EFFICACY OF SEED PRIMING FOR INDUCING STRESS TOLERANCE IN CHILLI (*Capsicum annum* L.) UNDER WATER STRESS CONDITION

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KERALA, INDIA

2023

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By

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THESIS

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2023

DECLARATION

I, hereby declare that this thesis entitled, **"Efficacy of seed priming for inducing stress tolerance in chilli (*Capsicum* annum L.) under water stress condition"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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LIST OF ABBREVIATIONS

CO_2	carbon-dioxide
ppm	parts per million
%	percent
˚C	degree celsius
cm	centimeters
m	meter
nm	nano meters
g	gram
kg	kilogram
µg	microgram
µl	microliter
ml	milliliter
Fv/Fm	variable to maximal fluorescence
NPQ	non-photochemical quenching
kDa	kilo daltons
rpm	rotations per minute
kPa	kilo pascals
µmol	micro-moles
mmol	milli-moles
s	seconds
mol	moles
h	hours

Introduction

1. INTRODUCTION

Chilli (*Capsicum annum* L.) is one of the important horticultural crops belonging to the family Solanaceae that is widely used by the human population because of its rich antioxidants, high pungency, rich flavour and vitamins. The majority of the world's chilli plants are grown in tropical regions and they are regarded as a good source of vitamin C, carotenoids, flavonoids and mineral components. India is the largest producer of chillies in the world with an annual production of 13.76 million tonnes followed by China. India's total global chilli production accounts for 36.57 per cent. Even though the production of chilli is high, still price volatility for agricultural commodities such as chilli leads to adverse economic impacts. Young chilli seedlings are not able to resist water shortages or excessive soil moisture, but older seedlings can (Ayoub, 1986). Hence adequate water management is required in the initial growth stages.

The water management schedule is critically vital for chilli plants at all stages of plant growth. For this reason, the crop needs to receive enough water to maintain healthy growth (Costa and Gianquinto, 2002). However, Climate change due to greenhouse gases from anthropogenic activities has both direct and indirect effects on agriculture. Throughout the growing phase and during developmental stages, plants are exposed to diverse biotic and abiotic stress due to erratic rainfall and climate variations (Zhou *et al*., 2007). High temperature, drought and salinity are the three main abiotic stresses, out of these droughts, those during the early stages of crop development delay and disrupt the uniform and timely sprouting of seedlings in chilli (Kaya *et al.,* 2006). Reduced water intake during the imbibition stage of seed germination reduces seedling emergence in chilli (Gowthami *et al.,* 2013). Plants increase various biochemical, molecular and physiological features as a result of ongoing water scarcity to adapt to unfavourable abiotic environments (Shebab *et al.,* 2010). Unfavourable abiotic environments like drought and other environmental conditions promote the prolific synthesis of reactive oxygen species (ROS) which gradually affects the lipids, RNA, DNA, carbohydrates and proteins (Gill and Tuteja, 2010).

One of the pre-germination strategies for overcoming drought circumstances is to prime the seeds with particular solutions. A simple, inexpensive and secure method for improving emergence, seedling growth, yield and crop tolerance to drought stress is the priming of seeds. When seeds are primed, pre-germination metabolic activities such as enhanced water uptake and reserve mobilisation of amylase, cellulase, and xylanase are stimulated without the seeds germinating (Zheng *et al.*, 2016). It has been demonstrated that priming seeds promote quicker germination rates, quicker seedling establishment and robust seedling growth. Farmers can

benefit from these biological priming processes since they shorten the time required for the emergence of seeds and lower the need for fertilisation, supplementary irrigation and recurring seedling costs (Acharya *et al.*, 2020).

Priming seed is a simple, affordable and practical method for encouraging quicker and uniform seedling emergence, increased seedling vigour and high output in a variety of field crops, especially in severe abiotic environments like salinity and drought (Jisha *et al.*,2013; Paparella *et al.*,2015). The key factors that cause primed seeds to germinate quickly and uniformly are enzyme activation, a reduction in the imbibition lag time, the production of metabolites that speed up germination, metabolic repair during imbibition, and osmotic adjustment. (Farooq *et al*.,2006, Hussain *et al*.,2015). Seed priming includes different methods like hydropriming, osmo priming, halo priming, nutrient priming, bio priming, chemical priming, and redox potential priming for various crops for different abiotic stress.

Chilli mostly grown in tropical climates faces frequent abiotic stress. Among them, the most common one is drought due to climatic changes. Chilli plants when subjected to drought stress prompts rapid stomatal closure and a drop-in photosynthetic activity, changes in concentration of carbohydrates, organic acids, as well as an osmolyte build up, small roots of the chilli i.e., up to 30 cm are particularly vulnerable to drought. Reduced leaf size, stem lengthening and root anchoring are the main effects of extreme drought stress which disrupt the crop's metabolic processes and water usage efficiency (Farooq *et al.,* 2009). Drought during the flowering stage in chilli affects flower survival percentage and pod formation stage which results in a decrease in fruit yield. Therefore, water stress condition is one of the main problems during the cultivation of chilli. There are too few studies on seed priming that focus on boosting crop establishment and yield in the chilli variety Vellayani Athulya. The benefits of KNO_3 and SiO_2 priming on the seedling stage, physiological and quality parameters during the flowering stage and yield attributes during the harvesting stage were therefore evaluated in this study.

Review of Literature

2. REVIEW OF LITERATURE

The berries or fruits of plants in the Solanaceae family include chillies (*Capsicum* spp.). Vitamin C, A, B complex, E, and minerals are all in great supply in chillies. Chillies are mostly grown in tropical and sub-tropical regions where they had been subjected to climatic changes. Water stress conditions significantly impacted crop establishment, growth and yield (Balakhina and Borkowska, 2013). Many pre-germination treatments like seed priming, seed fortification and seed infusion help to overcome unfavourable conditions. Seed priming primarily serves to monitor each seed's germination (Taylor and Harmon,1990).

The seeds are allowed to dry out in the shade and achieve a physiological condition known as the primed state after priming for a predetermined amount of time. This state aids in a better defence system (Beckers and Conrath, 2007). The methods used for seed priming include hydro priming, osmopriming, nutrient priming, hormonal priming, chemical priming, bio priming, solid matrix priming and redox priming depending on the effectiveness of the various priming agents under the various stresses experienced by the various crop species.

According to Farooq *et al. (*2005), Osmopriming also known as Osmo conditioning, is a priming technique that involves soaking seeds in low-water-potential solutions. KNO_3, KCl, $CaCl_2$, NaCl, and mannitol are a few of the compounds with low water potential. As per Foti *et al.* (2008); Moradi and Younsei (2009), osmotic seed priming is methodologically, technically and financially more demanding than hydropriming because it yields results more quickly and easily. It costs less than most water conservation techniques and provides farmers with highly appealing alternative for boosting crop establishment as well as yields. Being one of the most common cations in plants, potassium (K^+) is one of the most important micronutrients and has a significant impact on how crops grow and how their metabolism responds to biotic and abiotic stimuli (Wang *et al.,* 2013). Additional application of KNO_3 improves the antioxidant systems and prevents drought stress circumstances by increasing the photosynthetic pigments, lowering MDA levels and enhancing plant development (Fayez *et al.,* 2014). One useful agricultural micronutrient is silicon, which helps non-silicon-accumulating plants like the Solanaceae family better withstand drought (Shi *et al.,* 2016).

Watermelon germination can be boosted by seed priming with KNO_3 (Demir and Mavi, 2004). Osmopriming with KNO_3 enhanced the uniformity and rate of sorghum seedling emergence (Moradi and Younesi, 2009). In tomatoes, seed priming with KNO_3 improved root length, shoot length and seedling fresh weight (Nawaz *et al.,* 2011). In the current work, seeds

were primed with KNO_3, SiO_2 and unprimed. Drought stress was induced by withholding watering at the flowering stage. Priming seeds primarily aids in enhancing physiological, biochemical and yield features under challenging circumstances.

2.1 EVALUATION OF VARIOUS SEED PRIMING METHODS FOR WATER STRESS TOLERANCE IN CHILLI AT THE SEEDLING STAGE

2.1.1. Proline content

Farooq *et al. (*2013) recorded that hydro-priming of two wheat cultivars with ascorbic acid increased the proline content of the plants.

Increased proline content of *Nigella sativa* seedlings noticed when primed with KNO_3 (14 mg-1gram fresh weight), $ZNSO_4$ (17 mg $gram^{-1}$ fresh weight), PEG 6000 (20 mg $gram^{-1}$ fresh weight) and GA (21 mg $gram^{-1}$ fresh weight) under water stress conditions during the seedling establishment (Fallah *et al.*,2017).

Sahitya *et al.* (2018) disclosed that chilli genotypes were subjected to varying drought conditions and the proline concentration elevated in drought-tolerant genotypes like SHP4884-1-1 (1.69 µmoles g^{-1}) aiding in the maintenance of the RWC.

Rice seedlings primed with 2.5% KNO_3 (38.66 mg l^{-1}) and 3% SiO_2 (34.48 mg l^{-1}) when exposed to various types of stress such as mild, moderate and severe stress resulting in a steady rise in proline content (Ali *et al.,* 2021a).

When drought stress was applied to different chilli genotypes (Pusa Juala and Ghotki) during the flowering stage, the amount of proline in the leaves increased (2.7 µmoles proline g^{-1} of fresh weight and 3.5 µmoles proline g^{-1} of fresh weight) as an abiotic resistance mechanism (Mahmood *et al.,* 2021).

2.1.2 Malondialdehyde

Sairam and Saxena (1999) disclosed that there was increased lipid peroxidation content in wheat genotypes *i.e.,* PBW175, HD 2402 and WH 542 (221.50, 251.61 and 281.72 nmol g^{-1} fresh weight, respectively) when exposed to a water stress condition. On the other hand, decreased lipid peroxidation content was noted under irrigated conditions (137.63, 111.83 and 169 nmol g^{-1} fresh weight, respectively).

When maize genotype (Giza 2 and Trihybrid 321) seedlings were exposed to water stress through the application of PEG, tolerance genotypes (Giza 2) had lower MDA levels

(902 µmole g^{-1} of fresh weight) than sensitive genotypes (Trihybrid 321-1171 µmole g^{-1} of fresh weight) (Moussa and Abdel-Aziz, 2008).

Sahithya *et al. (*2018) reported that chilli genotypes grown under various levels of stress had minimal malondialdehyde content (0.83 mM g^{-1}) in drought-tolerant genotypes like SPH4884-1-1.

Khan *et al.* (2019) reported when rapeseed was primed with melatonin content during drought conditions (-0.4MPa PEG osmotic stress), recorded low level of malondialdehyde content (2800 nmol g^{-1} fresh weight).

When mustard seeds were exposed to water stress conditions throughout the early and late vegetative stages, seeds primed with Si showed less malondialdehyde concentration (38 nmol g^{-1} fresh weight) than non-treated seeds (69 nmol g^{-1} fresh weight) (Alamari *et al.*, 2020).

In response to drought stress, chilli genotype, Puerto showed lower MDA contents (39 µmoles g^{-1} fresh weight) (Kopta *et al.*, 2020).

In spinach, low MDA content was recorded in seeds treated with KNO_3 (1.8µmole g^{-1}) when compared to untreated seeds (2.1µmole g^{-1}) under the conditions of water stress (Bukhari *et al.*, 2021).

Decreased malondialdehyde content was noted even when the seed was primed with 2.5% KNO_3 (0.001mM mg^{-1} of fresh weight) and 3% SiO_2 (0.001mM mg^{-1} of fresh weight) during the stress condition (Ali *et al.,* 2021a).

Rice seedlings primed with 2.5% KNO_3, 3% SiO_2 and 2.5Mm SA under drought conditions, showed decreased MDA concentration (0.001, 0.002 and 0.024 µmol mg^{-1} fresh weight, respectively) when compared to unprimed seeds (0.080 µmol mg^{-1} fresh weight) (Ali *et al.*,2021b).

When the *Lathyrus odoratus* seeds were hydro-primed and halo primed with silicon and silicon nanoparticles and then exposed to saltwater levels, the lowered MDA concentration of 1.35 mg g^{-1} fresh weight was noted in well-established seedlings of halo and hydro-primed silicon nanoparticles (El-Serafy *et al.*, 2021).

2.1.3. Hydrogen peroxide

Moussa and Abdel-Aziz (2008) recorded that when maize genotype (Giza 2, Trihybrid 321) seedlings were exposed to water stress circumstances, the reduced value of hydrogen peroxide (4.78 µmole g^{-1} of fresh, 5.09 µmole g^{-1} of fresh) was noticed by applying PEG 6000.

Sahitya *et al.* (2018) reported a low level of lipid peroxidation (117.38 µmoles g^{-1}) in drought-tolerant genotypes (SPH4884-1-1) when exposed to various types of water regimes and their effect on water stress tolerance.

Bukhari *et al.* (2021) reported that lower hydrogen peroxide levels were displayed in the seedlings treated with KNO_3 (2.8 µmole g^{-1}) over untreated seeds (3.4 µmole g^{-1}) under several types of water stress settings.

El-Serafy *et al.* (2021) stated that when the hydro- and halo-primmed seeds of *Lathyrus odorants* were exposed to seal water levels, the well-established pea seedlings showed the least quantity of H_2O_2 (1.3 µmol g^{-1} fresh weight).

2.1.4 Seedling Vigour index 1& 2

Ali *et al.* (2021a) reported that slightly enlarged valves of SVI I and SVI II were recorded in the rice seedlings when seeds were primed with 2.5% KNO_3 (301.05 and 3573.12) and 3% SiO_2 (498.58 and 3069.7) under drought conditions when compared to control conditions (172.89 and 3128.65, respectively).

2.2 EFFECT OF THE SELECTED BEST TREATMENT FOR WATER STRESS TOLERANCE AND ITS EFFECT ON PHYSIOLOGICAL AND YIELD TRAITS IN CHILLI

2.2.1. Phenological traits

Water stress during the early flowering stage of several red chilli cultivars has resulted in fewer branches with less flowers $branch^{-1}$ and reduced fruit set, which directly impacts fruit quality and quantity (Abdulmalik *et al.,* 2012).

Low moisture and temperature variations brought by climate change, caused early blooming and other phenotypic changes that hurt plant growth and yield in chilli plants (Ayyogari *et al.,* 2014).

Yang *et al.* (2019) demonstrated that drought stress at key phases, such as the flowering period, affects grain yield and quality as well as physiological traits. When there was a drought

during critical stages, the ability to recover was strengthened, which gradually increased grain output when the conditions are right.

Maleki *et al.* (2013) stated that drought stress occurs in soybean during the flowering, podding and filling stages. The flowering and filling stages have reported the lowest seed production and oil content of any of them.

The Yard-long bean cv. BARI Borboti-1 were hydro primed at 12,18,24 and 30 hrs, halo priming with 1% $CaCl_2$ and 2% KNO_3 at 12, 18,24 and 30hrs. The seeds primed with 2%KNO_3 at 12 hrs, Hydro priming at 12hrs and unprimed seeds reached first flowering (41 days) (Karim *et al.*,2020).

2.2.2 Physiological traits

2.2.2.1 Relative water content (RWC)

When chilli genotypes were exposed to various stress conditions, the drought-tolerant genotypes (SPH4884-1-1) displayed a slightly lower rise in RWC (58%) compared to the sensitive genotypes (Teja-1-1-1-1) (47%) (Sahitya *et al.*, 2018).

When mustard seedlings treated with Si and NaCl were subjected to drought and salinity stress, the results demonstrated that Si+ NaCl treated seedlings (55%) had an effective role under both stress conditions when compared to non-primmed seeds (40%) (Alamari *et al.*, 2020).

Zhang *et al.* (2020) described that Si treatment of *Glycyrrhiza uralensis* plants reduced water loss by limiting the rate of stomatal transpiration.

Goto *et al*. (2021) reported that chilli peppers when exposed to varying amounts of soil moisture and an air vapour deficit, experienced reduced water absorption associated with higher transpiration, which resulted in a drop in their leaf water potential.

2.2.2.2 Cell membrane stability Index (CMSI)

Sairam and Saxena (1999) discovered that the wheat genotypes which were subjected to water stress tolerance *i.e.*, the tolerant genotypes like PBW 175 displayed an increase in membrane stability index (58%) and reduced lipid peroxidation (221.50 nmol $gram^{-1}$ fresh weight) in comparison to sensitive genotypes.

Moussa and Abdel-Aziz (2008) found that when maize genotypes were exposed to water stress conditions, there was a rise in relative water content with less membrane injury, directly demonstrating that tolerant genotypes exhibiting high RWC (83%) and were having high CMSI (75%).

2.2.2.3 Total proline content

Moussa and Abdel-Aziz (2008) revealed that when maize genotypes (Giza 2 and Trihybrid) were exposed to drought stress, the drought-tolerant genotype (Giza 2) had shown an increase in proline content (5.82 mM g^{-1} dry weight) when compared to drought-sensitive genotypes (Trihybrid 321) (3.96 mM g^{-1} dry weight).

Shafiq *et al.* (2015) disclosed that cotton seeds treated with 0.25%KNO_3 (1.3mg g^{-1} fresh weight) and 0.50% KNO_3 (1.5 mg g^{-1} fresh weight) and then exposed to drought stress *via* PEG 6000 had more chlorophyll than unprimed seeds.

Suwingo *et al.* (2017) reported that when red chilli types were subjected to several degrees of drought stress, the proline content continued to rise. When compared to other red chilli types, the Ferosa red chilli variety had been confirmed to have the greatest proline content (1.08 mM g^{-1}).

When rapeseed was primed with melatonin content under drought conditions (-0.4MPa PEG osmotic stress), it expressed an improved amount of proline content (590 µg g^{-1} fresh weight) (Khan *et al.,* 2019).

Proline content gradually rises with increasing stress. When *Lathyrus odoratus* were hydro-primmed and halo-primmed with silicon (Si) and silicon nanoparticles (SiNPs) under salt water stress, there was an increase in proline content (4.5 µmole g^{-1} of fresh) under hydro-primmed and halo-primmed silicon nanoparticles (SiNPs) conditions. This might be due to the direct impact of sea level on crop germination and establishment (El-Serafy *et al.,* 2021).

