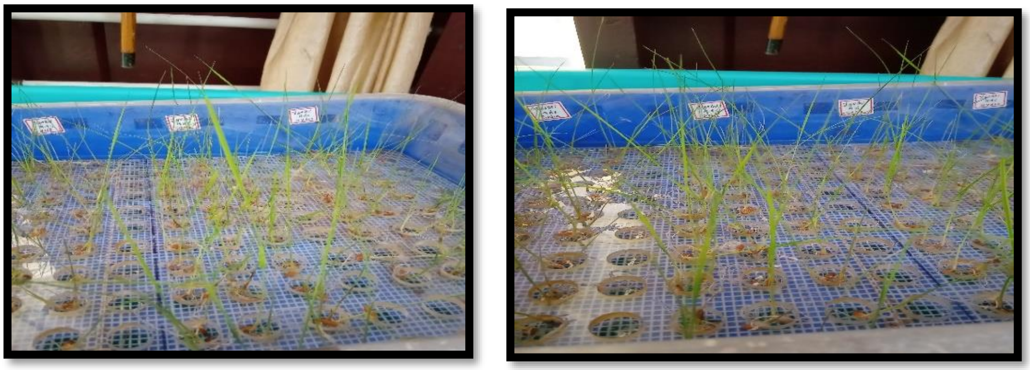
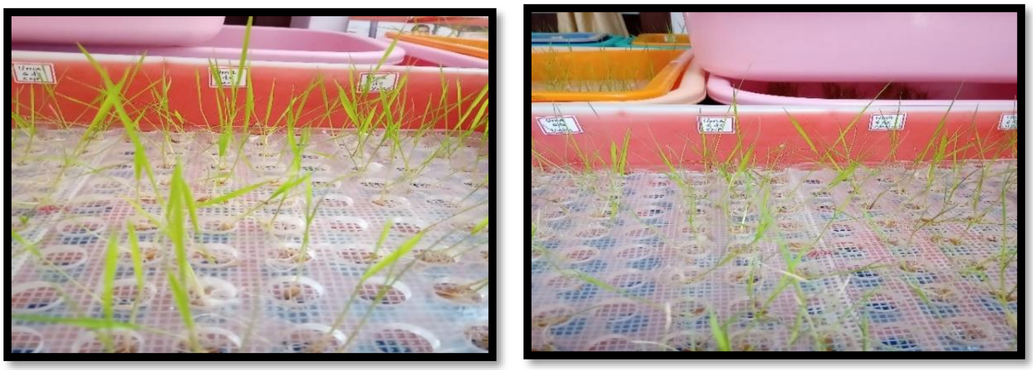


Plate 6. Variety Jyothi at salinity level 9 dS/m, seedlings from primed seeds

Uma 6 dS/m at 10 DAS



Uma 6 dS/m at 16 DAS

Plate 7. Variety Uma at salinity level 6 dS/m, seedlings from primed seeds

Uma 9 dS/m at 10 DAS

Uma 9 dS/m at 16 DAS

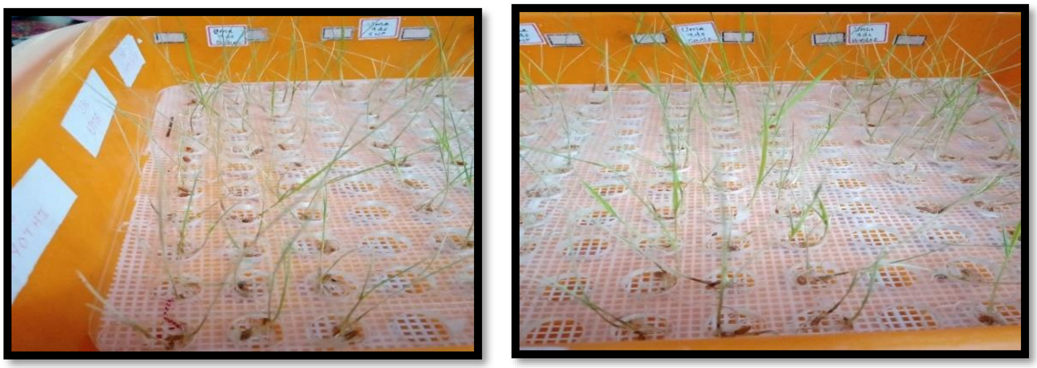


Plate 8. Variety Uma at salinity level 9 dS/m, seedlings from primed seeds

Vytilla-10, 9 dS/m at 10 DAS

Plate 9. Variety Vytilla-10 at salinity level 9 dS/m, 10 DAS after seed priming

Vytilla-10, 9 dS/m at 16 DAS

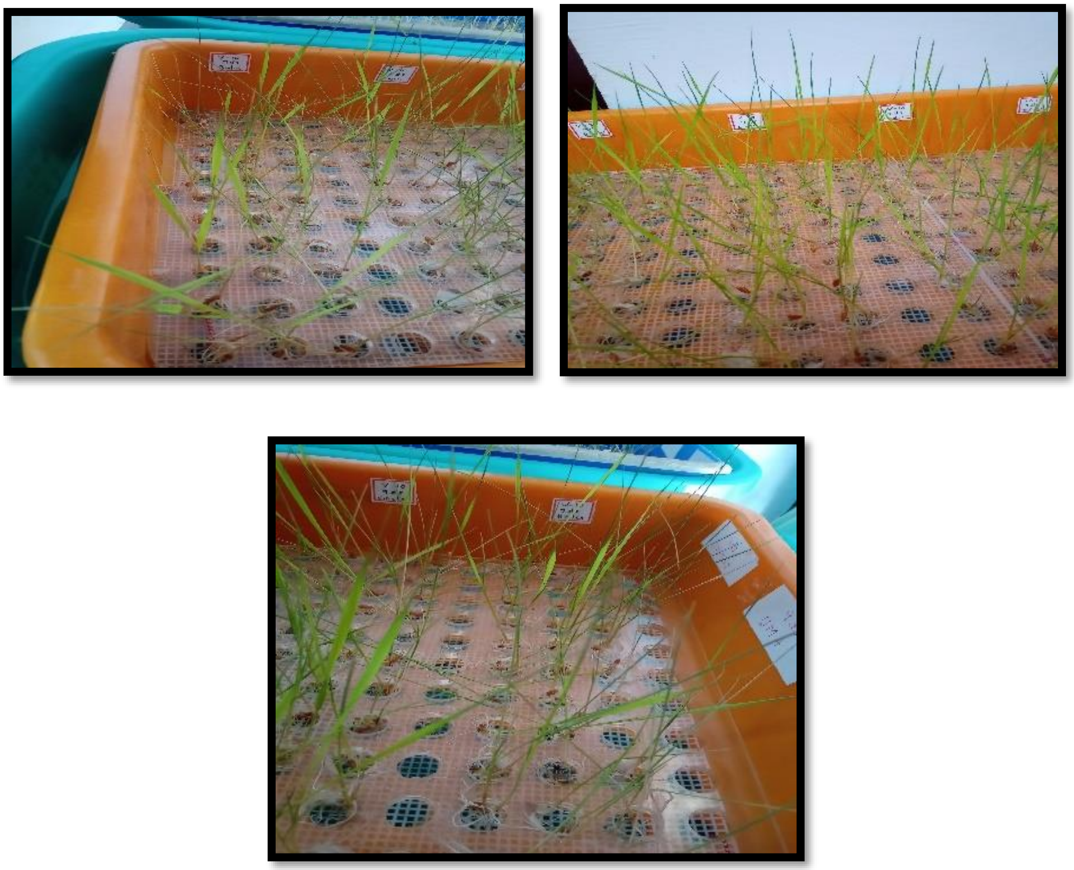
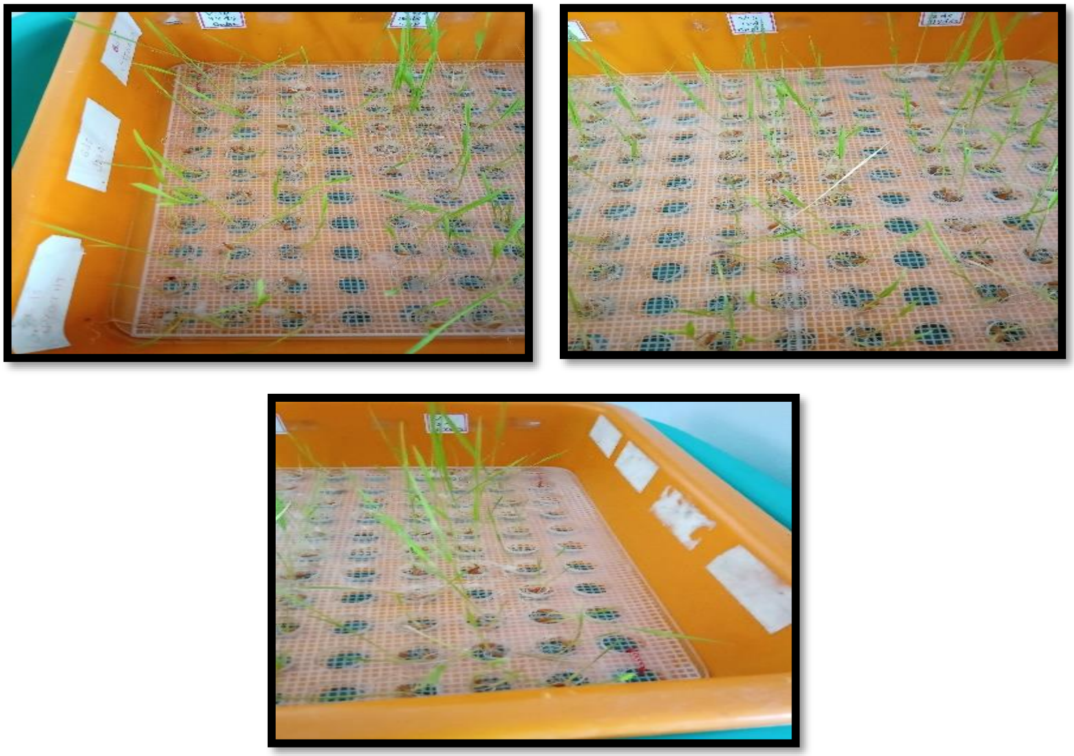