Goto *et al.* (2021) recorded that the chilli peppers exposed to varying amounts of soil water deficit and air vapour deficit experienced a decline in leaf water potential and a rise in total chlorophyll content (7.8 µChl ml^{-1}).

2.2.2.4 Total chlorophyll content

Sairam and Saxena (2000) reported that when wheat genotypes were exposed to water stress conditions, the tolerant genotypes like PBW 175 displayed the higher TCC (1.8 mg g^{-1} fresh weight) when compared to sensitive genotypes like HD 2402 (1.4 mg g^{-1} fresh weight).

Yasar *et al.* (2010) recorded TCC of 0.50 µmol g^{-1} when bean genotypes like S96 and SB were subjected to water stress by 10% PEG 6000 and TCC were recorded 6 days later after PEG implementation.

Pirzada *et al.* (2011) described that *Matricaria chamomilla* seedlings experience an increase in drought stress (100-55 field capacity%) followed by a decrease in chlorophyll content (3-5.8 mg g^{-1}).

Shafiq *et al.* (2015) reported that when cotton seeds treated with 0.25% and 0.50% KNO_3 were subjected to drought stress by PEG 6000 had shown an increase in chlorophyll content (1.25 and 1.48 mg g^{-1} fresh weight, respectively) when compared to non-primmed seeds (0.8 mg g^{-1} fresh weight).

Suwingo *et al.* (2017) noted that when red chilli varieties were subjected to different drought stress levels (50, 75 and 100% FC), the variety Ferosa recorded high TCC (65.61 cm^2 ml^{-1}) under irrigated conditions. Whereas, a decreasing trend of total chlorophyll content was noted with an increase in drought level. Low TCC (56.67 cm^2 ml^{-1}) was recorded in 50% FC conditions.

Sahitya *et al.* (2018) reported that chilli genotypes when subjected to different levels of drought lead to a decrease in chlorophyll content (12 mg g^{-1} fresh weight) in tolerant genotype (BSS355-1-1-1-1) when compared to the sensitive genotype (HPH1048-1-1-1-1) (11mg g^{-1} fresh weight).

Khan *et al*. (2019) expressed a greater amount of chlorophyll content (0.38 mg g^{-1} fresh weight) when rapeseed is primed with melatonin content under drought conditions (-0.4MPa osmotic stress).

When rice seed *var.* FARO44 primed with 2% and 2.5% KNO_3 and 3% and 3.5% SiO_2 were subjected to different kinds of drought levels like mild, moderate and severe. As the water stress condition increases, there was a decrease in chlorophyll content. Under severe conditions, seeds primed with 3.5% SiO_2 recorded a higher chlorophyll content value (18.39 mg ml^{-1}) when compared to control conditions (16.04mg ml^{-1}) (Ali *et al.,* 2021a).

Ali *et al.* (2021b) noted an increased amount of TCC under different levels of drought-like mild, moderate and severe, when rice seeds were primmed with 2.5% KNO_3 (25.05, 20.34 and 17.16 mg g^{-1} fresh weight, respectively), 3% SiO_2 (23.37, 21.95 and 18.01 mg g^{-1} fresh weight, respectively) and 2.5Mm SA (32.87,31.42 and 16.38 mg g^{-1} fresh weight, respectively) when compared to control seeds.

Bukhari *et al.* (2021) reported that seeds treated with KNO_3 (1.4 mg g^{-1}) containing chitosan/montmorillonite had shown a greater amount of TCC content during the water stress condition when compared to non-treated seeds (0.6 mg g^{-1}) in spinach.

2.2.2.5 Antioxidants

Salinity (1.5% NaCl for 24 hrs) and drought stress (PEG 6000 for 96hrs) were recorded to increase SOD (1.92 and 1.81 U μg^{-1} protein, respectively) levels in Liquorice seedlings (Pan *et al.*, 2006).

Mannivannan *et al.* (2007) recorded that at 30 DAS, cowpea was subjected to drought stress by propiconazole (PCZ) at the intervals of 3, 6 and 9 days which then caused an increase in CAT content in leaves (10 U mg^{-1} of protein, 11 U mg^{-1} of protein, 14 U mg^{-1} of protein, respectively).

Twelve sunflower cultivars when treated with silicon showed enhanced SOD content during drought conditions up to 7 days. (Gunes *et al.*, 2008).

Xiao *et al.* (2008) described that young, vegetatively propagated cuttings of *Populus cathayana* Rehder were subjected to drought stress for 12 weeks in a row, and it was noted to have increased activities of SOD (250 U g^{-1} fresh weight) in both the wet and dry climates and POD content (2.8 mmol guaiacol min^{-1} g^{-1}fresh weight) in the dry climate.

Ahmed *et al.* (2012) revealed that the catalase (19.28 U mg^{-1} of protein,19.20 U mg^{-1} of protein) and peroxidase activity values (5.65 U mg^{-1} of protein, 5.69 U mg^{-1} of protein) in maize seeds have grown larger after being primed with 20 mg l^{-1} salicylic acid and 20 mg l^{-1} ascorbic acid, respectively.

Chinese cabbage seeds bathed with 200 mmol l^{-1} KNO_3 subjected to (-5.0 Mpa) drought imposed by PEG 6000 reportedly produced increased levels of CAT (67.5 U g^{-1} fresh weight. min^{-1}), POD (116.9 U g^{-1} fresh weight min^{-1}) and SOD (67.5U g^{-1} fresh $weight^{-1}$ min (Yan, 2015).

Nigella sativa seedlings recorded an increase in CAT content when primed with KNO_3 (0.03 µmole mg^{-1} protein min^{-1}), $ZNSO_4$ (0.04 µmole mg^{-1} protein min^{-1}), PEG 6000(0.02 µmole mg^{-1} protein min^{-1}) and GA (0.04 µmole mg^{-1} protein min^{-1}) under water stress conditions during the seedling establishment (Fallah *et al.*, 2016).

Celik *et al.* (2017) revealed that when watering was withheld from 14-day-old tomato seedlings genotypes *i.e.,* X5671R and 5MX12956, POD content increased gradually (0.3 mmol H_2O_2 min^{-1} g^{-1} dry weight and 0.313 mmol H_2O_2 min^{-1} g^{-1} dry weight, respectively).

During the drought, the maize genotype Malka primed with 4 mM and 6 mM Si recorded higher levels of CAT (17 and 22 U mg^{-1} protein, respectively), POD (23 and 32 U mg^{-1} protein, respectively) and SOD (23 and 27 U mg^{-1} protein, respectively) (Praveen *et al.*, 2019).

Alamari *et al.* (2020) announced that when mustard seedlings primed with both Si and NaCl were subjected to salinity stress and water stress conditions throughout the early and late vegetative stages, there was an increase in the quantity of CAT (18 U min^{-1} mg^{-1} of protein) and SOD (14 U mg^{-1} of protein) contents under the treatment with Si+ NaCl with drought stress during late vegetative stages compared to the control seeds (8 and 6 U mg^{-1} of protein, respectively).

Ali *et al.* (2021a) reported that when rice seed *var.* FARO44 primed with 2%, 2.5%, and 3.5% KNO_3 and 3% and 3.5% SiO_2 were exposed to various levels of drought (mild, moderate and severe), there was an increase in SOD and CAT levels when compared to non-primed seeds with an increase in the water stress conditions. Among all the treatments, under severe drought 2.5% KNO_3 primed seed recorded higher CAT (0.56 Ug^{-1} min^{-1} fresh weight) over control (0.20 Ug^{-1} min^{-1} fresh weight) and 3% SiO_2 primed seeds had higher SOD valve (0.29 U^{-1} mg^{-1} fresh weight) when compared to control (0.06U^{-1} mg^{-1} fresh weight).

When seedlings were primed with 2.5% KNO_3 (0.03, 0.05 and 0.05 U mg^{-1} of protein), 3% SiO_2 (0.07, 0.10 and 0.10U mg^{-1} of protein) and 2.5mM SA (0.07, 0.08 and 0.06 U mg^{-1} of protein) exhibited higher levels of CAT activity than control seeds (0.02 U mg^{-1} of protein,0.01 U mg^{-1} of protein, 0.0 U mg^{-1} of protein) under drought conditions like mild, moderate and severe, respectively (Ali *et al.,* 2021b).

Ali *et al.* (2021b) reported that the seedlings exhibit higher levels of SOD activity when primed with 2.5% KNO_3 (0.07, 0.09 and 0.06 U mg^{-1} fresh weight), 3%SiO_2 (0.34, 0.34 and

0.29 U mg^{-1} fresh weight) and 2.5mM SA (0.58, 0.49 and 0.47 U mg^{-1} fresh weight) than control seeds (0.18, 0.25 and 0.15 U mg^{-1} fresh weight) under drought conditions like mild, moderate, and severe, respectively.

2.2.2.6 Total soluble sugars (TSS) and Total soluble protein (TSP)

Zhang *et al.* (2015) reported that the sorghum seeds primed with PEG in drought-stricken conditions produced more TSS than unprimed seeds.

Yan (2015) reported that compared to unprimed seeds, Chinese cabbage seedlings primed with 200mmol-1l KNO_3 subjected to drought (-5.0MPa osmotic potential) showed increased quantities of TSP (23 mg g^{-1}).

Fallah *et al.* (2016) found that the *Nigella sativa* seedlings showed rise in TSP content when primed with KNO_3 (1.38 µg g^{-1} fresh weight), $ZNSO_4$ (1.2 µg g^{-1} fresh weight), PEG6000 (1.09 µg g^{-1} fresh weight) and GA (1.07 µg g^{-1} fresh weight) under water stress conditions during seedling establishment than unprimed seeds.

Sewing *et al.* (2017) reported that when red chilli types were subjected to varying degrees of drought stress, displayed decreased TSS (23.55 mg g^{-1}) when compared to the control condition (23.55 mg g^{-1}).

Maize genotype Malka primed with 4- and 6-mM Si exhibited higher TSS levels (280 and 240 µmol g^{-1} fresh weight, respectively) during the drought condition. (Praveen *et al.*, 2019).

When rapeseed was primed with melatonin content in drought conditions (-0.4MPa osmotic stress), expresses an enlarged amount of soluble sugar content (28 mg g^{-1} fresh weight) and TSP (11 mg g^{-1} fresh weight) (Khan *et al.*, 2019).

Ali *et al*. (2021a) reported that rice seed *var.* FARO44 which were primed with 2%, 2.5%, and 3.5% KNO_3 and 3% and 3.5% SiO_2 was exposed to mild, moderate, and severe drought levels. As the water stress condition develops, there was increase in TSS and TSP when compared to non-primmed seeds. Among all the treatments, under severe drought 2.5% KNO_3 primed seed recorded higher TSP (0.83 mg ml^{-1}) over control (0.36mg ml^{-1}) and also had higher TSS (1.96mg ml^{-1}) when compared to control (1.17mg ml^{-1}).

When compared to untreated seeds (1.8 and 0.8mg g^{-1}) in spinach, seeds treated with KNO_3(2.5 and 1.5mg g^{-1}) containing chitosan-1montmorillonite showed higher quantities of TSS and TSP contents, respectively during the water stress condition (Bukhari *et al.*, 2021).

Seeds primed with 2.5% KNO_3, 3% SiO_2 and 2.5mM SA produced more TSS (2.91, 1.97 and 1.19 mg g^{-1} fresh weight, respectively) and TSP (1.33, 1.2933 and 0.7433 mg μg^{-1} fresh weight, respectively) than unprimed under mild, moderate, and severe droughts, respectively in rice crops (Ali *et al.*,2021b).

2.2.3. Quality traits

2.2.3.1. Capsaicin content

The capsaicin concentration of chilli peppers (*Capsicum chinense Jacq*.) decreased when they are subjected to drought conditions by watering every 7 (81mg-1 g dry weight of placenta) and 9 days (78 mg g^{-1} dry weight of placenta) with significant increase in their pungency when compared to daily watering (63 mg g^{-1} dry weight of placenta) in placenta (Ruiz-Lan *et al.*, 2011).

Kopta *et al.* (2020) reported that the chilli genotypes which were exposed to simulated drought stress displayed higher capsaicin concentration (75,000) as measured by Scoville Heat Units (SHU).

Mahmood *et al.* (2021) described that the drought-induced chilli peppers during the pod formation stage had a stronger impact on the capsaicin concentration, which showed a gradual decrease when compared to control conditions.

When the drought was applied to chilli genotypes such as Hungarian, Beauty Zest and home flavour at 50, 30, and 20 days after blooming, it was discovered that the creation of the pericarp in the fruits significantly affected the accumulation of capsaicin concentration. (Sung *et al.*, 2005)

2.2.3.2 Vitamin-C content

Kopta *et al.* (2020) found that the amount of ascorbic acid produced by chilli during simulated drought stress (1200-1600 mg kg^{-1} fresh weight) varied depending on the genotype and length of the stress.

When two wheat crop varieties, Buck Chambergo and Cooperative Maipun, were exposed to water stress conditions, only Buck Chambergo exhibited a notable drop in ascorbic acid content as compared to the control (Bartoli *et al.,* 2005).

When irrigation and nutrients were withheld from soybean, the ascorbic acid concentration decreased under water-stress circumstances (Seminario *et al.,* 2017).

2.2.4 Yield parameters

In *C. chinese,* maximum flowering occurred later and with a decreased number of flowers and fruit output under conditions of water stress (Jaimez *et al.,* 1999).

Gupta *et al.* (2001) reported that the drought-tolerant (C-306) and drought-sensitive (Kalyansona) wheat cultivars were both treated to water stress by withholding irrigation during the boot and anthesis stages. At the boot stage, the shoot dry weight (17.41 and 16.39g, respectively), the number of grains (348 and 325, respectively) and grain yield (9.49 and 9.41g, respectively) were recorded. It was noted that these parameters were significantly decreased in the anthesis stage with shoot dry weight (15.42 and 15.70g, respectively), number of grains (319 and 284, respectively) and grain yield (8.62 and 7.52g, respectively).

Samarah *et al.* (2006) reported that drought stress during the flowering stage had a greater impact on seed yield, which resulted in a decrease in the number of seeds per plant, number of fruits per plant and the weight of the seeds.

When drought conditions were applied to chilli sweet red cultivars, there were fewer flower buds and a greater percentage of flowers that fall off as a result of the stress, as well as less fruit set and fruit production (Abdulmalik *et al.,* 2012).

Khakwani *et al.* (2012) exposed six varieties to drought stress (35% FC) condition by withholding irrigation. Results conveyed that the wheat varieties, Damani, Hashim-8 and Zam-04 performed better with high grain yield per plant (2.43, 3.56g and 3.03g, respectively) under drought stress when compared to wheat varieties (Gomal-8, DN-73, Dera-98) with grain yield of 1.81, 1.89 and 1.87g per plant, respectively.

Maize hybrids like SP 13 were primed with 0.5%$ZNSO_4$ and 1.5% $ZNSO_4$, priming with 1.5% $ZNSO_4$ recorded higher grain yield (5.35 t ha^{-1}) followed by 0.5% $ZNSO_4$ (4.67 t ha^{-1}) and unprimed seeds (3.51 t ha^{-1}) (Afzal *et al.,*2013).

Makeli *et al.* (2013) subjected soybean cultivars to stress during the blooming, podding and seed-filling stages. Results showed that, compared to podding, the plants under stress during blooming and seed filling exhibited lower seed yields (2918 and 682kg, respectively), numbers of seeds produced per plant (128.1 and 152.8, respectively), 1000 seed weight (13.6 and 10.5g, respectively), seeds produced per pod (1.7 and 2.3%, respectively), biological yields (7951 and 7953kg-1ha, respectively), harvest indices (36.50 and 32.90%, respectively) and oil contents (22.27 and 20.80%, respectively).

Sibomana *et al.* (2013) imposed drought stress by reducing the percentage of pot capacity in tomato variety "money maker". The tomato plants that were under extreme stress (40% pot capacity) displayed a decrease in plant height (65.1cm), equatorial fruit diameter (27mm) and fruits plant^{-1} (34.5).

The soybean variety JS-9305 were primed with 1000ppm GA_3 (12 hrs) (1.45g, 47.37cm,53.40, 3.13 and 2078g) revealed an increase in dry matter content of seedling, plant height, number of pods plant, number of seeds plant and seed yield in contrast to unprimed seeds (1.12g, 40.04, 36.20, 2.62 and 1647g) (Agawane and Parhe,2015).

Das and Jana (2015), revealed that when lentil seeds (*Lens culinaris* medikus) were hydro-primed and two urea sprays at the branching and pod initiation stages recorded a higher number of pods plant $^{-1}$ (50), number of seeds pod $^{-1}$(1.889) and seed yield (1295 kg ha^{-1}), when compared to non-primed seeds without urea sprays (38,1.76, 1098 kg ha^{-1}).

When late sown wheat hybrids like HD-2189, Lok-1and Raj-3765 were hydroprimed. The hydro-primed varieties (HD-2189 – 2.38 and 1.69 t ha^{-1}, Lok-1– 2.19 and 1.49 t ha^{-1} and Raj-3765–2.75 and 1.86 t ha^{-1}, respectively) recorded higher grain yield when compared to unprimed varieties (HD-2189 – 2.03 and 1.40 t ha^{-1}, Lok-1– 1.96 and 1.24 t ha^{-1} and Raj-3765–2.40 and 1.56 t ha^{-1}, respectively during the year 2005 -2006 and 2006-2007 (Ramamurthy *et al.*,2015).

With the aid of PEG-8000, the cotton genotype (Bt-886) was subjected to drought condition and it is evident that changes in growth and biochemical features significantly decreased the number of flowers (0.666), dry weight of shoots (0.936 g) and roots (0.15) (Shafiq *et al.,* 2015).

Chilli plants have shallow roots with a depth of approximately 30cm, making them susceptible to long-term drought stress (Riduan *et al.,* 2015).

At 4 and 8 weeks after planting, chilli cultivars exposed to mild, moderate and severe drought conditions showed the average reduction (%) in branch number per plant (58.93%), shoot dry weight per plant (42.94%), fruit number per plant (29.50%) and fruit weight per plant (31.75%) (Suwignyo *et al.,* 2017).

Tomato seedlings were shown to be treated with Si, PEG+ Si, and PEG. When compared to seedlings exposed to control conditions, those treated with Si before sowing displayed more efficient root properties like root length (1.957cm), total root surface area (386 cm^2), total root volume (14.0 cm^3), average root diameter (0.68mm) (Yi *et al.,* 2018).

Two rice cultivars, Yangliangyou and Hanyou 113 were treated to water stress conditions during 2013-2014 at a flowering stage, which considerably impacts the grain filling stage. This resulted in significantly reduced grain yield (25.5 and 58.9%, respectively) and Chalkiness (8.40 and 15.6%, respectively) (Yang *et al.,* 2019.).

Karimi *et al.* (2021) revealed that when wheat seeds were primed with 2ppm IAA, 0.2% Mn solution, 0.2% Zn solution and non-primed seeds with or without *Azospirillum zeae* inoculation. of all these, seeds primed with 0.2% Zn solution recorded higher grain yield (2250 kg ha^{-1}), number of grains spike $^{-1}$(26.43) and the number of spikes per m^{-2}(250).

When chilli genotypes like hot pepper (Pusa Juala and Ghotki) were subjected to drought conditions during the flowering stage, drought conditions resulted in significant reductions in the flowering stage with flower survival percentage (5 and 7%, respectively), the number of fruits produced per plant (8 and 3%, respectively), fresh fruit weight (2.5 and 2.8g, respectively) and dry fruit weight (1 and 1.5g, respectively). They noted a similar trend during pod production stage also (Mahmood *et al.,* 2021).

Materials and methods

3. MATERIALS AND METHODS

The experiment was undertaken with the main objective to evaluate the various seed priming methods for water stress tolerance in chilli at the seedling stage and selected best treatment will be evaluated for phenological, physiological, quality and yield traits. Lab study and Pot culture study were conducted at Department of Seed science and technology, College of Agriculture, Vellayani, Thiruvananthapuram

3.1 EXPERIMENT DETAILS

3.1.1 Location

The experiments were conducted in Department of Seed Science and technology at College of Agriculture, Vellayani, located at $8^0$5' N latitude and $76^0$9'E longitude and an altitude of 29 m above mean sea level.

3.1.2 Seed material

Seeds of Vellayani Athulya were procured from Department of Vegetable Science, College of agriculture, Vellayani

3.1.3. Layout of the Experiment

The Lab experiment was conducted in Factorial CRD with 12 treatments and 3 replication and Pot culture experiment was conducted in Factorial CRD with 3 treatments and 5 replications.

3.2 EXPERIMENT I

Evaluation of various seed priming methods for water stress tolerance in chilli at the seedling stage

3.2.1. Treatments

Factor 1: Stress level (S) (3) (Alif,2019)

S1. Control

S2. Mild stress (PEG6000-5%)

S3. Moderate stress (PEG 6000-10%)

Factor 2: Nutrient and hormonal level(P) (4)

P1. Potassium nitrate (KNO_3)-2.5%

P2. Silicon dioxide (SiO_2)-3%

P3. Salicylic acid (SA)-2.5Mm

P4. Control

Replication -3

Number of seeds/replication-50

Design – Factorial CRD

Treatment details

T_1 (2.5 % KNO_3 + C)
T_2 (2.5 % KNO_3 + 5% PEG 6000)
T_3(2.5%KNO_3 + 10% PEG 6000)
T_4 (3% SiO_2+C)
T_5(3% SiO_2+ 5% PEG 6000)
T_6 (3% SiO_2+10% PEG 6000)
T_7(2.5mM SA+C)
T_8(2.5mM SA+5% PEG 6000)
T_9(2.5mM SA+10% PEG 6000)
T_{10}(C+C)
T_{11}(C+ 5% PEG 6000)
T_{12}(C+10% PEG 6000)

3.2.2 Methodology

Preliminary screening for water stress tolerance induced by seed priming was carried in chilli variety Vellayani Athulya. The experiment was carried out by sand tray method (Plate 1, 2 and 3.). surface sterilized seeds were used for the experiment. Seed priming: seeds were soaked in plant growth regulators and nutrient solution for 12 hours and dried in shade.

3.2.3 Observations recorded

The observations were recorded at Seedling stage (Plate 4.).

3.2.3.1 Seedling Vigour Index -1

Seedling Vigour Index -1 was estimated as per the formula given by Abdul -Baki and Anderson,1973.

Seedling Vigour Index -1 = Germination (%)×seedling length (cm)

3.2.3.2 Seedling Vigour Index -2

Seedling Vigour Index -2 was estimated as per the formula given by Abdul-Baki and Anderson,1973.

Seedling Vigour Index -2 = Germination (%) × Seedling dry weight (gm)

3.2.3.3 Proline content (µmoles of proline g $^{-1}$ of tissue)

The proline content was determined according to the methods of Bates *et al.* (1973). 0.2 g of fresh plant material was homogenized in 5 ml of 3 % aqueous sulfosalicylic acid and centrifuged at 4000 rpm for 20 minutes. The residue was re-extracted with 5 ml of 3 per cent sulfosalicylic acid and centrifuged. The supernatant was combined together and the volume was made to 10 ml. 2 ml of this aliquot was transferred into a test tube and 2 ml each of acid ninhydrin solution (1.25 g ninhydrin + 30 ml glacial acetic acid + 20 ml 6M orthophosphoric acid) and glacial acetic acid were added. The mixture was heated on boiling water bath at 100ºC for one hour after which the reaction was terminated by placing the tubes on ice bath. On cooling, the reaction mixture was shaken vigorously with 6ml of toluene and kept for one hour at room temperature. Chromatophore thus extracted in the toluene phase was separated. Absorbance was measured at 520 nm using toluene as blank. Standard curve was prepared with graded concentration of DL-proline and the quantity of proline was calculated in the sample.

3.2.3.4 Malonaldehyde (µmole ml^{-1})

As per the procedure described by wang *et al*. (2013) MDA was calculated

Extraction of MDA

Functional or senescent leaves (0.5g) were taken and ground in 5 ml extraction solution of 5% TCA then the centrifugation was performed at 5000g for 15 mints. The supernatant which is the MDA extraction solution, was stored at 4^0C.

Measurement of MDA

According to the corrected TBA method by Hodges *et al*. (1999). The level of MDA was estimated by taking 2 ml of extraction solution were mixed with 3 ml 0.5% TBA including 5% TCA vigorously. Then the mixture was heated at 95^0C for 30 min in a boiling water bath

and then cooled it to room temperature. Then it was centrifuged at 5000 g for 15 mints, the OD value of supernatant was detected at 450,532, and 600 nm. The concentration of MDA was determined using the formula.

CMDA (µMOL/ml) = 6.45 ×(D532-D600)-0.56 ×D450, where D450, D532, D600 are the absorbencies at 450,532,600 nm, respectively.

3.2.3.5 Hydrogen peroxide (µmoles g^{-1})

The procedure described by Velikova *et al*. (2000) and Elstner *et al. (*1975) were used to determine the content of H_2O_2. To determine the H_2O_2 content in sample, the extraction was performed in 5 ml of trichloroacetic acid solution.0.5 ml of supernatant was taken in TT containing 0.5 ml of 10 mM phosphate buffer (pH0.7) and 1 ml of 1 M potassium iodide. After vortexed, the absorbance was taken at 390 nm and the content of H_2O_2 was calculated using the molar extinction coefficient 0.28 μM^{-1}. The content of H_2O_2 was given on a standard curve.

T_{10}

T_{11}

T_{12}

Plate 1. Seed germination stage

T_1

T_2

T_3

T_4

T_5

T_6

T_7 T_8 T_9

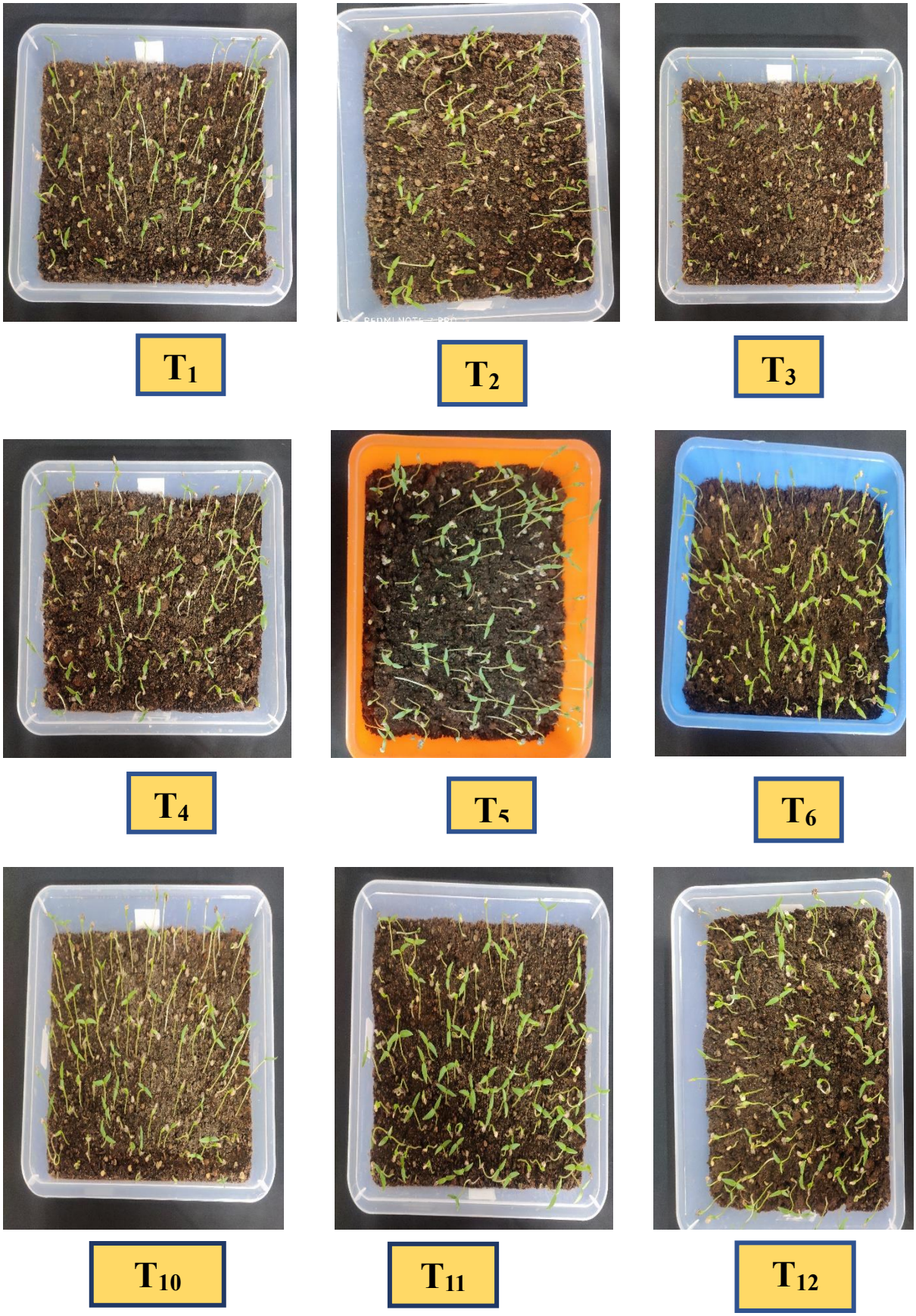

Plate 2. 7 Days after sowing (DAS)

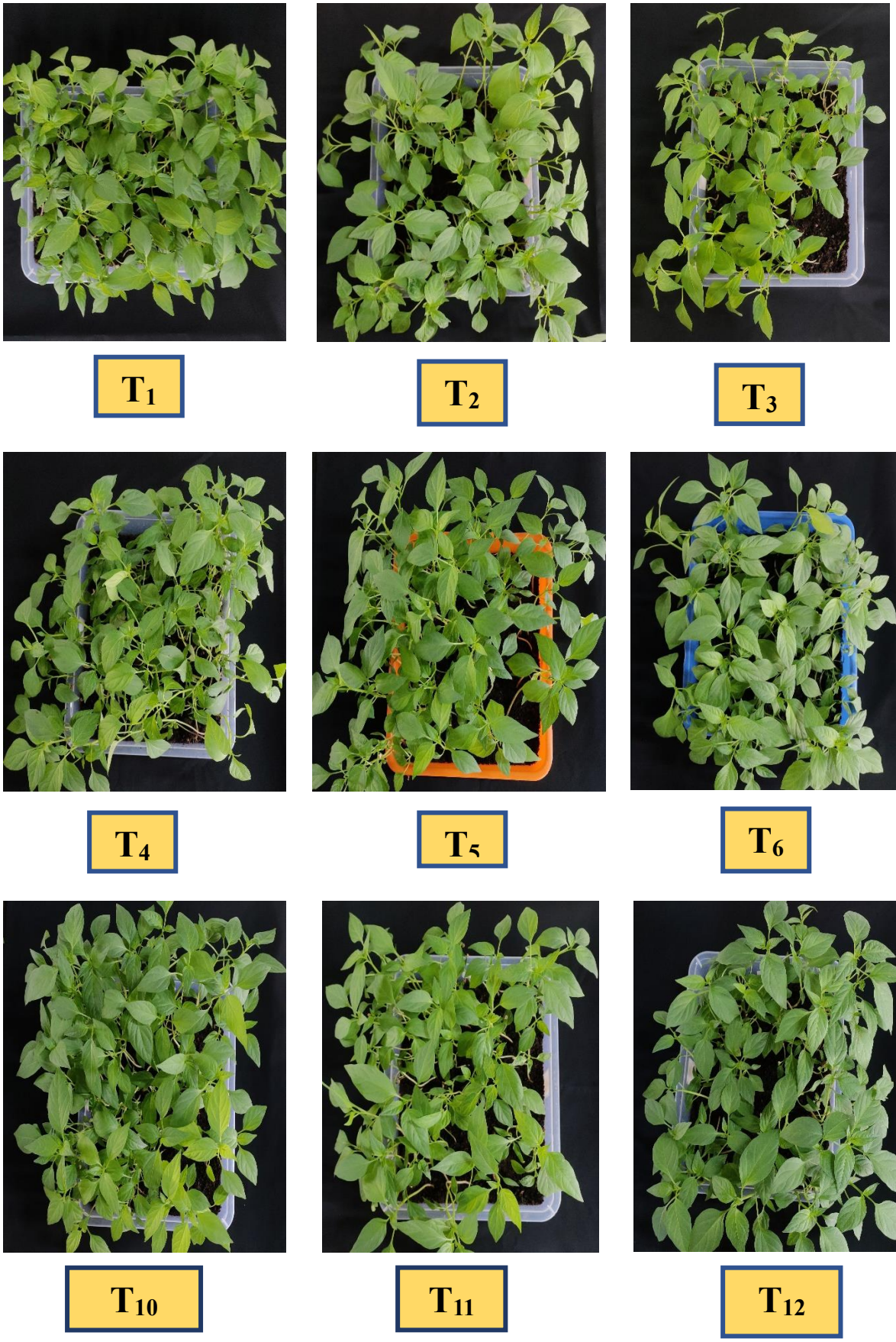

Plate 3. 30 Days after sowing (DAS)

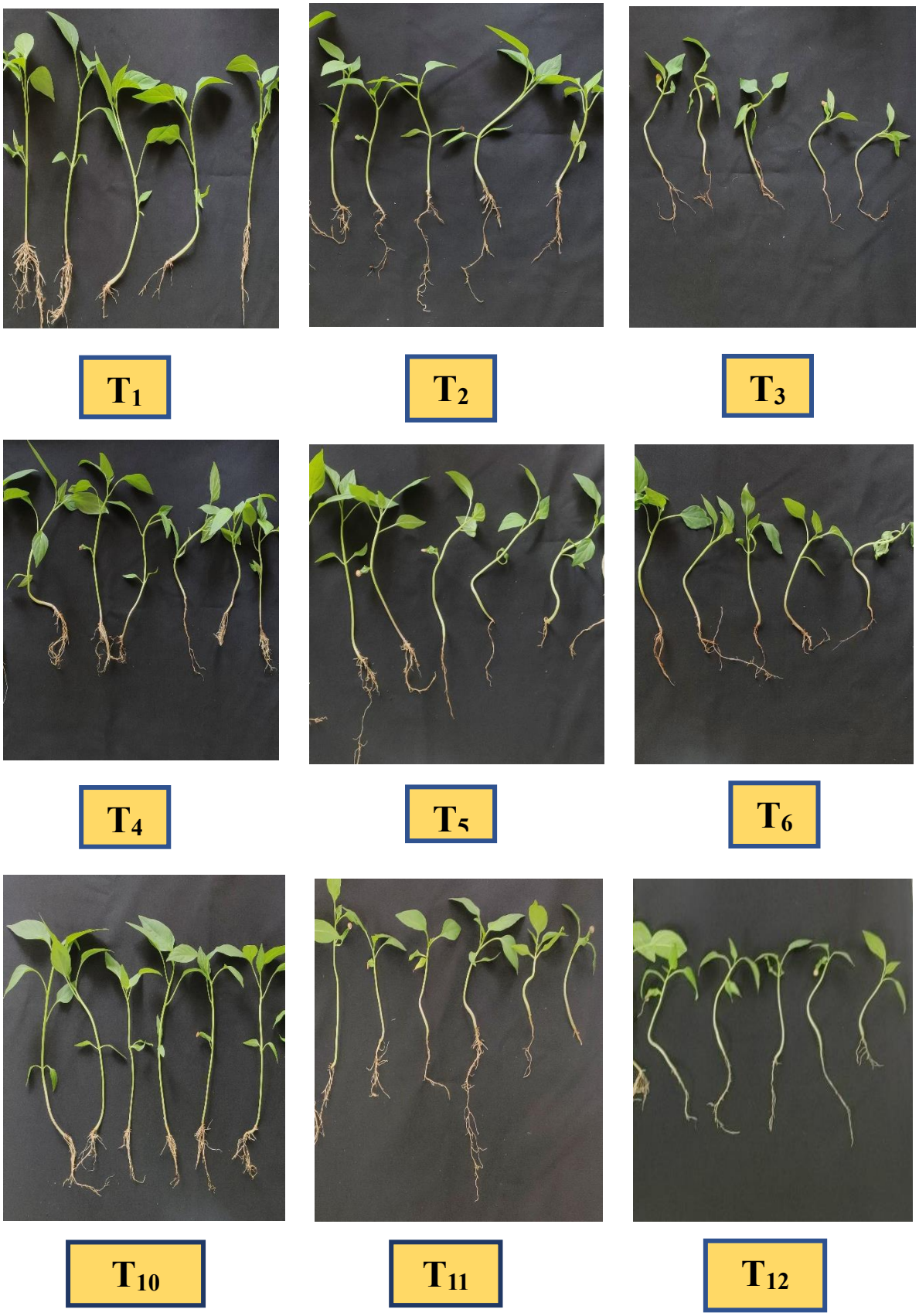

Plate 4. Effect of seed priming methods for water stress tolerance in chilli

3.3 EXPERIMENT II

Effect of selected best treatment for water stress tolerance and its effect on physiological and yield traits in chilli.