Plate 10. Vytilla-10 at salinity level of 9 dS/m, 16 DAS after seed priming

Vytilla-10, 12 dS/m at 10 DAS



Vytilla-10, 12 dS/m at 16 DAS

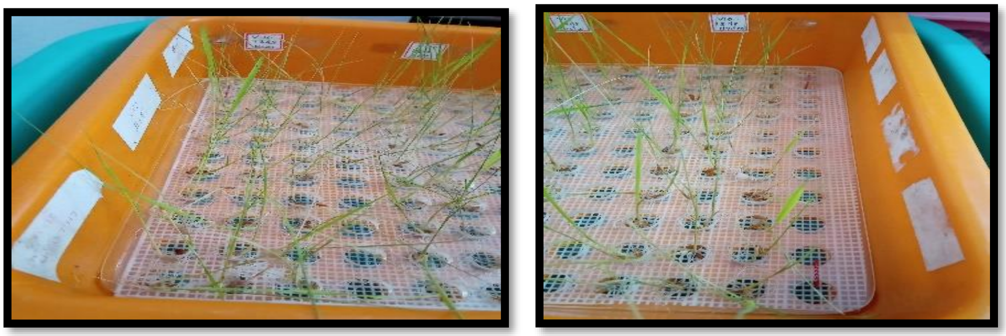


Plate 11. Variety Vytilla-10 at salinity level of 12 dS/m, seedlings from primed seeds

Part 3 Field experiment to assess the effect of priming in rice fields with saline water intrusion

Based on the results of experiment 2, study was conducted to find out the effect of chemicals as a foliar spray and to confirm the effect of seed priming in actual field situation. The experiment was continued with rice variety Vytilla-10. The results of the study are presented below.

4.9 Growth parameters

4.9.1 Plant height

There was no significant difference in plant height due to chemical priming at both 20 and 40 DAT, while at 60 DAT plant height was found high and comparable for seedlings primed with calcium chloride (110.78 cm) and BABA (108.33 cm). Interaction effect of chemical priming and foliar spraying were not significant. The analyzed data are presented as Table.19

Table.19 Effect of seed priming and foliar spray of priming chemicals on plant height of rice

Treatments	**Plant height (cm)**		
	20 DAT	**40 DAT**	**60 DAT**
BABA	60.33	82.92	108.33
$CaCl_2$	62.67	83.67	110.78
SNP	64.17	91.92	98.00
Control /water	63.67	80.25	100.56
C.D (0.05)	**NS**	**NS**	**10.06**

4.9.2 Number of tillers/m^2

At 20 DAT number of tillers per square metre for BABA (237 tillers/m^2), water (209.44 tillers/m^2) and sodium nitro prusside (202.78 tillers/m^2) were comparable, while the lowest number of tillers per sq.m was observed for calcium chloride. At 40 DAT, water (336.33 tillers/m^2) and sodium nitro prusside (334.45 tillers/m^2) had comparable and highest tiller number per square metre. At 60 DAT SNP (349.11 tillers/m^2) priming resulted in highest tillers per square metre. Foliar spraying of priming chemicals was not significant at 20 DAT and 40 DAT, while at 60 DAT weekly foliar spray (330.08 tillers/m^2) resulted in highest tillering per square meter.

Interaction effect was not significant at 20 DAT, but at 40 DAT interaction effect was comparable and high in seedlings primed with SNP given foliar weekly, water primed seedlings given foliar fortnightly, SNP primed seedlings given weekly water spray and calcium chloride primed seedlings with weekly foliar spray. At 60 DAT interaction effect was high in influencing tillering for sodium nitroprusside primed seedlings given weekly foliar spray.

The analyzed data is given as Table 20 and Table 21.

Table.20 Effect of seed priming and foliar spray of priming chemicals on number of tillers/m^2 in rice

Treatments	**Number of tillers/ m^2**		
	20 DAT	**40 DAT**	**60 DAT**
BABA	237.00	256.33	269.56
$CaCl_2$	135.78	273.33	287.11
SNP	202.78	334.45	349.11
Control /water	209.44	336.33	302.11
C.D (0.05)	**65.64**	**62.74**	**23.50**
Weekly interval	198.92	315.50	330.08
Fortnightly	202.75	292.33	292.33
Water weekly	187.08	292.50	283.50
C.D (0.05)	**NS**	**NS**	**20.35**

Table 21. Interaction effect of seed priming and foliar spray of priming chemicals on the number of tillers/m^2 at different stages of growth

Treatments	**No. of tillers/m^2**		
	20 DAT	**40 DAT**	**60 DAT**
BABA			
Weekly	221.33	245.67	277.67
Fortnightly	255.67	277.67	288.00
Water weekly	234.00	245.67	243.00
Calcium chloride			
Weekly	129.00	321.00	309.00
Fortnightly	129.00	266.33	319.00
Water weekly	149.33	232.67	233.33
SNP			
Weekly	232.67	407.33	434.67
Fortnightly	180.67	224.00	243.33
Water weekly	195.00	372.00	369.33
Hydro			
Weekly	212.67	288.00	299.00
Fortnightly	245.67	401.33	319.00
Water weekly	170.00	319.67	288.33
C.D (0.05)	**NS**	**108.66**	**40.71**

4.9.3 Leaf area index

LAI is an important parameter influencing yield. At 20 DAT comparable and high LAI was observed for BABA (2.94), water (2.81) and SNP (2.17) seed priming. The lowest LAI was observed in calcium chloride primed seedling (1.55). However, at 40 and 60 DAT calcium chloride priming had the highest leaf area index (5.74 at 40 DAT and 5.53 at 60 DAT). Leaf area index at 40 DAT for all other treatments were comparable. At 60 DAT the lowest leaf area index was observed in hydro primed seedlings. The highest LAI 20 DAT was observed for seedlings that received weekly foliar spray (3.07), while at 40 DAT the highest and comparable LAI was observed for weekly water spray and weekly foliar spray. At 60 DAT, LAI was high for weekly water spray.

At 20 DAT interaction effect was found high for seedlings primed with BABA given weekly foliar (5.34). However, at 40 DAT the highest LAI was observed for calcium chloride primed seedlings given water spray weekly. At 60 DAT priming seeds with calcium chloride followed by water spray, BABA primed seedlings given foliar weekly and calcium chloride primed seedlings given foliar weekly was observed to have highest and comparable LAI.

The analyzed data were presented as Table 22 and Table 23.

Table.22 Effect of seed priming and foliar spray of priming chemicals on leaf area index of rice

Treatments	**Leaf area index (LAI)**		
	20 DAT	**40 DAT**	**60 DAT**
BABA	2.94	3.39	4.09
$CaCl_2$	1.55	5.74	5.53
SNP	2.17	3.78	4.08
Control /water	2.81	2.97	3.46
C.D(0.05)	**0.81**	**0.78**	**0.04**
Weekly interval	3.07	4.30	4.80
Fortnightly	2.29	2.74	3.18
Water weekly	1.75	4.86	4.88
C.D(0.05)	**0.70**	**0.68**	**0.03**

Table 23. Interaction effect of priming chemicals and foliar spray on leaf area index at different stage of growth

Treatments	LAI at 20 DAT (cm)	LAI at 40 DAT (cm)	LAI at 60 DAT (cm)
BABA			
Weekly	5.34	4.99	5.47
Fortnightly	2.83	2.33	3.17
Water weekly	0.66	2.84	3.62
Calcium chloride			
Weekly	1.38	5.51	6.02
Fortnightly	1.82	4.01	4.49
Water weekly	1.45	7.70	6.07
SNP			
Weekly	2.80	3.79	4.34
Fortnightly	1.70	2.43	2.57
Water weekly	2.03	5.12	5.32
Hydro			
Weekly	2.77	2.93	3.37
Fortnightly	2.80	2.18	2.48
Water weekly	2.86	3.80	4.53
C.D(0.05)	**1.40**	**1.35**	**0.62**

4.10 Yield parameters

4.10.1 Number of panicles/m^2

Number of panicles/m^2 was significantly higher in calcium chloride primed seedlings (263.00) and the lowest was observed for hydro primed seedlings. Weekly foliar spray was found effective in increasing number of panicles/ m^2. The lowest number of panicles/ m^2 was observed in plants given fortnightly spray (199.00 panicles/m^2). Interaction effect was highest and comparable in calcium chloride primed seedlings which were given weekly foliar spray (305.00 panicles/m^2) and in sodium nitroprusside primed seedlings given weekly foliar spray (299.00 panicles/m^2). The lowest interaction effect of seed priming and foliar spraying was observed in sodium nitroprusside primed seedlings given fortnightly foliar spray (140 panicles/m^2).

4.10.2 Grains per panicle

Significantly higher grains per panicle was observed in seedlings primed using BABA (129.00) and the lowest grains per panicle was observed in SNP (117.00) and water primed seedlings (115.00). The grains per panicles were comparable in both SNP and water primed seedlings. Foliar spraying at fortnightly interval (139 grains/panicle) was helpful in increasing grains per panicle, while weekly spray of water was not effective (104 grains/panicle). SNP priming with fortnightly spray (148.00), seed priming using BABA (145.00) with weekly foliar spray and water priming with fortnightly spray (145.00) were effective interactions in significantly increasing grains per panicle.

4.10.3 Percentage of filled grains

Priming with calcium chloride (88%) increased the filling percentage in grains significantly. The least filling percentage (71%) was observed in plants primed using water. Weekly foliar spray increased the filling percentage of grains (81%), while spraying fortnightly (77%) and weekly spray of water (78%) gave the lowest result. There was no interaction effect.

4.10.4 Thousand grain weight (g)

Thousand grain weight was high for plants raised by calcium chloride seed priming (26.10 g) and the lowest was observed for plants raised using water primed seeds (21.57 g). Spraying of priming chemicals at fortnightly intervals (25.17 g) was effective over other priming chemicals, while there was only marginal effect due to the spraying of water weekly (22.06 g). Interaction effect of priming chemicals and foliar spray was observed in plants primed using calcium chloride (30.05 g) with fortnightly spray of calcium chloride 2% and the value was significantly higher. The lowest interaction effect on thousand grain weight was observed for plants raised by water seed priming with weekly spray of water (20.40 g).