3.3.1 Treatments

Factor 1: Stress level (C) (2)

C1. Control condition

C2. Stress condition

Factor 2: Nutrient and hormonal level (P) (3)

P1. Potassium nitrate (KNO_3)-2.5%

P2. Silicon dioxide (SiO_2)-3%

P3. Control

Replication -5

Number of pots/replication- 10

Design – Factorial CRD

3.3.2 Methodology

Selected best two treatments from experiment I (Plate 5.) were evaluated for water stress tolerance in chilli under pot culture condition (Plate 6.) for water stress indicators and yield.

Seeds of chilli variety was sown in pot trays. Irrigation and other cultural practices were followed according to KAU POP and were transplanted to pots 30 days after sowing. Water stress was induced by withholding irrigation for four days (Plate 7, 8, 9, 10, 11 and 12.) where the Relative Water Content (RWC) content of leaves reached at 70% during flowering initiation stage. The observations on physiological traits were taken when the RWC reached at 70%. Yield and Yield components were taken at harvesting stage.

Plate 5. General view of pot experiment

T_1 - 2.5% KNO_3

T_2 -3% SiO_2

T_3 - Unprimed seeds

Plate 6. Selected best treatments

Plate 7. T_1, T_2, T_3 before stress induction stage

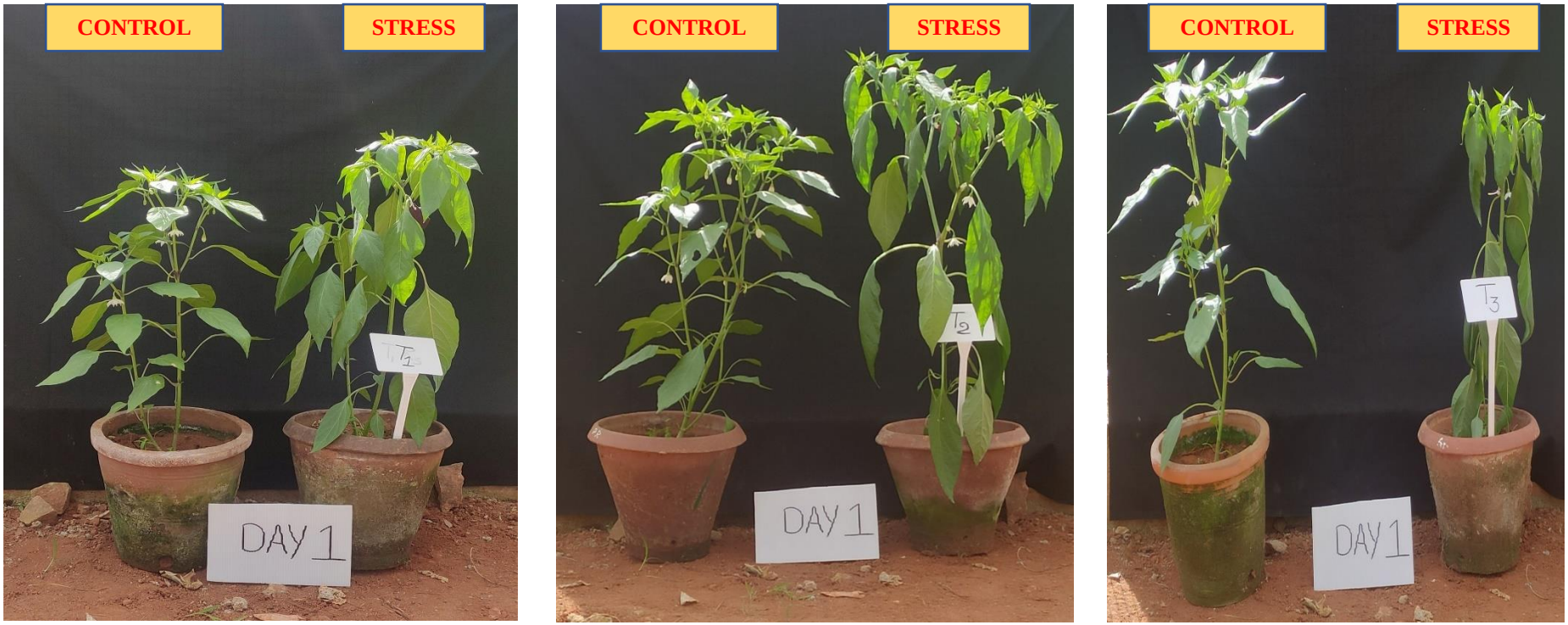

Plate 8. 1 day after stress induction stage

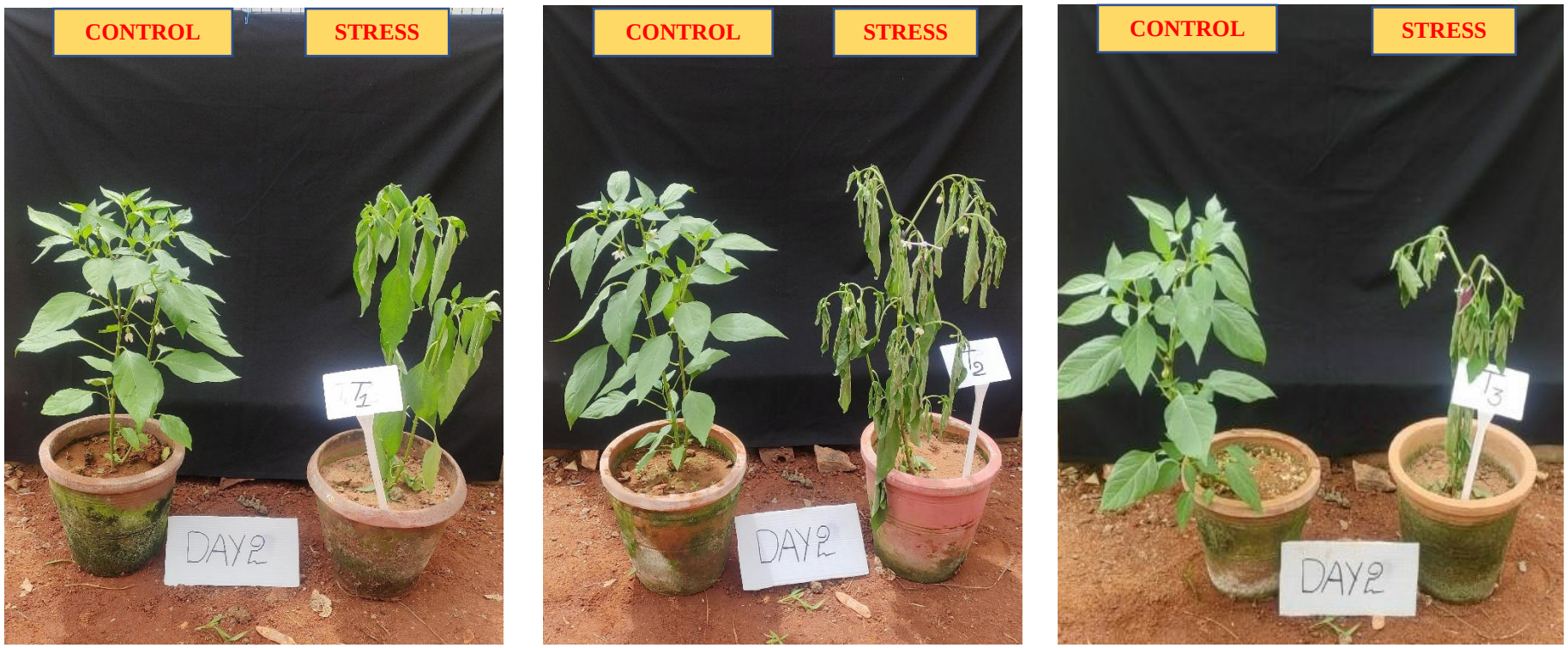

Plate 9. 2 days after stress induction stage

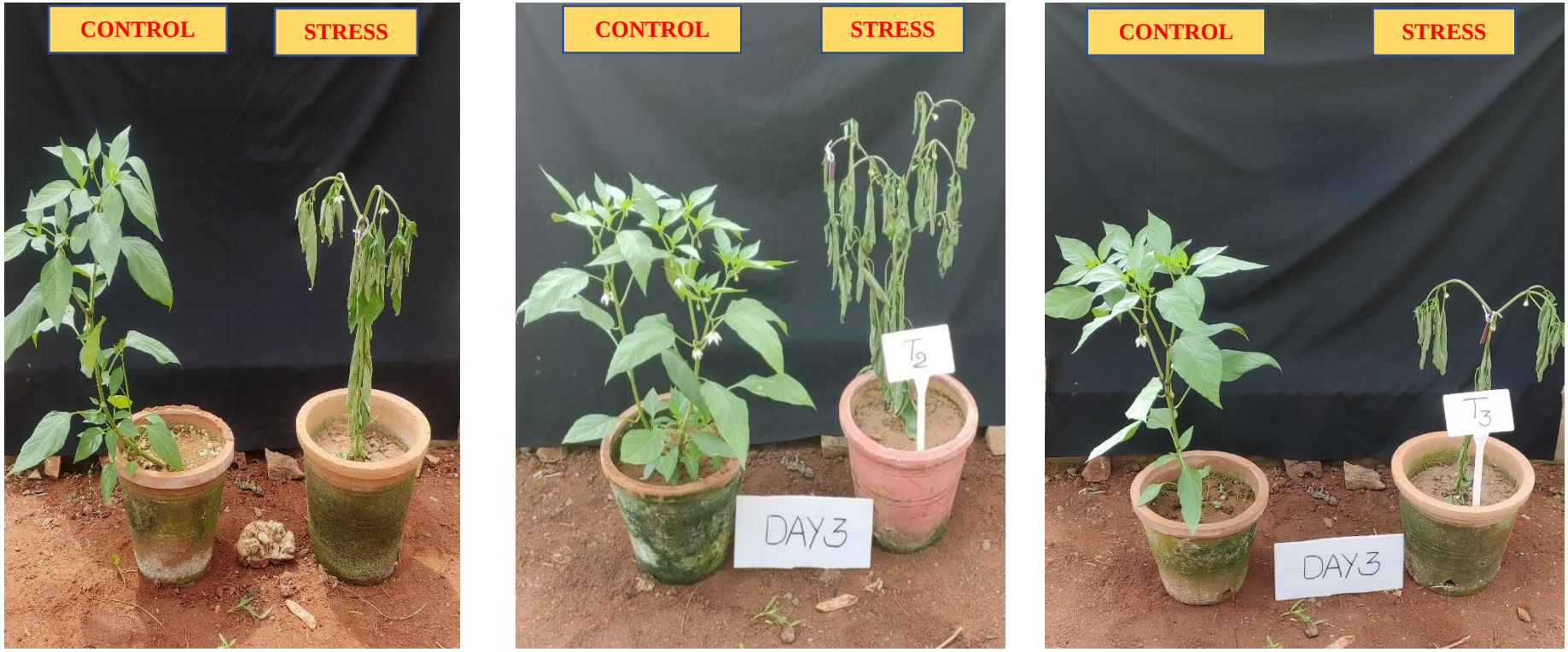

Plate 10. 3 days after stress induction stage

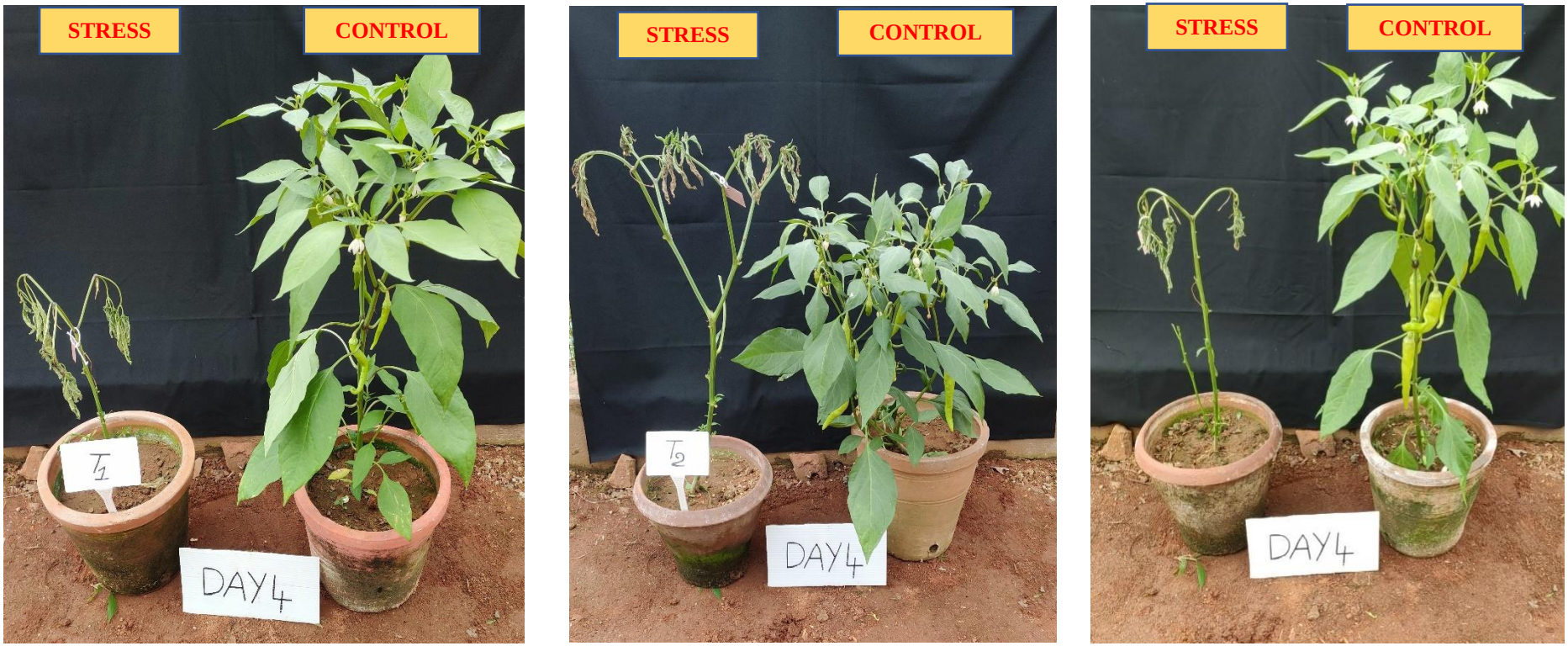

Plate 11. 4 days after stress induction stage

Plate 12. Recovery stage -7 days after adding water

3.3.3. Observations recorded

Phenological and Physiological parameters were recorded when the relative water content (RWC) of leaves reached 70% during the flower initiation stage. Yield and yield parameters were recorded at the harvesting stage.

3.3.4 Phenological traits

3.3.4.1 Days to first flowering

It was recorded as the number of days taken from the date of transplanting to the date when first flowering occurred in each treatment.

3.3.4.2 Days to first fruiting

It was calculated as the number of days from the date of transplanting to the date when plant produced the first fruit.

3.3.5 Physiological traits

3.3.5.1. Leaf Relative water content (%)

The Leaf Relative water content was evaluated by the method described by Barr and Weatherly,1962. Leaf discs were cut from the leaves, weighed and saturated them by floating on distilled water in Petri-dishes. After four hours, the discs were surface dried, weighed and then dry weight was determined after keeping in oven at 80ºC for 24 hours. Relative water content was calculated by the following formula.

$$\text{Relative water content (\%)} = \frac{\text{Fresh weight – Dry weight}}{\text{Turgid weight – Dry weight}} \times 100$$

3.3.5.2 Cell membrane stability Index (%)

The leaf Cell membrane stability index (CMSI) was calculated using Premachandran *et al.* (1990), as modified by Sairam,1994. Leaf discs (100 mg) were carefully rinsed in running tap water before being washed and again with distilled water. Following that, the discs were heated for 30 minutes in 10 mL of double distilled water at 40°C. After that, the electrical conductivity (C_1) was measured using an EC (Electrical Conductivity) metre. Following that, the same samples were immersed in a boiling water bath (100^0 C) for 10 minutes, and their electrical conductivity was also measured (C_2) and calculated by using following formula;

$$CMSI\ (\%) = [1- (C_1/C_2)] \times 100$$

3.3.5.3Proline content (µmoles of proline g $^{-1}$ of tissue)

The proline content was determined according to the methods of Bates *et al.* (1973). 0.2 g of fresh plant material was homogenized in 5 ml of 3 % aqueous sulfosalicylic acid and centrifuged at 4000 rpm for 20 minutes. The residue was re-extracted with 5 ml of 3 percent sulfosalicylic acid and centrifuged. The supernatant was combined together and the volume was made to 10 ml. 2 ml of this aliquot was transferred in to a test tube and 2 ml each of acid ninhydrin solution (1.25 g ninhydrin + 30 ml glacial acetic acid + 20 ml 6M orthophosphoric acid) and glacial acetic acid were added. The mixture was heated on boiling water bath at 100ºC for one hour after which the reaction was terminated by placing the tubes on ice bath. On cooling, the reaction mixture was shaken vigorously with 6ml toluene and kept for one hour at room temperature. Chromatophore thus extracted in toluene phase was separated. Absorbance was measured at 520 nm using toluene as blank. Standard curve was prepared with graded concentration of DL-proline and the quantity of proline was calculated in the sample.

3.3.5.4 Total Chlorophyll content (mg g^{-1} fresh weight)

The total chlorophyll concentration was obtained by using DMSO method was given by Reddy *et al*. (1992). Using the formulae, the absorbance at 645 and 663 nm was recorded, as well as the chlorophyll content, was determined.

$$\text{Total chlorophyll content (mg g}^{-1}) = (20.2 \times A645 + 8.02 \times A663) \times V/ (1000 \times W)$$

3.3.5.5 Catalase activity (n moles of H2O2 used min^{-1} g^{-1} weight of sample)

The catalase activity was obtained by using H_2O_2 was given by the Barber,1980.

Enzyme extraction:

Grind the sample (0.1g) with 0.1M phosphate buffer, pH 7.0 in a prechilled mortar and pestle. Centrifuge at 15,000g for 30min at 4˚C. Use the supernatant as enzyme source.

Enzyme assay:

Pipette out 3ml of phosphate buffer, 2ml of H_2O_2 and 1ml of enzyme extract into at 20˚C for 1min. Incubate at 20˚C for 1min. After 1min stop the reaction by adding 10ml of 0.7N H_2SO_4. Titrate the reaction mixture against 0.01N $KMNO_4$ to find out the residual H_2O_2 until a faint purple colour persists for at least 15sec. Prepare the blank by adding the enzyme extract

to an acidified solution of reaction mixture at zero time. Calculate the concentration of H_2O_2 using the extinction coefficient 0.036 $\mu mole^{-1}ml^{-1}$. The activity of the enzyme may be also expressed as nmoles of H_2O_2 used $min^{-1}g^{-1}$ weight of sample.

3.3.5.6 Peroxidase activity (units $min^{-1}mg^{-1}$ of protein)

The peroxidase activity was estimated by the procedure given by Reddy *et al. (*1985). The leaf material (about 200 mg) was ground with 10 ml of 0.1 M sodium phosphate buffer, pH 7.0, with a prechilled mortar and pestle. The homogenate was centrifuged at 10,000 Xg and 4°C for 30 min, and the supernatant was used as the source of the enzyme. Peroxidase activity was assayed by the method of Kar and Mishra (1976) with the following modifications. The assay mixture for peroxidase contained 2 ml of 0.1 M sodium phosphate buffer, pH 7.0, 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2, and 1 ml of 20 times diluted enzyme extract. After 5 min incubation at 25°C the reaction was stopped by adding 1 ml of 1.25 M H_2SO_4 to the reaction mixture, and the amount of purpurogallin formed was estimated by measuring A_{420}. The enzyme activity was expressed as mmol purpurogallin formed per g fresh weight per min.

3.3.5.7 Super oxide dismutase Activity (units mg^{-1} of protein)

The super oxide dismutase activity was estimated by the procedure given by Abedi and pakniyat,2010. Control and treated Leaf samples (0.5 g) were homogenized with 3.0 ml of potassium phosphate buffer and centrifuged at 2000 rpm for 10 min. To 0.2 ml of enzyme, 1.2 ml of sodium pyrophosphate buffer (0.0.25 M, pH-8.3), 0.1 ml of phenazine methosulfate (186 µM), 0.3 ml of nitro blue tetrazolium (NBT) (300 µM), and water in a volume of 2.8 ml were added. Reaction was initiated by adding 0.2 ml of NADH (780 µM). The reaction mixture was incubated at 30∘ C for 90 s. Then, 1.0 ml of glacial acetic acid was added to stop the reaction. Followed by reaction culmination, the reaction mixture was then shaken with 4.0 ml of n-butanol and then allowed to stand for 10 min and centrifuged. The intensity of chromogen in the butanol layer was read at 560 nm in a spectrophotometer. One unit of SOD enzyme activity was defined as the amount of enzyme that gave 50% inhibition of reduction of NBT.

3.3.5.8 Total soluble sugar (mg ml^{-1})

The total soluble sugar content was estimated by the procedure given by Hedge and Hofreiter,1962. weigh 100mg of the sample into a boiling tube. hydrolyse by keeping it in a boiling water bath for 3 hours with 5ml of 2.5N HCL and cool to room temperature. Neutralize

it with solid sodium carbonate until the effervescence ceases. Make up the volume to 100ml and centrifuge. Collect the supernatant and take 0.5 and 1ml aliquots for analysis. Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard.0 serves as blank. Make up the volume to 1ml in all the tubes including the sample tubes by adding distilled water. Then add 4ml of anthrone reagent. Heat for 8 min in a boiling water bath. Cool rapidly and read the green to dark green colour at 630 nm. Draw a standard graph by ploting concentration of the standard on the X-axis versus absorbance on the Y-axis. From the graph calculate the amount of carbohydrates present in the sample tube.

$$\text{Amount of carbohydrate present in sample (\%mg)} = \frac{\text{Sugar value from graph (mg)}}{\text{Aliquot sample used (0.5 or 1ml)}} \times \frac{\text{Total vol. of extract (ml)}}{\text{Weight of sample (mg)}} \times 100$$

3.3.5.9 Total soluble protein (mg ml^{-1})

The total soluble protein content was estimated by the procedure given by the Brandford,1976. Pipette out 0.01, 0.02, and 0.04, 0.1 ml of standard protein solution into a series of test tubes. Make up volume in each tube to 0.1 ml with an appropriate buffer, 0.1 ml of buffer alone serves as the blank. Add 5ml of protein reagent and mix thoroughly by inversion or vertexing. Measure the absorbance at 595 nm after 2 min and before 1 h against a reagent blank. Plot a standard graph and calculate the amount of protein in the un known sample treated in the same manner.

3.3.6 Quality traits

3.3.6.1 Capsaicin content (%)

The pungent principle reacts with Folin-Dennis regent to give a bluish complex which was estimated calorimetrically by Mathew *et al. (*1971). The fruits harvested at red ripe stage were dried at hot air oven at 50^{o}C and powdered finely in a mixer grinder.500mgs of each of the sample was weighed into test tubes. Added 10 ml acetone to it and kept overnight. Aliquots of 1 ml were pipetted into 100 ml conical flasks, added 25ml of Folin-Dennis Regent and allowed to stand for 30 minutes. Added 25 ml of freshly prepared sodium carbonate solution and shook vigorously. the volume was made up to 100 ml with distilled water and the optical density was determined after 30 minutes at 725nm against regent blank (1ml acetone + 25ml

Folin-Dennis regent +25ml aqueous sodium carbonate solution) using a UV spectrophotometer.

To determine the EI percent value for pure capsaicin, a stock solution of standard capsaicin (200mg-1) was prepared by dissolving 20 milligrams in 100 ml acetone. From this a series of solutions of different concentrations were prepared and their optical density measured at 725nm.standard graph was prepared and calculated the content of capsaicin in the samples.

3.3.6.2 Vitamin C content (mg 100g $^{-1}$ of sample)

This method was described by Harris and Ray, 1935.Take 5ml of the working standard solution into a 100ml conical flask. Add 10ml of 4% oxalic acid and titrate against the dye (V1 ml). End point is the appearance of pink colour which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid. Extract the sample (0.5-5g depending on the sample) in 4% oxalic acid and make up to a known volume (100ml) and centrifuge. Pipette out 5ml of this supernatant, add 10ml of 4% oxalic acid and titrate against the dye (V2 ml.)

$$\text{Amount of ascorbic acid (mg/100g sample)} = \frac{0.5\text{ mg}}{V1} \times \frac{V2}{15\text{ ml}} \times \frac{100\text{ml}}{\text{Wt. of the sample}} \times 100$$

3.7 Yield traits:

3.3.7.1 Plant height(cm)

The plant height was measured from the base of the plant (soil level) to the tip of the top leaf of the shoot at the vegetative stage and flowering stage and the average height was calculated for each treatment on per plant basis.

3.3.7.2 Number of Flowers per plant

The number of flowers present in the plant was recorded after transplanting.

3.3.7.3Number of Fruits per plant (g plant^{-1})

The number of fruits harvested at the green stage from five replication of each treatment at each picking was calculated and the total number of fruits per plant was calculated.

3.3.7.4 Fruit yield per plant (g plant^{-1})

The fruits harvested in the observation plants were weighed in each harvest and summed to record the fruit yield. The average yield from the observation plants in each treatment was recorded as the average fruit yield per plant.

3.3.7.5 Root volume(cm^3)

Root volume can be obtained by quantifying the volume of liquid a root displaces.

3.3.7.6 Number of seeds per fruit

The harvested fruits were cut open and several seeds were counted and noted.

3.3.7.7 Seed yield per plant (g $plant^{-1}$)

The harvested fruits of each plant were air-dried, threshing was carried out and seeds were separated from the threshed debris without damage to the seeds. The weight of seeds per plant from each replication of treatment was noted.

3.3.3.8 Dry matter (gram $plant^{-1}$)

Plants from five replication of each treatment were uprooted and transferred to a hot air oven at 80^0C for 72 hours and were recorded after harvesting. The mean value was calculated for each treatment and was expressed as gram $plant^{-1}$.

3.4 STATISTICAL ANALYSIS

The data were statistically analysed using the Analysis of Variance Technique (ANOVA) under Factorial Completely Randomized Design (CRD) for germination studies and pot culture experiments. GRAPES software was used for obtaining mean, Standard Error (SE) and Critical Difference.

Results

4. RESULTS

The objective of the present investigation entitled "Efficacy of seed priming for inducing stress tolerance in chilli (*Capsicum annum* L.) under water stress condition" was to evaluate the various seed priming treatments for water stress tolerance in chilli at the seedling stage and selected best treatments were evaluated for physiological and yield traits under pot culture study. The results of the present investigation were analysed statistically and presented in the following sections

4.1 EXPERIMENT 1 -Evaluation of various seed priming methods for water stress tolerance in chilli at seedling stage

Seeds were primed with 2.5%KNO_3, 3% SiO_2 and 2.5 mM for 12 hours and dried in shade. Then the primed seeds were evaluated under control, mild and moderate stress at seedling stage.

4.1.1 Seedling vigour index I

The effect of seed priming on the seedling vigour index-1 of chilli seedlings under water stress conditions is presented in table 1. The interaction of the two factors viz. priming agents and stress levels were found to be non-significant. In all the stress levels, the seeds treated with 2.5% KNO_3 (P1) recorded the highest SVI-1 with a mean of 1963.36 followed by seeds treated with the priming agent 3% SiO_2 (P2) recorded a mean of 1635.77 and the least SVI-1 was recorded in the unprimed seeds (P3) with a mean of 1201.48. As the stress levels increased from minimum to maximum, a proportional decrease in the SVI-1 was noted.

Table 1. Effect of seed priming on Seedling Vigour Index-1 in chilli seedlings under water stress conditions

Priming Treatment (P)	Seedling vigour index I			
	Control (S1)	Mild stress(S2)	Moderate stress (S3)	Mean
2.5% KNO_3 -P1	2285.23	1919.97	1684.87	**1963.36[a]**
3% SiO_2 -P2	1811.83	1504.50	1590.97	**1635.77[b]**
Non primed -P3	1300.13	1374.43	929.867	**1201.48[c]**
Mean	**1799.07[a]**	**1599.63[b]**	**1401.9[c]**	
CD ($\alpha \leq 0.05$)	NS			

4.1.2 Seedling vigour index 2

The effect of seed priming on the Seedling Vigour Index- 2 of chilli seedlings under water stress conditions has been listed in table 2. The effect of the priming agents 2.5% KNO_3 and 3% SiO_2 on the SVI-2 was significant over the non-primed seeds under the control (S1) and 10% PEG (S3) levels but not in the 5% PEG (S2) stress level where the priming treatments were on-par with one another with not much significant difference between them. Under the 10% PEG (S3) stress level, the seeds treated with 3% SiO_2 (P2 xS3) recorded the highest (3.8) SVI-2 value whereas under control (S1) condition the highest SVI-2 value (4.8) was recorded in the priming treatment with 2.5% KNO_3 (P1 x S1).

Table 2. Effect of seed priming on Seedling Vigour Index 2 in chilli seedlings under water stress conditions

Priming Treatment (P)	**Seedling vigour index II**			
	Control (S1)	**Mild stress (S2)**	**Moderate stress (S3)**	**Mean**
2.5% KNO_3 -P1	4.8^{a} ±0.4	4.1^{bc}±0.1	3.3^{d} ±0.1	**4.118^{b}**
3% SiO_2 -P2	4.5^{ab}±0.2	3.9^{c}±0.5	3.8^{cd}±0.1	**4.123^{a}**
Non primed -P3	3.8^{cd}±0.2	4.0^{bc}±0.1	2.5^{e} ±0.2	**3.276^{c}**
Mean	**4.401^{a}**	**4.048^{b}**	**3.276^{c}**	
CD (α≤0.05)	**P=0.31, S=0.3, PXS=0.537**			
SE(m)	0.181			
CV	7.2			

4.1.3 Malondialdehyde

The data on the malondialdehyde (MDA) content, which is an indicator of lipid peroxidation is listed in table 3. Significant difference between the priming treatments was not observed under the control condition (S1) regarding the MDA content, with all three treatments recording levels which were on par with one another. However, seeds primed with 3% SiO_2 (P2 x S2) recorded the least MDA content (0.42 mM/ml) under 5% PEG stress (S2) whereas, under 10 % PEG stress (S3) condition, the lowest MDA levels (0.28 mM/ml) were recorded in seeds primed with 2.5% KNO_3 (P1 x S3).

Table 3. Effect of seed priming on malondialdehyde content in chilli seedlings under water stress conditions

Priming Treatment (P)	Malondialdehyde (µmole ml^{-1})			
	Control (S1)	Mild stress(S2)	Moderate stress (S3)	Mean
2.5% KNO_3 -P1	0.34^{ef}±0.02	0.54^{b}±0.02	0.28^{f}±0.05	**0.39^{c}**
3% SiO_2 -P2	0.36^{de}±0.03	0.42^{cd}±0.03	0.42^{c}±0.02	**0.406^{b}**
Non-primed -P3	0.38^{cde}±0.03	0.5^{b}±0.03	0.6^{a}±0.02	**0.497^{a}**
Mean	**0.364^{c}**	**0.49^{a}**	**0.438^{b}**	**0.431**
CD (α≤0.05)	**P=0.033, S=0.033, PXS=0.057**			
SE(m)	0.019			
CV	7.01			

4.1.4 Hydrogen peroxide

The effect on Hydrogen peroxide content through priming treatments of chilli seedlings under water stress conditions is presented in table 4. The data reveals that in the control (S1) conditions, priming treatment with 2.5% KNO_3 (S1 x P1) recorded the highest (0.75 µmoles/g) amount of hydrogen peroxide, whereas under the 5% PEG (S2) and 10% PEG (S3) stress conditions, the highest amount of H_2O_2 was recorded in the unprimed seeds. The least amount of hydrogen peroxide was recorded by 3% SiO_2 (P2) treatment under all three stress conditions with a mean of 0.398 µmoles/g.

Table 4. Effect of seed priming on hydrogen peroxide (H_2O_2) content in chilli seedlings under water stress conditions

Priming Treatment	Hydrogen peroxide (µmolesg^{-1})			
	Control (S1)	Mild stress (S2)	Moderate stress (S3)	Mean
2.5% KNO_3 -P1	0.75^{c} ±0.01	0.51^{d}±0.01	0.53^{d}±0.007	**0.602^{b}**
3% SiO_2 -P2	0.38^{g}±0.01	0.4^{fg}±0.01	0.4^{f}±0.007	**0.398^{c}**
Non-Primed -P3	0.45^{e}±0.01	0.93^{a}±0.011	0.89^{b}±0.01	**0.759^{a}**
Mean	**0.532^{c}**	**0.616^{a}**	**0.611^{b}**	**0.586**
CD (α≤0.05)	**P=0.011, S=0.011, PXS=0.019**			
SE(m)	0.006			
CV	1.87			

4.1.5 Proline content

The data on the effect of seed priming on the proline content of chilli seedlings under water stress is presented in Table 5. In the control treatment (S1), i.e. which was not subjected to any water stress, it was found that the seeds primed with 2.5% KNO_3 (P1) and 3% SiO_2 (P2) had higher proline content with 0.13 µmoles/g and 0.14 µmoles/g respectively, compared to the non-primed seeds (P3) which recorded a significantly lower proline content of 0.09 µmoles/g. Similar trend was observed under both the stress conditions S2 (5% PEG) and S3 (10% PEG) where the seeds primed with 2.5% KNO_3 (P1) recorded significantly higher proline content followed by the priming treatment of 3% SiO_2 (P2) and the least being recorded in the non-primed (P3) seeds. On the whole, the mean of the proline content of all the priming treatments was recorded to be the highest (0.24 µmoles/g) under the 10% PEG (S3) stress treatment. On the other hand, 2.5% KNO_3 treated seeds averaged the highest proline content across all three stress treatments recording 0.201 µmoles/g.

Table 5. Effect of seed priming on the proline content in chilli seedlings under water stress conditions

Priming Treatment (P)	**Proline content (µmoles of proline g $^{-1}$ of tissue)**			
	Control (S1)	**Mild stress (S2)**	**Moderate stress (S3)**	**Mean**
2.5% KNO_3 -P1	0.13^{f} ± 0.007	0.22 ^{b} ± 0.006	0.24 ^{a} ± 0.005	**0.201^{a}**
3% SiO_2 -P2	0.14 ^{e} ± 0.006	0.20^{c} ± 0.006	0.20^{c} ± 0.006	**0.188^{b}**
Non-Primed -P3	0.09^{g} ±0.007	0.01^{d} ± 0.007	0.19 ^{c} ± 0.007	**0.154^{c}**
Mean	**0.123^{c}**	**0.2^{b}**	**0.22^{a}**	**0.181**
CD (α≤0.05)	**P=0.006, S=0.006, PXS=0.01**			
SE(m)	0.003			
CV	3.282			

4.2 EXPERIMENT II- Effect of selected best treatments for water stress tolerance and its effect on physiological and yield traits in chilli.