4.10.5 Yield (kg/ha)

Calcium chloride at 2 per cent was found to be superior among the priming chemicals in improving yield (4276 kg/ha), while the lowest yield (3272 kg/ha) was observed in plants raised using water priming. Foliar application at weekly intervals (4480 kg/ha) was found effective in improving yield while, weekly foliar spray of water (3684 kg/ha) was having the lowest yield. The application of calcium chloride as seed priming chemical was supplemented by the foliar spray at weekly interval (5804 kg/ha) and fortnightly intervals (5492 kg/ha), both of which produced comparable yield. Water seed priming followed by weekly water spray (3100 kg/ha), water priming with fortnightly water spray (3126 kg/ha) and BABA seed priming with weekly water spray (3232 kg/ha) were the lowest performers among the treatment combination. Data on yield parameters recorded are presented as Table 24 and Table 25.

Table.24 Effect of seed priming and foliar spray of priming chemicals on yield parameters and yield of rice

Treatments	No of panicles per sq.m	Grains per panicle	Filled grains (%)	1000 grain wt (g)	Yield (kg/ha)
Seed priming					
BABA	221.00	129.00	82.00	22.70	3737
$CaCl_2$	263.00	125.00	88.00	26.10	4276
SNP	220.00	117.00	74.00	24.05	3868
Control /water	192.00	115.00	71.00	21.57	3272
C.D (0.05)	**5.30**	**2.48**	**1.94**	**0.61**	**191**
Foliar spray					
Weekly	260.00	121.00	81.00	23.59	4480
Fortnightly	199.00	139.00	77.00	25.17	3952
Water weekly	213.00	104.00	78.00	22.06	3684
C.D (0.05)	**4.59**	**2.15**	**1.68**	**0.52**	**165**

Table.25 Interaction effect of seed priming and foliar spray of priming chemicals on yield parameters and yield of rice

Treatment	No of panicles per sq.m	Grains per panicle	Filled grains (%)	1000 grain wt. (g)	Yield (kg/ha)
BABA					
Weekly	230.00	145.00	85.00	23.00	4424
Fortnightly	194.00	143.00	81.00	23.05	3556
Water weekly	237.00	98.00	81.00	22.05	3232
$CaCl_2$					
Weekly	305.00	130.00	92.00	25.30	5804
Fortnightly	255.00	123.00	87.00	30.05	5492
Water weekly	230.00	123.00	86.00	22.95	4533
SNP					
Weekly	299.00	114.00	75.00	24.25	4100
Fortnightly	140.00	148.00	72.00	25.05	3632
Water weekly	222.00	91.00	74.00	22.85	3873
Hydro					
Weekly	207.00	95.00	72.00	21.81	3590
Fortnightly	207.00	145.00	68.00	22.51	3126
Water weekly	164.00	104.00	72.00	20.40	3100
C.D (0.05)	**9.18**	**4.30**	**NS**	**1.05**	**330**

4.11 Biochemical parameters

4.11.1 Total chlorophyll

Chlorophyll content was comparable for all priming chemicals except seedlings primed using calcium chloride at 20 DAT. BABA seed priming (2.79 mg/g), SNP seed priming (2.84 mg/g) and water priming (2.94 mg/g) were significantly superior. At 40 DAT seed priming with BABA (1.88 mg/g) and calcium chloride (1.70 mg/g) were comparable and superior. SNP (1.50 mg/g) and water (1.27 mg/g) seed priming did not increase the total chlorophyll content of the plant at 40 DAT. At 60 DAT use of SNP (3.18 mg/g) and calcium chloride (2.95 mg/g) seed priming improved the total chlorophyll content and the values were comparable. BABA (2.72 mg/g) and water priming (2.50 mg/g) were least effective in increasing total chlorophyll content. Effect of foliar spraying on total chlorophyll content was statistically insignificant at 20 DAT. However, at 40 DAT fortnightly spraying and weekly spray of water were effective in increasing total chlorophyll content. Effect of foliar spraying in improving total chlorophyll at 60 DAT was not significant.

Interaction effect of seed priming and foliar spraying of priming chemicals in improving chlorophyll content were high in water primed seedlings with weekly foliar spray at 20 DAT, while at 40 DAT comparable and high content of chlorophyll was observed in BABA primed seedlings with fortnightly foliar spray, BABA with weekly water spray, calcium chloride with fortnightly spray, calcium chloride with weekly water spray, SNP primed seedling with weekly spray and water primed seedling with weekly water spray. At 60 DAT total chlorophyll content was high in SNP primed seedlings with fortnightly foliar spray (3.74 mg/g).

4.11.2 Proline

At 20 DAT there was no significant difference in proline content in seedlings primed with chemicals, while at 40 DAT sodium nitroprusside primed seedlings (421.67 µg/g) had highest proline content. At 60 DAT seedlings raised using calcium chloride seed priming had significantly high proline levels (582.17 µg/g). Foliar spraying at 20 DAT was not significant. At 40 DAT fortnightly foliar spraying (243.75 µg/g) and weekly water spray (306.25 µg/g) were having high and comparable proline content. At 60 DAT weekly foliar spraying was having highest proline content (397.13 µg/g).

Interaction effect was comparable and high in calcium chloride primed seedling with weekly water spray (330.50 µg/g), SNP seed priming with weekly foliar spray (290.50 µg/g), SNP seed priming with fortnightly foliar spray (245.00 µg/g), SNP seed priming with weekly water spray (200.00 µg/g), water priming with weekly foliar spray (275.00

µg/g), water priming with weekly water spray (200 µg/g) and BABA with fortnightly foliar spray (250.00 µg/g). At 60 DAT calcium chloride with fortnightly foliar spraying (599.5 µg/g) had significant proline content in its leaves. The data related to total chlorophyll and proline at 20, 40 and 60 DAT are given below as Table 26 and 27.

Table 26. Effect of treatments on total chlorophyll (mg/g) and proline content in leaf (µg/g) at different growth stages of rice

Treatment	**Total chlorophyll content (mg/g)**			**Proline content in leaf (µg/g)**		
	20DAT	**40DAT**	**60DAT**	**20 DAT**	**40 DAT**	**60 DAT**
Seed priming						
BABA	2.79	1.88	2.72	200.00	163.33	151.33
$CaCl_2$	2.53	1.70	2.95	188.50	216.67	582.17
SNP	2.84	1.50	3.18	245.17	421.67	448.67
Control /water	2.94	1.27	2.50	196.67	211.67	143.00
C.D(0.05)	**0.26**	**0.37**	**0.23**	**NS**	**79.58**	**3.25**
Foliar spray						
Weekly	2.91	1.39	2.96	213.75	210.00	379.13
Fortnightly	2.74	1.80	2.83	183.75	243.75	287.00
Water weekly	2.66	1.58	2.72	225.00	306.25	327.75
C.D (0.05)	**NS**	**0.32**	**NS**	**NS**	**68.92**	**2.82**

Table 27. Interaction effect of treatments on total chlorophyll (mg/g) and proline content in leaf (µg/g) at different growth stages of rice

Treatment	**Total chlorophyll (mg/g)**			**Proline content in leaf (µg/g)**		
	20DAT	**40DAT**	**60DAT**	**20DAT**	**40DAT**	**60DAT**
BABA						
Weekly	2.69	1.28	3.32	180.00	265.00	170.00
Fortnightly	2.86	2.11	2.25	250.00	105.00	189.50
Water weekly	2.81	2.25	2.584	170.00	120.00	94.50
$CaCl_2$						
Weekly	2.49	1.54	3.26	110.00	150.00	587.50
Fortnightly	2.73	1.75	2.68	125.00	280.00	599.50
Water weekly	2.35	1.81	2.90	330.50	220.00	559.50
SNP						
Weekly	2.92	1.64	2.83	290.50	230.00	589.00
Fortnightly	2.57	1.47	3.74	245.00	470.00	209.50
Water weekly	3.02	1.40	2.97	200.00	565.00	547.50
Hydro						
Weekly	3.55	1.12	2.42	275.00	195.00	170.00
Fortnightly	2.80	1.87	2.64	115.00	120.00	149.50
Water weekly	2.46	0.84	2.44	200.00	320.00	109.50
C.D(0.05)	**0.45**	**0.63**	**0.40**	**119.70**	**137.80**	**5.63**

4.11.3 Chlorophyll a and Chlorophyll b

Chlorophyll a content was only significant at 60 DAT. Seedling raised using chemical primers calcium chloride (2.24 mg/g), sodium nitroprusside (2.16 mg/g) and BABA (2.15 mg/g) were having comparable chlorophyll a content. Water seed priming was having the lowest chlorophyll A content (1.95 mg/g). Chlorophyll b content was not significant at 20 DAT, while at 40 DAT, seedlings raised with BABA seed priming (0.64 mg/g) had the highest chlorophyll content. At 60 DAT seedlings raised with SNP (1.02 mg/g) had the highest chlorophyll B content. Data of the effect of chemical priming on chlorophyll a and b content are given in Table 28.

Table.28 Effect of treatments on chlorophyll a and chlorophyll b at 20 DAT, 40 DAT and 60 DAT

Treatments	**Chlorophyll a (mg/g)**			**Chlorophyll b (mg/g)**		
	20DAT	**40DAT**	**60DAT**	**20DAT**	**40DAT**	**60DAT**
Seed priming						
BABA	2.10	1.25	2.15	0.76	0.64	0.50
$CaCl_2$	1.95	1.46	2.24	0.61	0.24	0.70
SNP	2.10	1.49	2.16	0.58	0.38	1.02
Control /water	2.11	1.18	1.95	0.74	0.21	0.55
C.D (0.05)	NS	NS	0.125	NS	0.25	0.17

Interaction effect on chlorophyll a content was not significant at 20 DAT and 40 DAT, while at 60 DAT comparable and high chlorophyll a content was observed in seedlings raised from seeds primed using BABA with weekly foliar spray (2.31 mg/g), BABA with weekly water spray (2.28 mg/g), $CaCl_2$ primed seedlings with weekly foliar spray (2.17 mg/g), $CaCl_2$ with fortnightly foliar spraying (2.12 mg/g), $CaCl_2$ with weekly water spray (2.33 mg/g), seedlings raised using seed primer SNP with weekly foliar spraying (2.16 mg/ g) and seedlings primed using SNP as seed primer with fortnightly foliar spraying(2.27 mg/g). chlorophyll b at 20 DAT was found high in hydro primed seedlings given weekly foliar spray (1.103 mg/g).At 40 DAT chlorophyll b content was high in BABA primed seedlings given fortnightly spray (0.796 mg/g) and BABA primed seedlings given weekly water priming(0.717 mg/g). Data related to interaction effect of different seed priming chemicals and foliar spraying are given below as Table29.