4.2.1 Phenological traits

4.2.1.1 Days to first flowering

The data on the effect of seed priming on days to flowering in chilli under water stress conditions are presented in Table no 6. Among the three seed priming treatments, the seeds primed with 3% SiO_2 (P2) have shown earlier flowering, i.e., by 28 days whereas 2.5% KNO_3 (P1) and unprimed (P3) seeds attained flowering by 32 and 34 days respectively, under control conditions (C1).

Table 6. Effect of seed priming on Days to flowering in chilli under water stress conditions

Treatments	Days to first flowering (Days after Transplanting)		
	Control (C_1)	Stress (C_2)	MEAN (P)
2.5%KNO_3 - P_1	32	-	**32[b]**
3%Sio_2 - P_2	28	-	**28[a]**
Unprimed – P_3	34	-	**34[c]**
Mean (D)			
CD (α≤0.05)			
CV	**3.191**		

4.2.1.2 Days to first fruiting

The data on the effect of seed priming on Days to first fruiting in chilli under water stress conditions is presented in Table no 7. Of all the three seed priming methods under control (C_1) conditions, the seeds primed with 2.5% KNO_3 (C_1P_1) had started fruiting at 47 DAT followed by 3% SiO_2 (C_1P_2) and unprimed seeds (C_1P_3) at 46 DAT and 43 DAT respectively. Under stress condition (C_2), seeds primed with 2.5% KNO_3 (C_2P_1) had attained the fruiting stage at 56 DAT. The unprimed seeds (C_2P_3) and seeds primed with 3% SiO_2 (C_2P_2) required 55 DAT and 52 DAT respectively to attain fruiting. On the whole, of all the priming treatments, the seeds primed with 2.5% KNO_3 (P1) had a higher mean value of 51 DAT. Comparison between the two water stress conditions it was observed that plants subjected to stress (C_2) expressed a greater mean value of 54 DAT.

Table 7. Effect of seed priming on Days to first fruiting in chilli under water stress conditions

Treatments	Days to first fruiting (Days after Transplanting)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	47^c	56^a	**51.5^a**
3%Sio_2 – P_2	46^c	52^b	**49.333^b**
Unprimed – P_3	43^d	55^a	**49^c**
Mean (D)	**45.33^b**	**54.55^a**	
CD ($\alpha \leq 0.05$)	**P=1.391, D=1.136, P×D=1.967**		
SE(m)	**0.638**		
CV	**1.967**		

4.2.2 Physiological traits

4.2.2.1 Relative water content

The effect of seed priming on relative water content (RWC) in chilli under water stress conditions is presented in Table no 8. Under control conditions (C_1), the highest RWC was observed in the seeds primed with 2.5% KNO_3 (C_1P_1-94%), followed by 3% SiO_2 (C_1P_2 -71%) and unprimed seeds (C_1P_3-68%) RWC respectively. A similar trend was also observed under stress conditions (C_2), where 2.5% KNO_3 (C_2P_1) primed seeds exhibited higher RWC, while the unprimed seeds (C_2P_3) had the lowest value. The overall mean was highest in seeds primed with 2.5%KNO_3(P_1-80.5%) followed by 3%SiO_2(P_2 -71.667%) and unprimed seed (P_3-68.33%) among all the priming treatments. Comparing between the two water stress conditions, it was observed that mean of control conditions (C_1) was the highest with an RWC of 88.68%.

Table 8. Effect of seed priming on Relative water content (RWC) in chilli under water stress conditions

Treatments	Relative water content (%)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	94±1[a]	67±2[c]	**80.5[a]**
3%Sio_2 – P_2	87.33±1.528[b]	56±1[d]	**71.667[b]**
Unprimed – P_3	84.667±1.528[b]	52±2[e]	**68.33[c]**
Mean (D)	**88.667[a]**	**58.33[b]**	
CD (α ≤0.05)	**P=1.967, D=1.606, P×D=2.781**		
SE(m)	**0.903**		
CV	**2.127**		

4.2.2.2 Cell membrane stability index

The data on the effect of seed priming on the cell membrane stability index (CMSI) in chilli under water stress conditions is presented in Table no 9. Under control conditions (C_1), the seeds primed with 3% SiO_2 (C_1P_2) recorded CMSI of 91.66% followed by 2.5% KNO_3 (C_1P_1-82%) and the least being recorded in the unprimed (C1P3-77%) seeds. However, under stress conditions (C_2), a little change in trend was observed where the highest CMSI was observed in seeds primed with 2.5% KNO_3 (C_2P_1- 40.33%) followed by 3% SiO_2 primed seeds (C_2P_2-38.33%) and unprimed seeds (C_2P_3-24.33%). Overall, among the various seed priming methods (P), seeds primed with 3%SiO_2 (P_2-65%) had shown the highest mean value followed by 2.5%KNO_3 (P_1-61.167%) and unprimed seeds (P_3-50.833%). Comparing between the water stress conditions, it was observed that plants exposed to the control condition (C1) recorded a higher CMSI (83.667%).

Table 9. Effect of seed priming on Cell membrane stability index (CMSI) in chilli under water stress conditions

Treatments	CMSI (%)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	82±2[b]	40.33±1.528[d]	**61.167[b]**
3%Sio_2 – P_2	91.66±1.528[a]	38.33±1.528[d]	**65[a]**

Unprimed – P_3	77.33±2.082^{c}	24.33±1.528^{e}	**50.833^{c}**
Mean (D)	**83.667^{a}**	**34.33^{b}**	
CD (α ≤0.05)	**P=2.159, D=1.762, P×D=3.053**		
SE(m)	**0.991**		
CV	**2.908**		

4.2.2.3 Proline content

The data on the effect of seed priming for proline content in chilli under water stress conditions is presented in Table no 10. Under both the conditions, a similar trend had been observed, where seeds primed with 3% SiO_2 exhibited the highest proline content (C_1P_2 - 1.307 µM & C_2P_2 - 3.133 µM) followed by seeds primed with 2.5% KNO_3 (C_1P_1-0.98 µM & C_2P_1-2.977 µM) and unprimed seeds (C_1P_3-0.593 µM & C_2P_3-2.853 µM). Comparison between the means of the seed priming agents reveals that seeds primed with 3% SiO_2 (P_2) had recorded the highest mean value (2.22 µM) followed by 2.5%KNO_3(P_1) primed seeds (1.978 µM) and unprimed seeds (P_3-1.723 µM). Comparing between the two water stress conditions, it was observed that plants subjected to Stress condition (C_2) recorded the highest proline content (2.988 µM).

Table 10. Effect of seed priming on Total proline content in chilli under water stress conditions

Treatments	**Proline content (µmoles of proline g^{-1} of tissue)**		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	0.98±0.03^{e}	2.977±0.035^{b}	**1.978^{b}**
3%Sio_2 – P_2	1.307±0.031^{d}	3.133±0.015^{a}	**2.22^{a}**
Unprimed – P_3	0.593±0.031^{f}	2.853±0.015^{c}	**1.723^{c}**
Mean (D)	**0.96^{b}**	**2.988^{a}**	
CD (α ≤0.05)	**P=0.034, D=0.028, P×D=0.049**		
SE(m)	**0.016**		
CV	**1.382**		

4.2.2.4 Total chlorophyll content

The data on the effect of seed priming on total chlorophyll content in chilli under water stress conditions is presented in Table no 11. Under both the conditions, a similar trend had been observed i.e., the highest TCC was exhibited by the seeds primed with 2.5%KNO_3 (C_1P_1 -1.807mg/g & C_2P_1 - 4.577 mg/g), followed by 3%SiO_2 primed seeds (C_1P_2 -1.763mg/g & C_2P_2 -4.24mg/g) and unprimed seeds (C_1P_3-1.61mg/g & C_2P_3-4.11mg/g). The overall mean was highest in seeds primed with 2.5% KNO_3 (P_1-3.192mg/g) followed by 3%SiO_2 (P_2-3.002 mg/g) and unprimed seeds(P_3-2.86mg/g). Comparing between the water stress conditions, it was observed that plants exposed to the stress condition (C2) recorded the highest TCC (4.309 mg/g).

Table 11. Effect of seed priming on total chlorophyll content (TCC) in chilli under water stress conditions

Treatments	Total chlorophyll content (mg g^{-1})		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	4.577±0.169[a]	1.807±0.0254[d]	**3.192[a]**
3%Sio_2 – P_2	4.24±0.02[b]	1.763±0.025[d]	**3.002[b]**
Unprimed – P_3	4.11±0.026[c]	1.61±0.02[e]	**2.86[c]**
Mean (D)	**4.309[a]**	**1.727[b]**	
CD($\alpha \leq 0.05$)	**P=0.091, D=0.074, P×D=0.128**		
SE(m)	**0.042**		
CV	**2.39**		

4.2.2.6 Catalase activity

The data on the effect of seed priming on catalase activity in chilli under water stress conditions is presented in Table no 12. Under both the conditions, a similar trend had been observed by seeds primed with 2.5%KNO_3 had exhibited the highest catalase activity (C_1P_1 - 200 nM & C_2P_1 - 510 nM) followed by 3%SiO_2 primed seeds (C_1P_2 - 186.33 nM & C_2P_2 - 488.33 nM) and unprimed seeds (C_1P_3 -177.33 nM & C_2 P_3-324.66 nM). The overall mean was highest in seeds primed with 2.5%KNO_3(P_1-355 nM) followed by 3%SiO_2(P_2-337.33 nM) and unprimed seeds (P_3- 260 nM). Comparing between the water stress conditions, it was observed

that plants exposed to stress condition (C_2) had recorded the highest value of catalase activity (447 nM).

Table 12. Effect of seed priming on catalase activity (CAT) in chilli under water stress conditions

Treatments	Catalase activity (n moles of H2O2 used min^{-1} g^{-1} weight of sample)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	200±2^{d}	510±2^{a}	**355^{a}**
3%Sio_2 – P_2	186.33±1.528^{e}	488.33±2.082^{b}	**337.33^{b}**
Unprimed – P_3	177.33±1.528^{f}	324.66±2.517^{c}	**260^{c}**
Mean (D)	**187.889^{b}**	**447^{a}**	
CD (α ≤0.05)	**P=2.481, D=2.025, P×D=3.508**		
SE(m)	**1.139**		
CV	**0.621**		

4.2.2.6 Peroxidase activity

The data on the effect of seed priming on peroxidase activity in chilli under water stress conditions is presented in Table no 13. Under control conditions (C_1), the highest peroxidase content was recorded in the seeds primed with 2.5%KNO_3(C_1P_1-0.135units) followed by 3%SiO_2(C_1P_2-0.087 units) and unprimed seeds (C_1P_3-0.061 units). Under stress condition (C_2), the highest content of peroxidase activity was exhibited by seeds primed with 3%SiO_2 (C_2P_2 - 0.206 units) followed by 2.5%KNO_3 primed seeds (C_2P_1-0.194 units) and unprimed seeds (C_2P_3-0.123 units). The mean among seed priming methods (P), the highest value was recorded in the seeds primed with 2.5%KNO_3 (P_1) followed by 3%SiO_2(P_2) and unprimed seeds (P_3). Comparing between the two water stress conditions, it has been observed that the Plants exposed to stress(C_2) had recorded increased peroxidase content (0.174 units).

Table 13. Effect of seed priming on Peroxidase activity (POD) in chilli under water stress conditions

Treatments	Peroxidase activity (units min^{-1} mg^{-1} of protein)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	0.135±0.015^b	0.194±0.017^a	**0.165^a**
3%Sio_2 – P_2	0.087±0.008^c	0.206±0.012^a	**0.146^b**
Unprimed – P_3	0.061±0.007^d	0.123±0.012^b	**0.092^c**
Mean (D)	**0.094^b**	**0.174^a**	
CD (α ≤0.05)	**P=0.015, D=0.013, P×D=0.022**		
SE(m)	**0.007**		
CV	**8.1**		

4.2.2.7 Superoxide dismutase

The data on the effect of seed priming in superoxide dismutase (SOD) activity in chilli under water stress conditions is presented in Table 14. Under both the conditions, a similar trend had been followed. The highest value of SOD was recorded by the seeds primed with 2.5%KNO_3 (C_1P_1- 400.6 units & C_2P_1 - 492.6 units) followed by 3%SiO_2 (C_1P_2 - 385 units & C_2P_2 - 482 units) and unprimed seeds (C_1P_3 - 333.3 units & C_2P_3 - 414.66 units) respectively. The overall mean value was recorded by following descending order with seeds primed with 2.5%KNO_3(P_1 - 446.6units), 3%SiO_2(P_2 -433.5 units) and unprimed seeds (P_3 - 373 units). Comparing between the two water stress conditions, it was observed that plants exposed to stress condition (C_2 - 463.111 units) had shown increased SOD activity.

Table 14. Effect of seed priming on Superoxide dismutase activity (SOD) in chilli under water stress conditions

Treatments	Superoxide dismutase (SOD) (units mg^{-1} of protein)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	400.6±3.055^d	492.6±5.033^a	**446.6^a**
3%Sio_2 – P_2	385±2^e	482±2^b	**433.5^b**
Unprimed – P_3	333.3±3.512^f	414.66±2.517^c	**373^c**
Mean (D)	**373^b**	**463.111^a**	
CD (α ≤0.05)	**P=4.022, D=3.284, P×D=5.688**		

SE(m)	**1.846**
CV	**0.765**

4.2.2.8Total soluble sugars

The data on the effect of seed priming on total soluble sugars in chilli under water stress conditions is presented in Table 15. Under both conditions, a similar trend had been observed. The highest value was recorded by the seeds primed with 2.5%KNO_3 (C_1P_1 - 3.347mg/ml & C_2P_1- 1.573mg/ml) followed by 3%SiO_2 (C_1P_2 - 2.007mg/ml & C_2P_2- 0.74mg/ml) and unprimed seeds (C_1P_3- 0.37mg/ml & C_2P_3 - 0.183mg/ml) respectively. Comparing between the water stress conditions, it was observed that plants exposed to Stress condition (C_2) recorded decreased (0.832mg/ml) TSS. The overall highest mean value of TSS is also observed by the seeds primed with 2.5% KNO_3(P_1 - 2.46 mg/ml).

Table 15. Effect of seed priming on Total soluble sugars (TSS) in chilli under water stress conditions

Treatments	Total soluble sugars (mg ml^{-1})		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	3.347±0.142[a]	1.573±0325[c]	**2.46[a]**
3%Sio_2 – P_2	2.007±0.189[b]	0.74±0.115[d]	**1.373[b]**
Unprimed – P_3	0.37±0.085[e]	0.183±0.045[e]	**0.277[c]**
Mean (D)	**1.908[a]**	**0.832[b]**	
CD (α ≤0.05)	**P=0.22, D=0.18, P×D=0.312**		
SE(m)	**0.101**		
CV	**7.4**		

4.2.2.9 Total soluble protein

The data on the effect of seed priming on Total soluble protein (TSP) in chilli under water stress conditions is presented in Table 16. Similar trend had been observed under both conditions. The highest TSP was recorded by the seeds primed with 2.5%KNO_3 (C_1P_1 - 2.012mg/ml & C_2P_1 - 1.073mg/ml) followed by 3%SiO2(C_1P_2-1.81mg/ml &C_2P_2 - 0.972mg/ml) and unprimed seeds (C_1P_3 - 1.51mg/ml & C_2P_3 -0.757 mg/ml) respectively. Comparing between the water stress conditions, it was observed that plants exposed to control

(C_1) recorded the highest TSP level TSP(1.777mg/ml). The overall mean was highest in the 2.5%KNO3 primed seeds (P_1-1.542mg/ml).

Table 16. Effect of seed priming on Total soluble protein (TSP) in chilli under water stress conditions

Treatments	Total soluble protein (mg ml^{-1})		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	2.012±0.01[a]	1.073±0.129[d]	**1.542[a]**
3%Sio_2 – P_2	1.81±0.02[b]	0.972±0.02[e]	**1.391[b]**
Unprimed – P_3	1.51±0.02[c]	0.757±0.021[f]	**1.133[c]**
Mean (D)	**1.777[a]**	**0.934[b]**	
CD (α ≤0.05)	**P=0.069, D=0.057, P×D=0.098**		
SE(m)	**0.0302**		
CV	**4.072**		

4.2.3 Quality traits

4.2.3.1 Capsaicin content

The data on the effect of seed priming on capsaicin content in chilli under water stress conditions is presented in Table no 17. The capsaicin content under both the conditions follows a similar trend, the highest to lowest value was recorded by seeds primed with 2.5% KNO_3(C_1P_1 -0.129 % & C_2P_1 -0.022%), 3%SiO_2(C_1P_2-0.102% & C_2P_2- 0.019 %) and unprimed seeds (C_1P_3 -0.074 % & C_2P_3-0.018%) respectively. The overall highest mean was observed in seeds primed with 2.5%KNO_3(P_1 - 0.075%) followed by 3%SiO_2 primed seeds (P_2 - 0.06%) and unprimed seeds (P_3-0.046%). Comparing between the water stress conditions, it was observed that plants exposed to control (C_1) had recorded highest capsaicin content (0.102%).

Table 17. Effect of seed priming on Days Capsaicin content in chilli under water stress conditions

Treatments	Capsaicin content (%)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	0.129±0.015[a]	0.022[d]	**0.075[a]**
3%Sio_2 – P_2	0.102±0.007[b]	0.019[d]	**0.06[b]**
Unprimed – P_3	0.074±0.001[c]	0.018±0.001[d]	**0.046[c]**
Mean (D)	**0.102[a]**	**0.019[b]**	
CD (α ≤0.05)	**P=0.008, D=0.007, P×D=0.012**		
SE(M)	**0.004**		
CV	**8.047**		

4.2.3.2 Ascorbic acid content

The data on the effect of seed priming on vitamin -C content in chilli under water stress conditions is presented in Table no 18. Under control conditions (C_1), the seeds primed with 2.5%KNO_3(C_1P_1) had recorded the highest Vit-C content (483.083mg) followed by 3%SiO_2 primed seeds (C_1P_2- 474.3 mg) and unprimed seeds (C_1P_3 - 397.133 mg) respectively. Under stress condition (C_2) seeds primed with 3%SiO_2(C_2P_2 - 393.567 mg) had recorded highest in contrast to control conditions(C_1). As for mean value (D), 3%SiO_2 primed seeds (P_2) had recorded the highest vit-C content (433.95 mg) followed by 2.5%KNO_3(P_1 - 416.433 mg) and unprimed seeds (P_3 - 380.433 mg). Comparing between the water stress conditions, it was observed that plants exposed to the control condition (C_1) had recorded the highest capsaicin content (451.517 mg) which drastically reduced during the stress condition (C_2 - 363.733 mg).

Table 18. Effect of seed priming on Vitamin -C content in chilli under water stress conditions

Treatments	Vitamin -C content (mg $100g^{-1}$ of sample)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	483.083±0.186[a]	349.803±0.589[f]	**416.443[b]**
3%Sio_2 – P_2	474.3±1.137[b]	393.567±1.106[d]	**433.95[a]**
Unprimed – P_3	397.133±0.172[c]	363.733±1.069[e]	**380.433[c]**

Mean (D)	**451.517[a]**	**369.034[b]**	
CD (α ≤0.05)	**P=1.037, C=0.847, P×C=1.467**		
SE(m)	**0.476**		
CV	**0.201**		

4.2.4 Yield parameters

4.2.4.1 plant height

The data on the effect of seed priming on plant height in chilli under water stress conditions is presented in Table no 19. A Significant difference was not observed under the both conditions between the seeds primed with 2.5%KNO_3 (C_1P_1 - 58.667cm & C_2P_1 - 57cm). However, 3%SiO_2 primed seeds (C_1P_2 -53.33cm & C_2P_2 - 46cm) had shown a significant variation between control and stress conditions. In unprimed seeds, a difference can be observed (C_1P_3 -48.33cm & C_2P_3 - 43cm). The overall mean values(D) were highest in 2.5%KNO_3 primed seeds (P_1-57.88cm) followed by 3%SiO_2 primed seeds (P_2-49.667) and unprimed seeds (P_3- 45.667). Comparing between the water stress conditions, it was observed that plants exposed to control (C_1) have recorded the highest plant height(53.44cm) as stress condition (C_2) recorded a decreased plant height (48.667).

Table 19. Effect of seed priming on Plant height in chilli under water stress conditions

Treatments	**Plant height (cm)**		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	58.667±1.528[a]	57±2[a]	**57.833[a]**
3%Sio_2 – P_2	53.33±1.528[b]	46±1[cd]	**49.667[b]**
Unprimed – P_3	48.33±2.082[c]	43±2[d]	**45.667[c]**
Mean (D)	**53.44[a]**	**48.667[b]**	
CD (α ≤0.05)	**P=2.179, D=1.779, P×D=3.081**		
SE(m)	**1**		
CV	**3.392**		

4.2.4.2 Number of Flowers plant $^{-1}$

The data on the effect of seed priming on No. of flowers/plants in chilli under water stress conditions is presented in Table 20. Under control conditions, seeds primed with 3% SiO_2 treatment (C_1P_2 - 33 flowers/plant) had recorded highest number of flowers/ plant followed by 2.5% KNO_3 (C_1P_1 - 30 flowers/plant) and unprimed seeds (C_1P_3 - 27 flowers/plant). Under stress condition(C_2), unprimed seeds (C_2P_3 - 22 flowers/plant) recorded the highest value followed by the seeds primed with 2.5% KNO3 (C_2P_1 - 21 flowers/plant) and 3% SiO_2(C_2P_2 - 19 flowers/plant). Both 2.5% KNO_3(P_1) and 3% SiO_2(P_2) are on par to each other (26), while unprimed seeds(P_3) have a lesser mean value (24.5 Flowers/ plant) when compared to 2.5%KNO_3(P_1) and 3% SiO_2 (P_2). The control (C_1) condition recorded the highest number of flowers/plant with a mean value (30 flowers/plant) among the water stress conditions.

Table 20. Effect of seed priming on Number of flowers plant^{-1} in chilli under water stress conditions

Treatments	No flowers plant^{-1} (Number)		
	Control (C_1)	Stress (C_2)	MEAN (P)
2.5%KNO_3 – P_1	30±1.528^{b}	21±1.528^{d}	**26^{a}**
3%Sio_2 – P_2	33±1^{a}	19±1^{e}	**26^{a}**
Unprimed – P_3	27±1^{c}	22±2^{d}	**24.5^{b}**
Mean (D)	**30.111^{a}**	**20.889^{b}**	
CD ($\alpha \leq 0.05$)	**P×D=2.481**		
SE(m)	**0.805**		
CV	**5.1**		

4.2.4.3 Number of fruits plant^{-1}

The data on the effect of seed priming on No. of fruits/ plants in chilli under water stress conditions is presented in Table 21. Under control condition(C_1) both the seeds primed with 2.5% KNO_3 (C_1P_1) and 3% SiO_2 (C_1P_2) recorded an equal number of fruits/ plant (C_1P_1-27 & C_1P_2 - 27) followed by unprimed seeds (C_1P_3 - 19) respectively. Under stress conditions(C_2) also the fruits/plants in both priming methods are on par with each other (C_2P_1-16 & C_2P_2- 15) followed by unprimed seeds (C_2P_3 -11.667). Among the various seed priming methods, the

mean of both 2.5% KNO_3 (P_1) and 3% SiO_2 (P_2) (21) was on par whereas the unprimed seeds (P_3) recorded a lower number of fruits/ plant (15.33). Comparing between the two water stress conditions, it was observed that plants under the control conditions (C_1) recorded a higher number of fruits/plants (24.44) compared to plants subjected to stress condition (C_2) (14.33).

Table 21. Effect of seed priming on Number of fruits plant^{-1} in chilli under water stress conditions

Treatments	No fruits plant^{-1} (Number)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	27±1^{a}	16±1^{c}	**21.5^{a}**
3%Sio_2 – P_2	27±2.082^{a}	15±0.577^{c}	**21.33^{b}**
Unprimed – P_3	19±1^{b}	11.667±0.577^{d}	**15.33^{c}**
Mean (D)	**24.44^{a}**	**14.333^{b}**	
CD (α ≤0.05)	**P=1.453, D=1.186, P×D=2.054**		
SE(m)	**0.667**		
CV	**5.55**		

4.2.4.4 Fruit yield plant^{-1}

The data on the effect of seed priming on fruit yield/plant in chilli under water stress conditions is presented in Table 22. Under control conditions (C_1) seeds primed with 2.5%KNO_3 treatment (C_1P_1), had recorded a greater number of fruit yield /plant (86.723g) followed by 3%SiO2 primed seeds (C_1P_2-76.381g) and unprimed seeds (C_1P_3- 68.523g) respectively. Under stress condition (C_2), the highest fruit yield was recorded by the seeds primed with 2.5% KNO_3 (C_2P_1 -44g) followed by 3%SiO_2 (C_2P_2-38.723g) and unprimed seeds (C_2P_3- 34.747g). The overall mean was highest in seeds primed with 2.5% KNO_3(P_1 - 65.508g) while other treatments like 3%SiO_2(P_2 - 57.552g) and unprimed seeds (P_3- 51.635g) had recorded reduced fruit yield/plant. Plants exposed to control conditions (C_1) had recorded the highest fruit yield/plant (77.209g) compared to stress condition (C_2- 39.254g).

Table 22. Effect of seed priming on fruit yield plant^{-1} in chilli under water stress conditions

Treatments	Fruit yield plant^{-1} (grams)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	86.723±2.358^a	44.293±0.883^d	**65.508^a**
3%Sio_2 – P_2	76.381±0.804^b	38.723±1.306^e	**57.552^b**
Unprimed – P_3	68.523±0.82^c	34.747±0.931^f	**51.635^c**
Mean (D)	**77.209^a**	**39.254^b**	
CD (α ≤0.05)	**P=1.643, D=1.341, P×D=2.323**		
SE(m)	**0.754**		
CV	**2.243**		

4.2.4.5 Root volume

The data on the effect of seed priming on root volume in chilli under water stress conditions is listed in Table 23. Under control condition (C_1), both seeds primed with 2.5%$KNO_3$and 3%SiO_2 are on par (C_1P_1-69 cm^3 & C_1P_2-67.33cm^3) while unprimed seeds (C_1P_3) had reduced root volume (53.667cm^3). Under stress condition (C_2), lowest root volume (36cm^3) was recorded by unprimed seeds (C_2P_3) and it increases as 3%SiO_2 (C_2P_2 - 39.667cm^3) and 2.5% KNO_3(C_2P_1-cm^3) have the highest root volume. The overall highest mean had exhibited by the seeds primed with 2.5%KNO_3 (P_1-55.5cm^3) followed by 3%SiO_2(P_2- 53.5cm^3) and unprimed seeds (P_3- 44.833cm^3). Comparing the water stress condition, the control condition (C_1) recorded the highest root volume (63.33cm^3) as it consecutively decreases (39.22cm^3) in stress conditions(C_2).

Table 23. Effect of seed priming on root volume in chilli under water stress conditions

Treatments	Root volume (cm^3)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	69±1^a	42±1^c	**55.5^a**
3%Sio_2 – P_2	67.33±1.528^a	39.667±1.528^c	**53.5^b**
Unprimed – P_3	53..667±1.528^b	36±1^d	**44.833^c**
Mean (D)	**63.333^a**	**39.222^b**	
C.D.(p≤0.05)	**P=1.852, D=1.512, P×D=2.619**		

SE(m)	**0.85**
CV	**2.53**

4.2.4.6 number of seeds plant^{-1}

The data on the effect of seed priming on the number of seeds/fruit in chilli under water stress conditions is presented in Table 24. Under control condition (C_1), both 3%SiO_2 (C_1P_2) and unprimed seeds (C_1P_3) are on par with each other (74.667) where 2.5%KNO_3(C_1P_1) treatment had recorded a little less no of seeds/ fruit (73.33). Under stress condition (C_2), both 2.5%KNO_3(C_2P_1- 40) and 3%SiO_2(C_2P_2 -41) are on par with each other while for unprimed seeds (C_2P_3) recorded a little less number (36). The overall highest mean value was recorded in seeds primed with 3%SiO_2 (P_2-57.883) treatment value followed by 2.5%KNO_3(P_1 - 56.667) and unprimed seeds (P_3 - 55.333). Comparing between the water stress conditions, Control condition (C_1) recorded a greater number of seeds/fruit (74.22).

Table 24. Effect of seed priming on Number of seeds fruit^{-1} in chilli under water stress conditions

Treatments	**No of seeds fruit^{-1} (Number)**		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	73.33±1.528^a	40±2^b	**56.667^b**
3%Sio_2 – P_2	74.667±1.528^a	41±1^b	**57.833^a**
Unprimed – P_3	74.667±1.528^a	36±1^c	**55.333^c**
Mean (D)	**74.222^a**	**39^b**	
CD (α ≤0.05)	**P=1.852, D=1.512, P×D=2.619**		
SE(m)	**0.85**		
CV	**2.6**		

4.2.4.7 Seed yield plant^{-1}

The data on the effect of seed priming on seed yield/chilli in chilli under water stress conditions is listed in Table 25. Under both the conditions, a similar trend had been recorded, seeds primed with 3%SiO_2(P_2) have greater seed yield/fruit (C_1P_2 - 4.117g & C_2P_2 - 3.067g) followed by 2.5%KNO_3(C_1P_1- 3.767g & C_2P_1 - 2.857g) and unprimed seeds (C_1P_3 -3.25g & C_2P_3 - 2.61g) respectively. The overall mean value was highest in seeds primed with

3%SiO_2(P_2 - 3.592g) followed by 2.5% KNO_3(P_1-3.312g) and unprimed seeds(P_3-2.93g). Comparing between the water stress conditions, it was observed that plants exposed to control conditions (C_1) recorded the highest seed yield/plant (3.711g) compared to stress conditions (C_2 - 2.844g).

Table 25. Effect of seed priming on seed yield plant^{-1} in chilli under water stress conditions

Treatments	Seed yield plant $^{-1}$ (gram)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	3.767±0.117^{b}	2.857±0.08^{e}	**3.312^{b}**
3%Sio_2 – P_2	4.117±0.035^{a}	3.067±0.049^{d}	**3.592^{a}**
Unprimed – P_3	3.25±0.036^{c}	2.61±0.026^{f}	**2.93^{c}**
Mean (D)	**3.711^{a}**	**2.844^{b}**	
CD (α ≤0.05)	**P=0.082, D=0.067, P×D=0.117**		
SE(m)	**0.038**		
CV	**1.998**		

4.2.4.8 Dry matter

The data on the effect of seed priming on the dry matter in chilli under water stress conditions is listed in Table no 26. Under both the conditions (C_2), higher dry matter content was recorded by the seeds primed with 2.5%KNO_3(C_1P_1 - 46.22g & C_2P_1 - 31.6g), followed by 3%SiO_2 primed seeds (C_1P_2 -37.687g & C_2P_2 - 26.573g) and unprimed seeds (C_1P_3-33.517g & C_2P_3 - 24.573g) respectively. Comparing between the water stress conditions, the control condition (C_1) recorded a higher amount of dry matter content (39.141g) The overall mean was highest in seeds primed with 2.5%KNO_3(P_1 - 38.91g) followed by SiO_2(P_2 - 32.13g) and unprimed seeds (P_3- 29.135g).

Table 26. Effect of seed priming on Dry matter in chilli under water stress conditions

Treatments	Dry matter (gram)		
	Control (C_1)	**Stress (C_2)**	**MEAN (P)**
2.5%KNO_3 – P_1	46.22±1.005^{a}	31.6±0.66^{d}	**38.91^{a}**
3%Sio_2 – P_2	37.687±0.879^{b}	26.573±0.797^{e}	**32.13^{b}**
Unprimed – P_3	33.517±0.572^{c}	24.573±0.94^{f}	**29.135^{c}**

Mean (D)	**39.141[a]**	**27.642[b]**	
CD (α ≤0.05)	**P=1.035, D=0.845, P×D=1.464**		
SE(m)	**0.475**		
CV	**2.465**		

Discussion

5. DISCUSSION

Chilli is a tropical plant that is primarily grown in southern India. Major production of chilli was from India accounting for 36% of the world's share and it is mainly sold to India's neighbours, including Thailand, Bangladesh, Sri Lanka, the United States, and Vietnam (Anonymous, 2018). Climate change may cause changes in temperature, relative humidity, precipitation, wind direction and sunshine intensity, leading to drought, soil salinity, overheating, erratic rainfalls, and wind storms. Due to climate changes, most tropical and subtropical crops must deal with a significant problem of water stress during the critical stages of growth. This eventually results in lower biomass and vigour, which directly affects the quality and quantity of fruit size and fruit production. The productivity of crop chilly is severely impacted by temperature fluctuations, excessive rains, acidity, salinity of the soil, disease and insect pest attacks (Sarada *et al.*, 2015).

Water stress during the crop period increases the salt concentration in the soil, which has a direct impact on the reverse osmosis of water loss from plant cells. Due to physiological, biochemical, quality, and yield features that simultaneously impair the photosynthesis and respiration process, this may result in decreased plant growth and output (Penna and Hughes, 2007). Water stress is one of the most critical factors that affect the growth and development of chilli and is a key factor of climate change has a greater impact on crop yield decline in dry and semi–dry areas of cropping systems of the world (Yan, 2015). Drought stress is reported to inhibit seed germination and growth of seedlings through the creation of low osmotic potential which inhibits the uptake of water by plants (Kaya *et al.*, 2006).

Seed priming can be considered one of the pre-germination techniques which enhances rapid and uniform seedling establishment, with high vigour and yield in vegetables and field crops (Basra *et al.*, 2005; Kaur *et al.*, 2005). After priming with respective solutions like distilled water, nutritive solutions and bio-inoculants are allowed to shade dry for a particular period. Shade dry has a good effect on germination when compared to improperly dried seeds under storage conditions.

Under environmental stress conditions, the physiological mechanism of seeds can be improved by seed priming which provides resistance to stress (Demirkaya *et al.*, 2006). Macromolecular and nucleic acid damage, oxidative reactions which show a lesser effect in primed seeds when compared to unprimed seeds.

5.1 EVALUATION OF VARIOUS SEED PRIMING METHODS FOR WATER STRESS TOLERANCE IN CHILLI AT THE SEEDLING STAGE

The seeds primed with 2.5% KNO_3 under moderate stress condition recorded high SVI I (1684.87) followed by 3% SiO_2 (1590.97) and unprimed seeds (929.867). Similar trend was recorded under control conditions (Figure 1.). Ali *et al.* (2021a) reported that KNO_3 and SiO_2 priming minimized the effect of drought and stimulated seedling growth and emergence. Priming of seeds with any of the chemicals enhances pre germination process such as increased water imbibition, cell division, and hydrolytic enzymes in rice, maize and wheat that boost up germination and seedling emergence which were affected under stressful conditions (Varier *et al.,* 2010, Wojtyla *et al.,* 2016). Enhancement of rice seedlings growth could be due to the faster cell division and cell elongation and activation of ROS scavenging enzymes in priming seeds with 2%, 2.5% KNO_3 and 3%, 3.5 SiO_2 under drought conditions which significantly boost the SVI I (Ali *et al.,* 2021b). Javed *et al.* (2020) stated that two rice cultivars primed with 0.75% KNO3 recorded significant seedling growth, biomass, and vigour.