Table.29 Interaction effect of seed priming and foliar spraying of chemicals on chlorophyll A and chlorophyll B at 20 DAT, 40 DAT and 60 DAT

Treatments	**Chlorophyll A (mg/g)**			**Chlorophyll B (mg/g)**		
	20DAT	**40DAT**	**60DAT**	**20DAT**	**40DAT**	**60DAT**
BABA						
Weekly	2.18	0.88	2.31	0.518	0.398	1.011
Fortnightly	2.05	1.32	1.97	0.605	0.796	0.075
Water weekly	2.10	1.54	2.17	0.708	0.717	0.417
Calcium chloride						
Weekly	1.91	1.30	2.28	0.578	0.243	0.979
Fortnightly	2.05	1.48	2.12	0.683	0.274	0.567
Water weekly	1.88	1.59	2.33	0.471	0.214	0.567
SNP						
Weekly	2.16	1.44	2.16	0.755	0.199	0.668
Fortnightly	2.05	1.50	2.27	0.521	0.080	1.470
Water weekly	2.09	1.54	2.04	0.933	0.867	0.931
Hydro						
Weekly	2.24	1.02	1.93	1.103	0.140	0.491
Fortnightly	2.07	1.83	2.07	0.729	0.349	0.570
Water weekly	2.02	0.71	1.84	0.440	0.131	0.600
C.D (0.05)	**NS**	**NS**	**0.22**	**0.411**	**0.432**	**0.289**

DISCUSSION

5.DISCUSSION

5.1 Screening rice varieties for salinity tolerance

An experiment to assess the salinity tolerance threshold of three varieties namely Jyothi, Uma and Vytilla -10 was conducted. Salinity tolerance in rice was assessed based on parameters like germination percentage, plant height, root length etc. The results are discussed in this chapter and discussions are based on similar experiments conducted elsewhere.

5.1.1 Effect of salinity on germination and growth parameters of rice varieties

Germination percentage was found to be affected by salinity level of 12 dS/m in variety Jyothi and at salinity levels below 12 dS/m germination was unaffected. This is in conformity with the result of several studies in rice that it is tolerant to salinity during germination and it becomes very susceptible during seedling and reproductive stage (Ali *et al*., 2014). Alam *et al.* (2002) also reported that rice is highly tolerant to salinity. The reduced germination percentage at 12 dS/m suggests the possibility that in hyper saline solutions the highly susceptible varieties show decreased germination. In varieties Uma and Vytilla-10 germination was not affected upto salinity level of 12 dS/m. Zeng *et al*. (2001) also reported that rice germination was not affected by salinity. Similar result was reported by Heenan *et al*. (1988) in their work on salinity tolerance in rice at different growth stages. In variety Vytilla-10 germination percentage was found to be independent of the level of salinity.

In Jyothi plant height was affected by salinity of 3 dS/m. However, the initial shock at 3 dS/m was overcome by the plant later on. This is evident from plant height values at 16 DAS and 21 DAS. A salinity level of 6 dS/m was found to affect the growth of the plant from 3 days of sowing. Similar was the case with variety Uma where plant height was found to be affected at salinity level above 3 dS/m. Both in the case of Jyothi and Uma, there was a progressive decrease in plant height with increase in salinity, while root length increased with increase in salinity. General decline in shoot length in rice varieties subjected to above threshold salinity level can be further understood from similar findings by several researchers.

Alam *et al*. (2004) reported that a negative correlation existed between shoot length and salinity level. The authors found that this decline in plant height depended on the salinity level and varied between cultivars. Four reasons attributed for reduced plant height in rice under salt stress were reduction in photosynthesis, reduced turgor due to reduced water potential, the root response to salt stress to down regulate shoot length and root length via long distance signals and lastly the changed scenario that might have created nutrient imbalance in plants. The increased level of salinity affected final cell size and rate of cell production in plants. This led to reduced shoot length (Kurth *et al*., 1986; Zidan *et al*., 1990; Azaizeh *et al*., 1992). The plant height, dry weight of root and shoot was affected by salinity (Khatun and Flowers, 1995; Munns and Testor, 2008; Hakim *et al*., 2010; Gupta and Huang, 2014; Reddy *et al*., 2017).

Root length on the other hand was found to increase with increase in salinity for all the three varieties. A possible reason for this may be that plants increased their root length under stress conditions to increase the volume of soil they could explore.

The typical symptoms of salinity stress were categorized based on standard evaluation score and the score obtained by each variety denoted salinity tolerance level. Higher the score greater was the damage due to salinity stress. Standard evaluation score (SES) for salinity level above 6 dS/m showed that variety Jyothi was highly susceptible at 16 DAS and 21 DAS. The phenotypic score of 5 increased to 7 at 16 DAS and to 9 at 21 DAS in Jyothi. In a study conducted at Rice Research Station, Vyttila, it was observed that Jyothi is salt sensitive rice (KAU, 2014). Thitisaksakul *et al*. (2015) also reported that the threshold salinity level for rice was 3 dS/m. Based on the result it was concluded for variety Jyothi the threshold salinity level is 3 dS/m.

In Uma decrease in shoot length and higher score was registered for salinity level of 6 dS/m. Salinity score progressed from 3 at 10 DAS to 5 at 16 DAS and to 7 at 21 DAS.

At salinity level of 9 dS/m variety Uma became highly susceptible with a score 7 at 9 dS/m. The increased salinity tolerance in Uma compared to Jyothi might be due to its parentage from *Pokkali* as the latter had in it the most saline tolerant *Saltol* gene. Alam *et al*. (2004) reported that the salt injury symptoms varied according to the cultivar, due to differences in genetic makeup.

The variety Vytilla-10 was found tolerant to salinity upto 6 dS/m. Scoring at 21 days suggested Vytilla-10 to be susceptible at 9 dS/m and highly susceptible at 12 dS/m. Salinity tolerance in *Pokkali* varieties were mainly due to the presence of *Saltol* gene (Bonilla *et al*., 2002), Na^+ extrusion into vacuoles (Kader and Lindberg, 2005), and increased activity of ROS scavengers like catalase and antioxidants (Vaidyanathan *et al*., 2003). Above 9 dS/m the growth parameters were found to be affected by salinity. Vytilla-10 being a tolerant variety was found to show rapid growth at salinity levels below 9 dS/m, where most of the modern varieties succumbed to salt injuries. The increased plant vigour resulted in dilution effect. Dilution effect reduced the concentration of Na^+ per unit biomass. Kumar *et al*. (2013) and Platten *et al.* (2013) reported that plant vigour was a mechanism to avoid toxic effect of salinity.

It should be noted that the growth at salinity levels reduced compared to actual stage of the crop, this may be due to the reduced nutrient content in the medium. Such drawback of the growth medium was reported by different researchers worldwide and studies on rectifying this condition was taken up at IARI by Sharma and Pandey (2018) with the development of PusaRich medium for rice screening. Scientist worldwide continue with Yoshida solution as standard medium despite the new development, and the same was followed in this study also. To justify our conclusions a field study was also undertaken.

5.2 Effect of seed priming on germination and growth parameters of rice varieties under salt stress conditions

Effect of seed priming on salinity tolerance of Jyothi, Uma and Vytilla-10 was studied. The salinity level was fixed based on the threshold salinity level of each variety based on results of experiment 1. Jyothi and Uma were given salinity stress of 6 dS/m and 9 dS/m, while Vytilla-10 was given a salinity stress of 9 dS/m and 12 dS/m. The overall effect of priming on germination and growth is discussed in this chapter.

As already discussed, salinity upto 9 dS/m was not found to hinder germination in Jyothi. However, it was observed that use of beta amino butyric acid (BABA) as a seed primer increased the germination percentage significantly over other primers. The effect of priming chemicals was dependent on the level of salinity and duration of salt exposure. BABA was found to be effective at lower salinity level of 6 dS/m, while at higher salinity level of 9 dS/m $CaCl_2$ was effective. Similar influence of beta amino butyric acid as seed primer in enhancing seed germination in rice was reported by Jisha and Puthur (2016). The positive effect of calcium chloride was attributed to the role of calcium in inhibiting sodium uptake as reported by several authors that calcium inhibited sodium uptake (Bonilla *et al.*, 2004; Marcar, 1986; Nayyar and Walia 2003). Many workers have reported increased plant growth by calcium chloride application in rice under salinity stress (Colmer *et al.*, 1996; Ebert *et al.*, 2002; Kinraide, 1999; Kurth *et al.*, 1986; Marschner, 1995; Munns, 2002; Rengel, 1992; Suhayda *et al.*, 1992; Tobe *et al.*, 2003).

There was no significant difference in plant height at 10 DAS but at 16 DAS seedlings primed using calcium chloride and BABA were found to be comparable in plant height. It can be assumed that the effect of seed priming was more after 10 DAS. This phenomenon explained that salt injury in plants was also dependent on exposure time to salinity. Rice response to salt stress depends on duration and type of salt, developmental stage and many other factors (Bernardo *et al.*, 2000; Cramer *et al.*, 2001).

Bado *et al*. (2018) suggested that scoring should be carried out after 12 days of salt treatment as at this stage, growth retardation became more apparent in sensitive varieties and tolerant ones would continue to grow in spite of salinity. In the present study priming with calcium chloride and BABA were found to significantly increase plant height and root length in Jyothi. Root length was not significant at 10 DAS, but after 16 days of sowing root length was highest for calcium chloride at both levels of salinity. The value was comparable with control, which mean root growth in calcium chloride primed seedlings proceeded at the same phase as in non- saline condition. Galahitigama and Wathugala (2016) in their study reported that 2 % calcium chloride increased the plant height, number of leaves and shoot dry weight. Calcium treated salt-stressed plants displayed amplified chlorophyll content and suppressed the accumulation of H_2O_2 and thereby reduced oxidative damage (Arif *et al*., 2018). The reduced oxidative stress might have improved the root and shoot growth.

Both calcium chloride and sodium nitroprusside were effective in increasing plant growth as indicated by data on dry weight. These two priming chemicals were more effective at higher salinity level. The effect of SNP was due to the release of nitic oxide. It was reported that pretreatment of NO improved the dry weight of rice genotypes exposed to salinity stress (Adamu *et al*., 2018). Dry weight value of SNP primed seedlings indicated that it was equally useful in increasing shoot and root length up to 10 DAS. The use of calcium chloride as a seed primer was also equally effective. Use of calcium chloride and sodium nitroprusside were comparable at 9 dS/m in increasing biomass production at 10 DAS. The effect of primers like calcium chloride and sodium nitroprusside were more significant at higher salinity level. Increased dry weight suggested that calcium chloride priming improved the overall growth (both root and shoot length) of the plant and was helpful at higher salinity.

Among the different priming chemicals tried, germination in variety Uma was found to be enhanced by calcium chloride and water priming. Calcium enhanced ion exchange activities in seed (Mokhtari *et al*., 2008), while, hydro priming was also found to increase seed germination, which may be due to prolonged lag phase in seed germination. Priming prolonged the lag phase in seed germination, that allowed some pre germinative physiological and biochemical processes to take place and the same time prevented germination (Bradford, 1986). Water priming was effective at salinity level of 6 dS/m and calcium chloride was effective at 9 dS/m. At 16 DAS and 10 DAS plant height was found to be influenced by priming, seedlings treated with calcium chloride recorded the greatest increase in plant height and was comparable with seedlings treated using SNP. Similar findings were reported by Galahitigama and Wathugala (2016) and Arif *et al*. (2018) that 2 % calcium chloride increased the plant height in rice. At 10 DAS plant height was found to increase in 6 dS/m, interaction between salinity levels and priming chemicals on plant height was not significant. This may be due to reduced salt stress during initial stages. But at 16 DAS the plants maintained at salinity level of 9 dS/m registered more plant height. Activity of calcium chloride was found enhanced at salinity level of 9 dS/m, probably due to combined effect of both these factors.

At 16 DAS and 10 DAS root length increased in calcium chloride treated plants. The highest increase in root length at 16 DAS was for seedlings primed using BABA and calcium chloride. The increase in root length was significant in seedlings maintained at salinity level of 6 dS/m (10 DAS), while at 16 DAS increase in root length was noted for seedlings maintained at salinity level of 9 dS/m. As in case of variety Jyothi, in Uma also plant height and root length increased in seedlings primed with calcium chloride.

The dry matter production at 10 DAS was found high for calcium chloride and SNP treated seedlings and highest dry weight was found in seedlings maintained at salinity level of 9 dS/m. At 16 DAS highest dry weight was recorded by calcium chloride primed seedlings. Calcium chloride can be said to be a chemical capable of increasing plant height, root length and dry weight under higher level of salinity. Galahitigama and Wathugala (2016) reported that 2 % calcium chloride increased the dry weight in rice seedlings. Increased root and shoot growth as a result of calcium chloride priming increased the dry weight. Soil application of calcium chloride was reported to provide a direct source of calcium and replace Na^+ (Gupta and Abrol, 1990; Mace *et al*., 1999; Qadir *et al*., 2001). Its effectiveness as a soil amendment might have contributed to the overall performance in plant.

Seed priming with sodium nitroprusside (SNP) increased the germination percentage in Vytilla-10. Hammed *et al*. (2013) reported that SNP priming improved seed germination in wheat. Interaction effect was also high for seedlings primed using SNP at salinity level of 9 dS/m. No definite answers to the interaction effects between salinity level and certain priming chemicals such as calcium chloride, sodium nitroprusside are known.

Plant height though comparable at 10 DAS, at later stage seedlings primed with calcium chloride had increased height. Interaction effect was observed for SNP at 9 dS/m. The same pattern of growth was observed in root length, at 10 DAS root length was almost comparable but at 16 DAS root length was high in calcium chloride primed seedlings. Interaction effect was observed for seedlings primed using calcium chloride and sodium nitroprusside at salinity level of 12 dS/m, hydro and calcium chloride at salinity level of 9 dS/m. Calcium chloride and sodium nitroprusside were found more effective at higher salinity level, while BABA was mainly effective at lower salinity level.

Highest dry weight at 10 DAS and 16 DAS was for seedlings raised from calcium chloride priming. Galahitigama and Wathugala (2016) reported that supply of calcium as calcium chloride increased the plant height and dry weight in rice.

5.2.1 Visual symptoms of salt injury in rice varieties

Phenotypic scoring for variety Jyothi showed that at 16 DAS priming chemicals BABA and calcium chloride at salinity level of 6 dS/m had a score of 3 (nearly normal growth, but leaf tips white and rolled and was considered tolerant) and hydro priming was having a score of 7 (suceptible), while at higher salinity levels $CaCl_2$ scored 3 (tolerant) while BABA (moderately tolerant, score 5). Calcium chloride was effective in imparting salt tolerance in Jyothi. Jyothi falls under highly susceptible category, but upon seed priming with calcium chloride tolerance level of Jyothi got improved. Seedlings from primed seed could tolerate salinity upto 6 dS/m. This may be due to multiple reasons like reduced ROS level, dilution effect as a result of increased dry matter production and improved nutritional balance in plants. Arif *et al*. (2018) reported that salt stressed plants treated with calcium showed increased chlorophyll content, as well as reduced the accumulation of H_2O_2, which protected the cells from oxidative damage.

At salinity level of 6 dS/m and 9 dS/m, all priming chemicals were highly tolerant (score1) at 10 DAS while hydro priming had a score of 3 (tolerant) in rice variety Uma. As the crop stage progressed to 16 DAS growth remained comparable, but the score increased to 3. At 9 dS/m the chemicals were equally effective up to 10 DAS, but at 16 DAS, $CaCl_2$, SNP and BABA were found to be effective. Yousof (2013) reported that priming seeds with calcium reduced salt injury. Similar effect of nitric oxide in alleviating salinity stress was reported by Adamu *et al*. (2018) in their study on the effects of SNP in rice.

In rice variety Vytilla-10, seedlings from seeds treated with BABA, $CaCl_2$ and SNP had normal growth without any visual salt injuries at salinity levels of 9 dS/m or 12 dS/m upto 10 DAS. The injury level for hydropriming was more at salinity level of 12 dS/m (score 5) than at salinity level of 9 dS/m (score3), however at 16 DAS, seedlings from BABA, $CaCl_2$ and SNP primed seeds were found to be affected by salt injury (score-3, tolerant category). It can be inferred that Vytilla-10 was tolerant to salinity up to 10 DAS and could not tolerate long term exposure to higher salinity. At 16 DAS priming with BABA was less effective compared to SNP (score-3, tolerant) and $CaCl_2$ (score-3, tolerant) in overcoming salt injury. In all the three varieties calcium chloride improved the salinity tolerance compared to water priming. The effect of calcium chloride in improving salt tolerance in all the varieties may be due to altered physiological mechanisms in plants. Seed priming with calcium chloride priming is reported to increase chlorophyll content of seedlings, suppress the accumulation of hydrogen peroxide and increase relative water content (Arif *et al*., 2018). The increased presence of calcium in plant cell would have balanced the Na^+/K^+ ratio. Afzal *et al*. (2012) also reported that seed priming with calcium chloride brought about small leaf Na^+ accretion at the expense of K^+ uptake into two rice varieties, but the effect was more pronounced in tolerant genotype.

5.3 Field trial to assess the effect of priming in rice in fields with saline water intrusion

The result of field trial conducted in *Pokkali* land with rice variety Vytilla-10 is discussed below. The priming chemicals treated were BABA, $CaCl_2$, SNP and water

a) Plant height

Plant height generally reduces under salinity stress. The role of chemical primers in improving plant height could be observed only when the seedlings were exposed to salinity stress. The seeds primed with $CaCl_2$ and beta amino butyric acid were found to increase plant height after 60 DAT, while plant height was unaffected by priming during the initial stages of the crop. Alam *et al*. (2004) in their study on rice growth under different salinity levels reported the possible role of calcium in improving salt tolerance in rice by its cellular functions in plant. The increase in plant height coincided with peak field salinity peak (Figure 1). It should also be noted that the effect of $CaCl_2$ was helpful for the crop at its most sensitive phenophase of early seedling and reproductive stages. Zeng *et al*, (2001) and Moradi *et al*. (2007) also found that these two are the most sensitive stages. A similar finding substantiating the effect of $CaCl_2$ was reported in rice. Yousof (2013) reported that seed priming increased the shoot length, germination parameters, dry weight in rice compared with control (distilled water) under salt stress. Increase in plant height, number of leaves and shoot dry weight was found by the application of 2 % calcium chloride in a study conducted by Galahitigama and Wathugala (2016). Similar effect of beta amino butyric acid (BABA) in improving seedling growth was reported by Jisha and Puthur (2016). They reported that increased seedling growth was observed in stressed and unstressed plants treated with BABA.

b) Number of tillers/m^2

Many reports suggested that tillering in rice was affected by salinity. In the initial stages (20 DAT and 40 DAT) it was observed that there was no improvement in tiller number due to different priming chemicals. However, seed priming with sodium nitroprusside was found effective in increasing tillers per metre square at 60 DAT. Nitric oxide released from SNP was found to scavenge superoxide free radicals produced in plants in response to increased salinity (Thomas *et al*., 2003) which could otherwise have resulted in reduced tillers. In salt stress, plants especially rice loses its tillers as a mechanism to cope with salinity. Zhu (2001) reported that excess accumulation of ROS lead to inhibition of cell division and cell expansion, this ultimately leading to death. The role of nitic oxide in alleviating oxidative damage due to salinity was reported in several plants (Uchida *et al*., 2002; Zhang *et al*.,2006; Guo *et al*.,2009)

Nabi *et al*. (2019) reported that application of nitric oxide improved the antioxidant activity in plants. Foliar spraying of priming chemicals at weekly interval was effective to an extent in increasing number of tillers at 60 DAT. Interaction effect was noticed between SNP and weekly foliar spraying in improving tillering. This implied that enough release of NO may not occur by seed priming alone, it has to be supplemented on a weekly basis.

5.3.3 Leaf Area Index

Leaf area index is an important parameter influencing yield in rice. In this experiment initially LAI was found high for BABA, hydropriming and SNP but at later stage of crop growth treatment with calcium chloride at 2% registered maximum leaf area. Alam *et al*. (2004) reported that there was a negative correlation between leaf area and salinity level. They continued that increase in salinity to 4.5 dS/m brought about 50 % reduction in leaf area in rice. Youssef *et al*. (2017) reported that in lettuce that foliar application of calcium chloride at 20 mM increased the growth parameters especially height, fresh weight, dry weight and LAI. Increased leaf area would help the plant in increasing the photosynthetic capacity. Also increase in leaf area has a diluting effect in the context of salinity tolerance.

The lowest leaf area was found for water priming at 60 DAT. In the initial stages of crop growth all the priming chemicals except calcium chloride were having comparable leaf area index. But it was observed that leaf area index at later stages of the crop growth increased for calcium chloride treated plants. Priming with $CaCl_2$ was found to increase leaf area index at 40 DAT and 60 DAT. Meneguzzo *et al*. (2000) in their studies with durum wheat found that under saline stress the Ca^{2+} content in cell was found decreasing and this lowered the calcium signaling required for salt tolerance. Hasegawa *et al*. (2000) and Parre *et al*. (2007) reported that calcium acted as a secondary messenger and the decrease in calcium could be compensated through external application of calcium sources. The increased presence of calcium in plant may be due to foliar application of $CaCl_2$.Weekly foliar spraying of priming chemicals before 40 DAT was found effective. Although at 20 DAT BABA seed priming with weekly foliar spray was effective, at later stages calcium chloride with weekly water spray was effective.

5.3.4 Yield parameters

Effect of salinity on yield parameters of rice is a widely researched topic. Salinity stress is known to increase spikelet sterility, reduced tillers, reduced grain filling and grain weight etc. Application of calcium chloride was effective in increasing number of panicles. Seed priming was found effective in improving panicles per square meter. Karrem *et al*. (2019) reported that highest tillering efficiency was obtained by the use of 150 mM calcium chloride. Tillering efficiency indicated the number of productive tillers. It needs to be noted that weekly foliar spraying was effective and strong interaction between weekly foliar and calcium chloride seed priming was observed. This suggest that application of calcium chloride on weekly basis improved the number of panicles per meter square.

Number of grains per panicle was high for seedlings primed with BABA. The exogenous application of BABA was found effective at fortnightly interval. Exogenous application of BABA was reported to increase silique length and seed production in mustard (Jakab *et al*. 2001) and Wu *et al*. (2010) and Zimmerli *et al*. (2008). The exact pathway leading to increased grain number is not known. Interaction effect was found for SNP application at fortnightly intervals, BABA seed priming with weekly foliar sprays and hydropriming with fortnightly foliar sprays. The increase in grains per panicle in SNP at fortnightly intervals might have been due to the ability of NO to prevent cell death due to oxidative damage as discussed earlier. BABA seed priming with weekly foliar spray and hydro priming with fortnightly spray were effective in increasing grains per panicle.

Priming with calcium chloride increased the filling percentage. This may be due to increased presence of calcium in plants, which essentially has a role in grain development and filling. Applying foliar spray at weekly interval was found effective. Weekly application ensured that calcium was available to the plant throughout its life cycle.

Thousand grain weight was high in plants treated with calcium chloride. Grains of calcium chloride treated plants had high weight (26 g), probably due to the role of Ca in grain filling. Moreover, *Pokkali* soils are deficient in calcium, so foliar application at fortnightly intervals would have negated calcium deficiency in plants.

As didcussed in this chapter, grains per panicle, number of panicles and thousand grain weight were high in $CaCl_2$ treated plants, so obviously yield would be high for plants treated with calcium chloride. A yield increase of 61 % was noticed in calcium chloride primed plants.

5.3.5 Biochemical parameters

Increased chlorophyll in leaves is vital for increased photosynthesis in plants. Ashraf (1994) reported that reduction in chlorophyll content in rice under salt stress was due to increased degradation of chlorophyll pigments as a result of metabolic imbalance. Priming seeds with beta amino butyric acid and calcium chloride was found to increase chlorophyll content in Vytilla 10 at 40 DAT. Priming with calcium chloride and BABA were equally effective (comparable). Jisha and Puthur (2016) in rice, reported that the chlorophyll content in seedlings was more when BABA seed priming was done than in non- primed seedlings. They also reported that BABA seed priming got carried over to seedlings, and in this way BABA priming enhanced tolerance to saline stress in rice seedlings. In our study weekly foliar spray was found effective at 40 DAT. Cohen *et al*. (2010) reported that there was clear evidence that by foliar or soil drenching of BABA, the chemical enters inside the cells and resulted in a cascade of signalling process which are mediated through hydrogen peroxide. The effect of calcium chloride in increasing chlorophyll content was observed at 40 DAT and 60 DAT. Suriyan *et al*. (2012) in their work on role of calcium in alleviating salinity stress in indica rice reported that application of calcium chloride enhanced the chlorophyll content in *Pokkali* and salt sensitive IR 29. At 60 DAT the effect of sodium nitroprusside in increasing chlorophyll content was noticed. Fortnightly application of SNP was found effective. Sodium nitroprusside is used as a donor of nitric oxide. The exogenous application of nitric oxide improves plant biomass and leaf chlorophyll by improving the activities of antioxidants that help scavenge free radicals (Dong *et al*., 2014; Kausar *et al*.,2013). Use of beta amino- butyric acid was found to be useful at low salinity level, but at higher salinity level calcium chloride and sodium nitroprusside had relevance. Moreover, fortnightly spray of priming chemical was effective. While chlorophyll a content was found to increase at 60 DAT by the application of calcium chloride, SNP and BABA, chlorophyll b content was improved in SNP primed seedlings. In SNP primed seedlings both chlorophyll a and chlorophyll b contents increased.

The role of proline in plants under stress cannot be underestimated. A wide array of activities is performed by proline under stress. Some are known and some are largely unknown. They help overcome osmotic stress experienced by plants under salt stress. Their association with ABA in stress tolerance is a widely researched topic and still it remains a mystery. Proline is known to indue the expression of salt responsive genes in plants (Ashraf and Foolad, 2007). Proline also acts as a free radical scavenger. Increased plant proline levels are said to be found in tolerant plants. So, increase in proline levels is an index to increased salt tolerance. In our study increase in proline content was observed at 40 DAT in SNP primed plants. The next best chemical found effective in increasing proline level was calcium chloride. At 60 DAT the proline content was found high for calcium chloride treatment. The next best chemical found effective was use of sodium nitroprusside. Jday (2016) observed that in *Cakile maritima* seedlings, pre-treatment of SNP significantly enhanced proline levels and this was due to enhanced activity of P5CS, which is known as an enzyme involved in proline synthesis. He continued that SNP application modified the signalling activity. Suriyan *et al*. (2012) reported that exogenous application of calcium chloride (7.92mM) increased the proline level in *Pokkali*. All the above findings by different authors in different crops are in conformity with our obtained result, that both $CaCl_2$ and SNP are effective in increasing proline and thereby salinity tolerance in plants.

SUMMARY

6. SUMMARY

A study was conducted at RRS, Vytilla during 2019, Kharif season to study the effect of chemical priming in rice for salinity tolerance. The study consisted of three experiments as summarized below:

In the first experiment, salinity tolerance of two ruling rice varieties and variety Vytilla-10 to different salinity levels at early growth stage was assessed under laboratory condition using float culture technique by providing Loshida nutrient solutions of salinity levels of 3 dS/m, 6 dS/m, 9 dS/m and 12 dS/m.

In variety Jyothi, growth retardation in terms of seedling height was noticed for salinity level above 6 dS/m at 10 DAS. At 16 DAS, complete cessation of growth and plant death was noticed for salinity level above 6 dS/m and at 21 DAS, almost all seedlings dried up completely at salinity level above 6 dS/m, while plants subjected to salinity level of 3 dS/m remained tolerant showing leaf rolling and white tip symptoms. Threshold salinity level for Jyothi was identified as 6 dS/m.

In variety Uma growth retardation was noticed at salinity levels of 6 dS/m and 9 dS/m at 16 DAS and at 10 DAS in plants maintained at salinity levels 9 dS/m and 12 dS/m. Complete cessation of plant growth was observed at 16 DAS in salinity level of 12 dS/m. By 16 DAS all plants dried.

In Vytilla-10 normal growth was observed for plants at all stages of observation at salinity levels of 3 dS/m and 6 dS/m. Leaf rolling was observed in seedlings at salinity level of 9 dS/m at 10 DAS, growth retardation was noticed at 12 dS/m at 10 DAS and all plants dried at 16 DAS in 12 dS/m. At 21 DAS cessation of growth was observed at salinity level of 9 dS/m.

In another experiment seed priming using BABA, SNP, $CaCl_2$ and water as control was used to assess the effect of seed primers on the tolerance level of rice varieties Vytilla-10, Uma and Jyothi to salinity. The germinated seedlings were raised in Yoshida solution.

Seed priming using chemicals enhanced the salinity tolerance in all the three varieties. Priming with calcium chloride (2%) increased the plant height and root length at 16 DAS, in all the three varieties.

In variety Jyothi at 16 DAS priming chemicals BABA and calcium chloride at salinity level of 6 dS/m had a score of 3 (tolerant) and water priming was having a score of 7 (susceptible). At higher salinity level of 9 dS/m $CaCl_2$ was found tolerant (score 3) while BABA was found as moderately tolerant (score 5). Salinity tolerance in variety Jyothi was enhanced by calcium chloride seed priming at a dose of 2 %.

In variety Uma at 16 DAS, priming with $CaCl_2$, BABA and SNP had a score of 3 (tolerant) at salinity levels of 6 dS/m and 9 dS/m.

For variety Vytilla-10 at 16 DAS, the plants pre -treated with BABA, $CaCl_2$ and SNP were found be tolerant (score-3). Water priming at 9 dS/m had a score of 5 (moderately tolerant) compared to the other treatments. At 12 dS/m, salt injury was higher (highly susceptible) for water primed plants (score-9). At both salinity levels calcium chloride and SNP increased the salinity tolerance level in Vytilla-10.

In the field experiment seeds of variety Vytilla-10 was primed using SNP, calcium chloride, BABA and water. The germinated seedlings were transplanted to *Pokkali* field and the effect of priming chemicals as foliar spray at weekly (30, 37 and 44 DAT), fortnightly (30,45,60 DAT) and weekly water spray as control was assessed.

In Vytilla-10, the effect of priming was prominent in the later stages of crop growth. Seed priming and foliar spray of $CaCl_2$ at 2 % resulted in highest LAI, more number of panicles, filled grain percentage and thousand grain weight, ultimately resulting in the highest yield of rice. Weekly spraying of chemicals (30, 37 and 44 DAT) was found to perform better than spraying at fortnightly intervals (30, 45 and 60 DAT) resulting in more yield. Seed priming and foliar spraying of sodium nitroprusside (SNP) resulted in more chlorophyll content in leaf, while calcium chloride enhanced proline content in the leaf at 60 DAT.

Priming seeds with 2 % calcium chloride and foliar spraying of calcium chloride (2%) at weekly interval (30, 37 and 44 DAT) was found effective in improving salinity tolerance in rice.

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APPENDIX

APPENDIX

Appendix 1. Weather parameters in *Pokkali* during the cropping period

Month	Mean maximum temperature^{0}C	Mean minimum temperature^{0}C	Mean evaporation (mm)	Mean sunshine hours(hrs/day)	Monthly rainfall (mm)	Monthly E.C
September	33.1	25.5	2.79	3.52	534	2.1
October	33.9	26	2.77	5.05	772.5	3
November	29.8	24.5	2.7	5.5	178	4.5
December	29.1	21.7	2.52	5.3	129	6

Appendix 2. Data on maximum temperature (^{0}C) and minimum temperature (^{0}C)

Date	**Maximum temperature ^{0}C**	**Minimum temperature ^{0}C**	**Date**	**Maximum temperature ^{0}C**	**Minimum temperature ^{0}C**
1/9/2019	30.1	22.3	24/9/2019	30.5	22.4
2/9/2019	32	23	25/9/2019	31	22.4
3/9/2019	31.2	22.5	26/9/2019	33	23.5
4/9/2019	31.2	23	27/9/2019	32	24.5
5/9/2019	33	6	28/9/2019	31.5	22
6/9/2019	32	22.5	29/9/2019	31	23
7/9/2019	32.5	22.5	30/9/2019	32.5	22.5
8/9/2019	33.5	22	1/10/2019	32	23
9/9/2019	32	23	2/10/2019	32.1	23.1
10/9/2019	32.5	24	3/10/2019	31.1	22.5
11/9/2019	33	24	4/10/2019	31.2	22
12/9/2019	33	24.5	5/10/2019	33	22.6
13/9/2019	33	22.4	6/10/2019	32	22
14/9/2019	32	22.4	7/10/2019	32.5	22.5
15/9/2019	32.4	23	8/10/2019	33.3	22.3
16/9/2019	32.4	22.5	9/10/2019	32	23.1
17/9/2019	32.1	22	10/10/2019	32.5	24.1
18/9/2019	32	21.5	11/10/2019	33.1	24.3
19/9/2019	32.3	22.8	12/10/2019	33	24.5
20/9/2019	32.3	23	13/10/2019	33	22
21/9/2019	30	23.5	14/10/2019	32.1	22.4
22/9/2019	32.8	22.5	15/10/2019	32.4	23
23/9/2019	32	24	16/10/2019	32.4	22.5

17/10/2019	32.2	22	14/11/2019	32	22.4
18/10/2019	32	22.5	15/11/2019	32.4	23
19/10/2019	32	22.8	16/11/2019	32.4	22.5
20/10/2019	32.3	23.3	17/11/2019	32	22
21/10/2019	30	24.5	18/11/2019	32	22.5
22/10/2019	32.8	25	19/11/2019	32	22.8
23/10/2019	32.8	24.5	20/11/2019	32.3	23.3
24/10/2019	30	22.4	21/11/2019	30	24.5
25/10/2019	31.2	22.3	22/11/2019	32.8	22.5
26/10/2019	33.1	23.4	23/11/2019	32.8	24.5
27/10/2019	32.2	22.5	24/11/2019	30	22.1
28/10/2019	31.5	22	25/11/2019	31.2	22.3
29/10/2019	31	22.5	26/11/2019	33	23
30/10/2019	32.5	22.5	27/11/2019	32	22.5
31/10/2019	33	23	28/11/2019	31.5	22
1/11/2019	32.1	22	29/11/2019	31	22.5
2/11/2019	32	23.1	30/11/2019	32.5	23.5
3/11/2019	31	22.5	1/12/2019	32.1	22
4/11/2019	31.2	22	2/12/2019	32	23
5/11/2019	33	22.6	3/12/2019	31	22.5
6/11/2019	32	22	4/12/2019	31.2	22
7/11/2019	32.5	22.5	5/12/2019	33	24.4
8/11/2019	33	22.3	6/12/2019	32	22
9/11/2019	32	23.1	7/12/2019	32.5	22.5
10/11/2019	32.5	24.1	8/12/2019	33	22
11/11/2019	33	24.3	9/12/2019	32	23
12/11/2019	33	24.5	10/12/2019	32.5	24
13/11/2019	33	22	11/12/2019	33	24

12/12/2019	33	24.5
13/12/2019	33	22
14/12/2019	32	22.4
15/12/2019	32.4	23
16/12/2019	32.4	22.5
17/12/2019	32	22
18/12/2019	32	21.5
19/12/2019	32	22.8
20/12/2019	32.3	23.3
21/12/2019	30	23.5
22/12/2019	32.8	22.5
23/12/2019	32.8	24.5
24/12/2019	30	22
25/12/2019	31.2	22.4
26/12/2019	33	23
27/12/2019	32	22.5
28/12/2019	31.5	22
29/12/2019	31	22.5
30/12/2019	32.5	23.5
31/12/2019	32	22.5

Chemical priming for improving salinity tolerance in rice

by

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(2018 – 11 - 065)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the
requirement for the degree of

Master of Science in Agriculture

(AGRONOMY)

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2020

Abstract

Measures to improve abiotic stress tolerance in plants is gaining importance due to raised concerns on climate change and food security issues all over the world. Though different methodologies for combating abiotic stress like conventional breeding and molecular breeding have been in existence, they are often limited by time and biosafety concerns respectively. Chemical priming is a cost-effective approach in managing abiotic stress. It is reported that use of chemicals, both synthetic and natural, prior to stress events can enhance tolerance to abiotic stresses such as salinity and drought. Application of chemical agents externally can impart a minor stress which will help to generate stress signals that can lead to certain biological changes resulting in increased stress tolerance in later stages of the crop. Rice is a crop badly affected by salinity. Use of chemicals such as beta amino butyric acid (BABA), sodium nitroprusside and calcium chloride as seed primers to improve salinity tolerance may turn promising. With this objective a study was formulated with three experiments. The experiments were screening rice varieties for salinity tolerance, effect of seed priming on germination of rice varieties under salt stress condition and field trial to assess the effect of priming in rice fields with saline water intrusion.

The First experiment was a lab study to screen three rice varieties namely Jyothi, Uma and Vytilla-10 at five salinity levels *i.e.,* non-saline, 3 dS/m, 6 dS/m, 9 dS/m and 12 dS/m. Based on the results it was concluded that at 16 DAS with salinity level of 6 dS/m variety Vytilla-10 was highly tolerant (score 1). Variety Jyothi was found susceptible at 6 dS/m (score 7) whereas variety Uma was moderately tolerant (with score 5). At 21 DAS, varieties Uma and Vytilla-10 were found to be susceptible at salinity levels of 6 and 9 dS/m respectively with a score of 7.

Objective of second experiment was to assess whether chemical priming could improve salinity threshold of the three varieties. The design used was two factorial CRD with three replications. The treatments were four priming chemicals and two salinity levels. Based on the results of the first experiment, threshold salinity level for Jyothi, Uma and Vtyilla-10 were estimated. Jyothi and Uma were susceptible at 6 dS/m, while Vytilla-10 was susceptible at 9 dS/m. Therefore, in second experiment variety Jyothi, salinity levels of 6 dS/m and 9 dS/m, for variety Uma salinity levels of 6 dS/m and 9 dS/m and for Vytilla-10 salinity levels 9 dS/m and 12 dS/m were taken. Each variety was primed using three chemicals, namely beta amino butyric acid (BABA 1 Mm/L), sodium nitroprusside (SNP,100 µM) and calcium chloride ($CaCl_2$, 2%), water priming was included as control. The efficacy of each priming chemical was analyzed based on germination percentage, growth parameters and visual salt injury symptoms. Priming seeds with calcium chloride was found to influence both the growth parameters and salinity tolerance of seedlings.

Based on the results of second experiment, a field trial was undertaken to assess the efficacy of priming chemicals as seed primers and as foliar spray. An experiment was laid out at Rice Research Station, Vytilla in factorial RBD with three replications. The treatments were three priming chemicals namely beta amino butyric acid (BABA), sodium nitroprusside (SNP) and calcium chloride ($CaCl_2$), with water priming as control and three foliar sprays at weekly intervals, fortnightly intervals and weekly water spray. Rice variety Vytilla-10 was used for the study. The seedlings were transplanted to main field and foliar spraying of priming chemicals was done at weekly intervals at 30, 37 and 45 DAS, fortnightly intervals at 30, 45 and 60 DAS as well as weekly water spray. Calcium chloride seed priming was found effective in increasing plant height at 60 DAT, leaf area index, number of panicles, percentage of filled grains, thousand grain weight, yield, proline content in leaf and chlorophyll content (at 60 DAT). Most of the growth and yield parameters were influenced by weekly spray of calcium chloride. More tillers/m^2 was observed in seedlings primed using SNP and weekly foliar spraying of SNP was effective in increasing tillering. Total chlorophyll content was found to increase with SNP seed priming and foliar spraying of SNP at fortnightly intervals was found most effective at 60

DAT to increase chlorophyll content. Grain filling was found to increase with BABA seed priming and weekly foliar spray of the same chemical was highly effective.

Priming rice seeds with 2 % calcium chloride and foliar spraying of calcium chloride at 2 % on a weekly basis was found to be a reliable method to combat salinity stress and for improving growth, yield and yield attributes of rice.

Chemical priming for improving salinity tolerance in rice

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സംഗ്രഹം

സസ്യങ്ങളിൽ അബയോട്ടിക് സ്ട്രെസ് ടോളറൻസ് മെച്ചപ്പെടുത്തുന്നതിനുള്ള പരമ്പരാഗത മാർഗ്ഗങ്ങളായ ബ്രീഡിംഗ്, മോളിക്യുലർ ബ്രീഡിംഗ് പോലുള്ള വ്യത്യസ്ത രീതികൾ നിലവിലുണ്ടെങ്കിലും, അവയുടെ പ്രായോഗികത സമയദൈർഘ്യവും ബയോ സേഫ്റ്റി ആശങ്കകളും കൊണ്ട് പരിമിതമാണ്.

അബയോട്ടിക് സ്ട്രെസ് കൈകാര്യം ചെയ്യുന്നതിനുള്ള ചെലവ് കുറഞ്ഞ സമീപനമാണ് കെമിക്കൽ പ്രൈമിംഗ്. ചില പ്രത്യേക രാസവസ്തുക്കളുടെ ഉപയോഗം ചെടികളിലെ അബയോട്ടിക് സ്ട്രസ്സ് കുറക്കുന്നതിന് കാരണമാകുമെന്ന് പല മുൻകാലപഠനങ്ങളും വെളിപ്പെടുത്തിയിട്ടുണ്ട്. രാസവസ്തുക്കളുടെ ബാഹ്യപ്രയോഗങ്ങൾ ചെടിയിൽ മാററങ്ങൾക്ക് കാരണമാകുന്ന സ്ട്രസ്സ് സിഗ്നലുകൾ ഉളവാക്കുകയും അവയുടെ സഹിഷ്ണുത വർദ്ധിപ്പിക്കുന്നതിന് കാരണമാവുകയും ചെയ്യുന്നു.

ചെടികളുടെ പ്രകൃതിദത്തമായ സ്ട്രെസ് ടോളറൻസ്, രാസവസ്തുക്കളുടെ ഉപയോഗം മൂലം സ്ട്രെസ് ടോളറൻസിൻറെ വർദ്ധന എന്നീ കാര്യങ്ങളിൽ പരീക്ഷണങ്ങൾ നടത്തുകയും ,ഫീൽഡു ട്രയസിലൂടെ അവ സ്ഥിരീകരിക്കുകയും ചെയ്തു. ബീററാ അമിനോബ്യൂററാറിക് ആസിഡ്, സോഡിയംനൈട്രോപ്രുസൈഡ്, കാൽസ്യം ക്ളോറൈഡ് എന്നീ രാസവസ്തുക്കളാണ് പരീക്ഷണങ്ങൾക്ക് ഉപയോഗപ്പെടുത്തിയത്.

നെൽചെടിയുടെ പ്രകൃതിദത്തമായ സ്ട്രെസ് ടോളറൻസ് കണ്ടെത്തുന്നതിനുള്ള പരീക്ഷണത്തിൽ ജ്യോതി- 6 ഡിഎസ്, ഉമ- 6 ഡി എസ്

വൈററില-10- 9 ഡിഎസ് എന്നീ ലവണാംശ റേററിംങ് ടോളറേററു ചെയ്യുമെന്ന് കണ്ടെത്തി.

കെമിക്കൽ പ്രൈ മിംഗിന് മൂന്ന് ഇനം നെൽചെടികളുടെയും ലവണാംശ റേററിംങ് മെച്ചപ്പെടുത്താൻ കഴിയുമോ എന്ന് വിലയിരുത്തുന്നതിനായിരുന്നു രണ്ടാമത്തെ പരീക്ഷണം.

മൂന്ന് റെപ്ലിക്കേഷനുകളുള്ള രണ്ട് ഫാക്റ്റോറിയൽ സിആർഡിയായിരുന്നു ഉപയോഗിച്ച ഡിസൈൻ. മൂന്ന് പ്രൈമിംഗ് രാസവസ്തുക്കളും രണ്ട് ലവണാംശ അളവുകളുമാണ് പരീക്ഷണത്തിന് ഉപയോഗപ്പെടുത്തിയത്.

ആദ്യ പരീക്ഷണത്തിന്റെ ഫലത്തെ അടിസ്ഥാനമാക്കി ജ്യോതി, ഉമ, വൈറ്റില -10 എന്നിവയ്ക്കുള്ള ലവണാംശത്തിന്റെ അളവ് തിട്ടപ്പെടുത്തി. . അതിനാൽ, രണ്ടാമത്തെ പരീക്ഷണത്തിൽ ജ്യോതിയിൽ, ലവണാംശത്തിന്റെ അളവ് 6 dS / m, 9 dS / m, ഉമാ- ലവണാംശം 6 dS / m, 9 dS / m, വൈറ്റില -10 ലവണാംശം 9 dS / m, 12 dS / m എന്നീ തോതിലാണ് എടുത്തത്. ബീറ്റ അമിനോ ബ്യൂട്ടിറിക് ആസിഡ് (BABA 1 Mm / L), സോഡിയം നൈട്രോപ്രൂസൈഡ് (SNP, 100 μM), കാൽസ്യം ക്ലോറൈഡ് ($CaCl_2$, 2%) എന്നിങ്ങനെ മൂന്ന് രാസവസ്തുക്കൾ ഉപയോഗിച്ചാണ് ഓരോ ഇനം നെൽവിത്തിനെയും പ്രൈംചെയ്തത്. കൂടാതെ എല്ലാ ഇനങ്ങളുടെയും സാമ്പിളുകൾ , വാട്ടർ പ്രൈമിംഗ് വഴി റെഫറൻസിനായി ഉപയോഗപ്പെടുത്തി. മുളയ്ക്കുന്ന ശതമാനം, വളർച്ചാ പരാമീറ്ററുകൾ, കാണപ്പെടുന്ന ലക്ഷണങ്ങൾ എന്നിവ അടിസ്ഥാനമാക്കി ഓരോ പ്രൈമിംഗ് രാസവസ്തുക്കളുടെയും ഫലപ്രാപ്തി വിശകലനം ചെയ്തു. കാത്സ്യം ക്ലോറൈഡിൻറെ ഉപയോഗം തൈകളുടെ വളർച്ചയുടെ മാനദണ്ഡങ്ങളെയും തൈകളുടെ ലവണാംശ സഹിഷ്ണുതയെയും സ്വാധീനിക്കുന്നതായി കണ്ടെത്തി.

കെമിക്കൽ പ്രൈമിംഗ് ഉപയോഗിച്ചുള്ള രണ്ടാമത്തെ പരീക്ഷണത്തെ അടിസ്ഥാനമാക്കി, ചെടികളുടെ വളർച്ചാ വ്യതിയാനത്തെയും സവിശേഷതകളെയും ഫീൽഡു ട്രയൽ മുഖേന സ്ഥിരീകരിച്ചു ഫീൽഡു ട്രയസിന് ഫോളിയർ സ്പ്രേയും നൽകിയിരുന്നു (ആഴ്ച, രണ്ടാഴാച ഇടവിട്ട് -കൂടാതെ വാട്ടർ കൺട്രോൾ).

ഫീൽഡ് ട്രയൽ ക്രമീകരിച്ചത് വൈററില നെല്ല് ഗവേഷണകേന്ദ്രത്തിലാണ്. ഫീൽഡു ട്രയസിന് വൈററില 10 ഇനം നെൽചെടികളാണ് ഉപയോഗിച്ചത്.

ചെടികളുടെ ഉയരം 60 DAT, ഇല വിസ്തീർണ്ണ സൂചിക, പാനിക്കിളുകളുടെ എണ്ണം, ധാന്യങ്ങളുടെ ശതമാനം, ആയിരം ധാന്യ ഭാരം, വിളവ്, ഇലയിലെ പ്രോലിൻ ഉള്ളടക്കം, ക്ലോറോഫിൽ ഉള്ളടക്കം (60 DAT) എന്നിവ കാൽസ്യം ക്ലോറൈഡ് പ്രൈമിംഗ് മുഖേന വർദ്ധിപ്പിക്കുന്നതായി കണ്ടെത്തി. കാൽസ്യം ക്ലോറൈഡിന്റെ പ്രതിവാര സ്പ്രേയാണ് വളർച്ചയുടെയും വിളവിന്റെയും പാരാമീറ്ററുകളെ കൂടുതലായി സ്വാധീനിച്ചത്.

എസ്എൻപി ഉപയോഗിച്ച് പ്രൈം ചെയ്ത തൈകളിൽ കൂടുതൽ ടില്ലറുകൾ /m2 നിരീക്ഷിക്കപ്പെട്ടു, എസ്എൻപിയുടെ പ്രതിവാര ഫോളിയർ സ്പ്രേയും ഫലപ്രദമായിരുന്നു. കൂടാതെ ക്ലോറോഫിൽ ഉള്ളടക്കം വർദ്ധിക്കുന്നതായി കണ്ടെത്തി, രണ്ടാഴ്ച ഇടവേളകളിൽ എസ്എൻപി ഇലകളിൽ തളിക്കുന്നത് 60 DAT യിൽ ക്ലോറോഫിൽ ഉള്ളടക്കം വർദ്ധിപ്പിക്കുന്നതിന് ഏറ്റവും ഫലപ്രദമായി കണ്ടെത്തി. ബാബ പ്രൈമിംഗ് വഴി പതിരുകൾ കുറഞ്ഞതായി കണ്ടെത്തി, അതേ രാസവസ്തുവിന്റെ പ്രതിവാര ഫോളിയർ സ്പ്രേയും വളരെ ഫലപ്രദമായിരുന്നു.

നെൽ വിത്തുകൾക്ക് ആഴ്ചയിൽ 2% കാൽസ്യം ക്ലോറൈഡ് തളിക്കൽ അവയുടെ ലവണാംശ ടോളറൻസ് വർദ്ധിക്കുന്നതിനും ഫലസമൃദ്ധിക്കും കാരണമാകുമെന്നും ഈ പരീക്ഷണത്തിലൂടെ സ്ഥിരീകരിച്ചു.