Under moderate water stress conditions, SVI II recorded high in seeds primed with 3% SiO_2 (3.8), while low value recorded by unprimed seeds (2.5), but under control conditions 2.5% primed seeds (4.8) recorded high value followed by 3% SiO_2 (4.5) and unprimed seeds (3.8) (Figure 2). Farooq *et al.* (2019b) reported in rice that improved biomass in rice because of cell division, elongation and nucleic acid synthesis which simultaneously increases the SVI II. Farooq *et al.* (2013) stated that two wheat cultivars when subjected to hydro primed and primed to ascorbic acid recorded increased biomass weight owing to enhancement of proline contents.

under water stress conditions, the minimum MDA content was recorded by the seeds primed with 2.5% KNO_3 (0.28 µmole/ml) and the maximum was recorded by the unprimed seeds (0.60 µmole/ml) under moderate stress (Figure 3.). Chowdhury *et al.* (2017) also reported that the accumulation of proline and low MDA content are essential features of stress tolerance exhibited by plants under drought. The measurement of MDA is a valuable tool for measuring oxidative lipid injury (Mollar *et al.,* 2007).

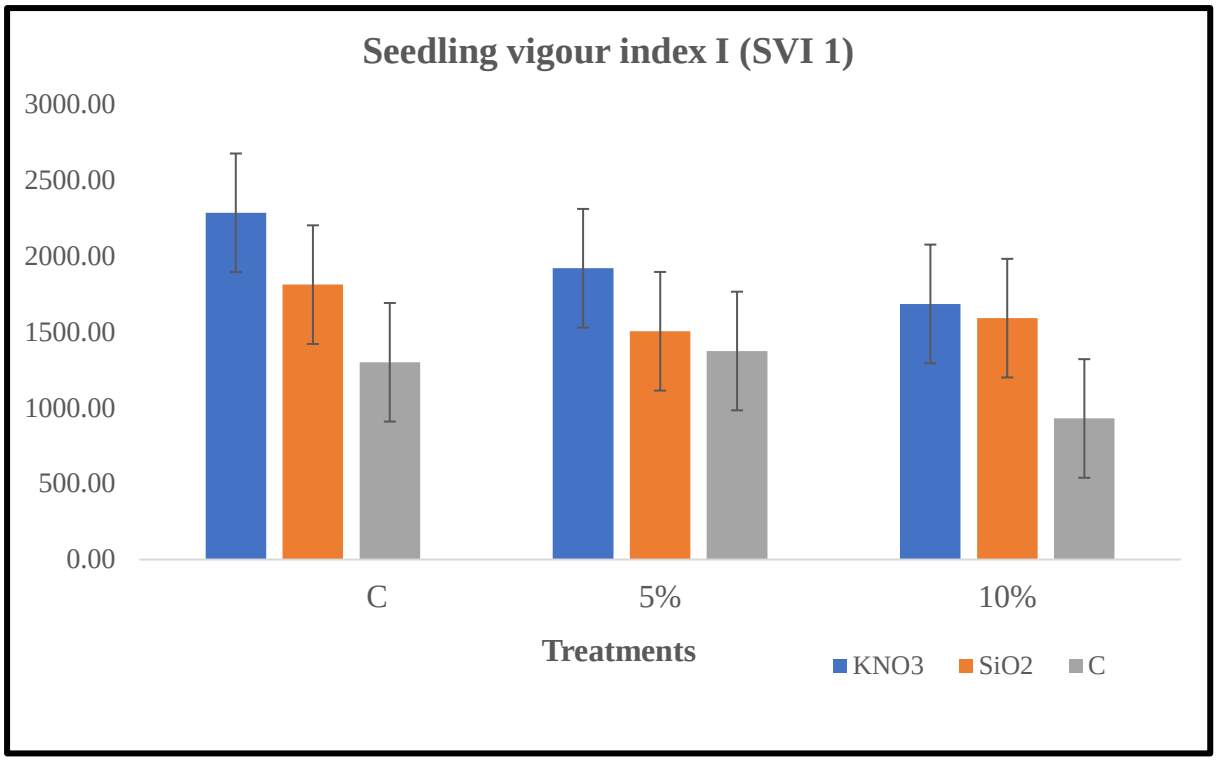

Figure 1. Effect of seed priming on Seedling vigour Index I in chilli under water stress conditions

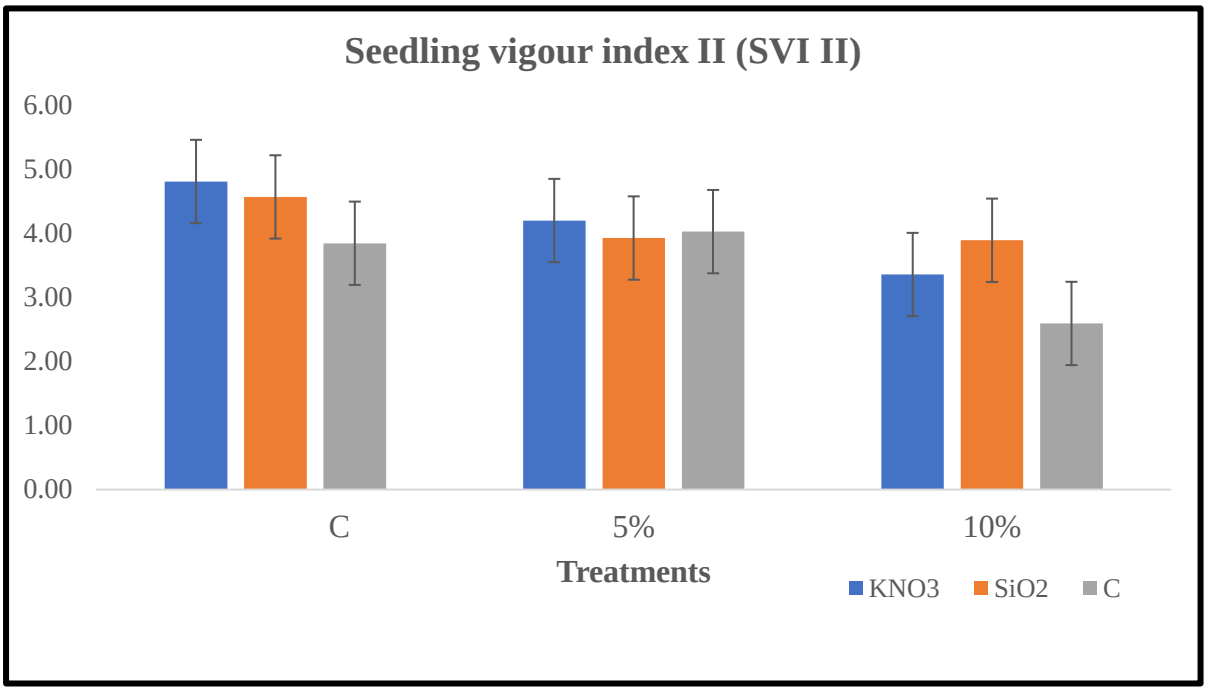

Figure 2. Effect of seed priming on Seedling vigour index II in chilli under water stress conditions.

Under moderate water stress conditions, seeds primed with 3% SiO_2 recorded low H_2O_2 content (0.4 µmole/g) followed by 2.5% primed seeds (0.53 µmole/g) and unprimed seeds (0.89 µmole/ml) whereas under control conditions, 3% primed seeds recorded low value (0.38 µmole/g) followed by unprimed seeds (0.45 µmole/g) and 2.5% KNO_3 primed seeds(0.75 µmole/g) (Figure 4.).In agreement to these studies, Chilli seedlings subjected to extreme stress conditions were reported to have a decrease in the concentration of H_2O_2 along with the concentration of MDA (Sahithya *et al.,* 2018). Yang and Mioa (2010) stated that there will be a simultaneous decrease of H_2O_2 along with MDA when species are subjected to continuous drought treatments.

The results of this study show that 2.5% KNO_3 (0.24µ moles of proline /g of tissue) and 3% SiO_2 (0.20µ moles of proline /g of tissue) result in increased proline content in comparison to unprimed seeds (0.19µ moles of proline /g of tissue) (Figure 5.). In support to these findings, seeds of Chinese cabbage pre-soaked in KNO_3 and urea exhibited improved content of proline (Yan, 2015). Seeds primed with KNO_3 and SiO_2 have shown high proline content compared to unprimed seeds. As stress levels increase proline is reported to enhance tolerance towards stress by the accumulation of buffers cytosolic pH thereby maintaining the constant redox status in cell (Choudhary *et al.,* 2005). This helps in maintaining the osmotic potential which is one of the plant adaptation techniques towards water deficiency conditions.

Seeds primed with salicylic acid (SA) 2.5 mM did not germinate. It may be due to high concentration. Agreement to the current studies, tomato seeds primed with SA (0, 50, 100, 150 mg/L) recorded a decrease in germination when concentration increases under drought conditions (Ghoohestani *et al.,* 2012). Sorghum seeds primed with SA 50 ppm and GA 50 ppm had shown improved germination percentage, germination index and seedling dry weight under drought stress (Sheykhbaglou *et al.,* 2013). Wheat seeds primed with 50 ppm ascorbic acid and 50 ppm SA improved the final germination count and also reduced the germination time (Afzal *et al.,* 2006). Seed priming with either of the chemical helps for better growth and good establishment for the entire crop period.

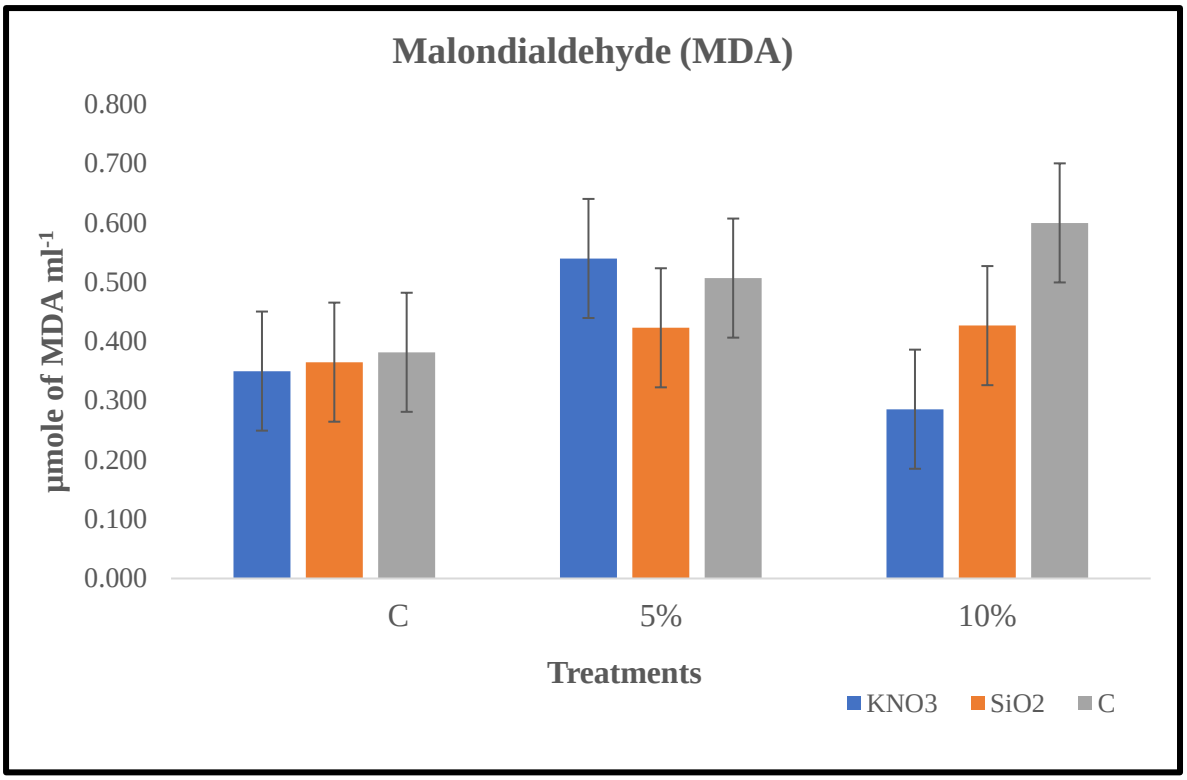

Figure 3. Effect of seed priming on Malondialdehyde (µmoles ml^{-1}) in chilli under water stress conditions.

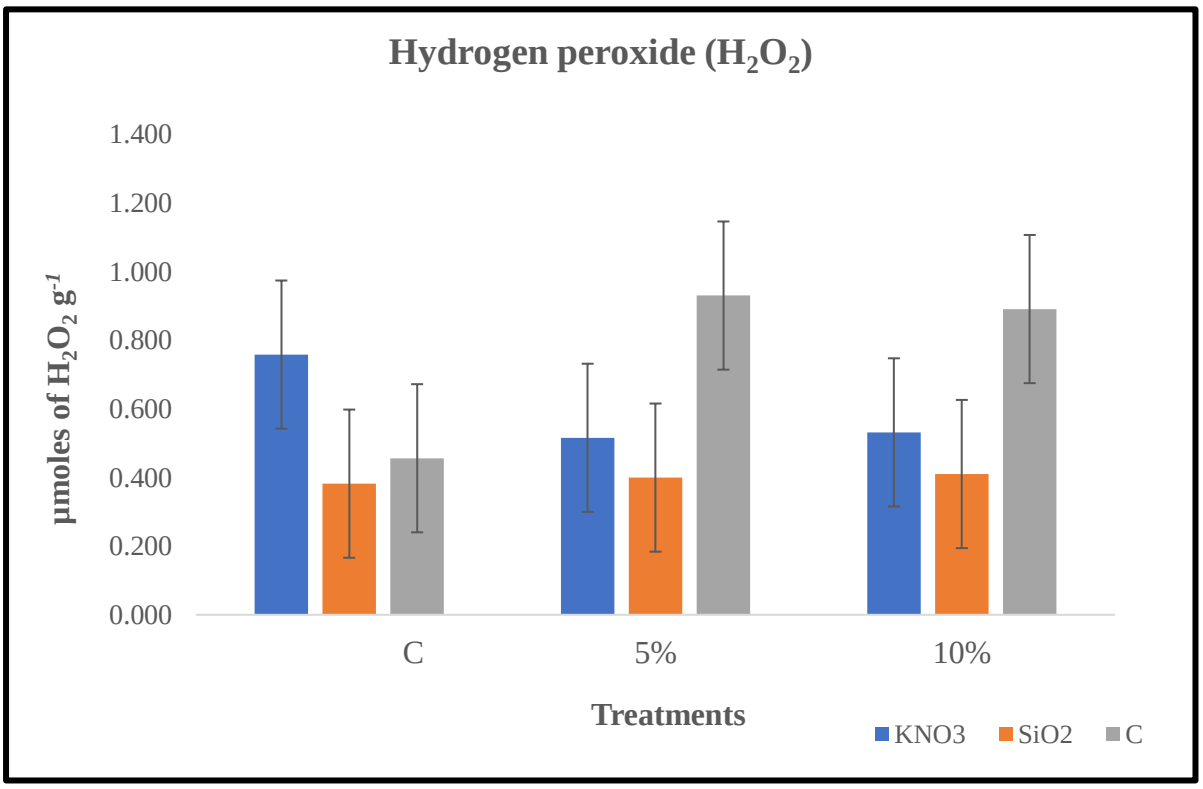

Figure 4. Effect of seed priming on Hydrogen peroxide (µmoles g^{-1}) in chilli under water stress conditions.

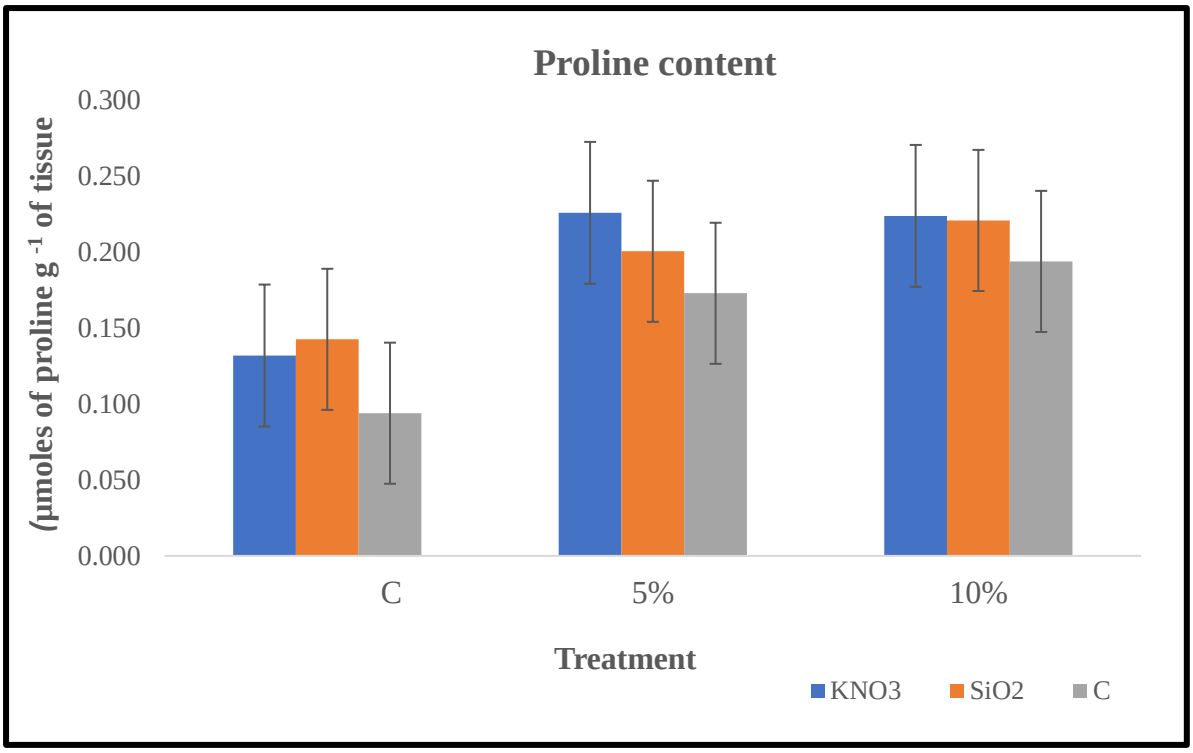

Figure 5. Effect of seed priming on proline (µmoles of proline g $^{-1}$ of tissue) in chilli under water stress conditions.

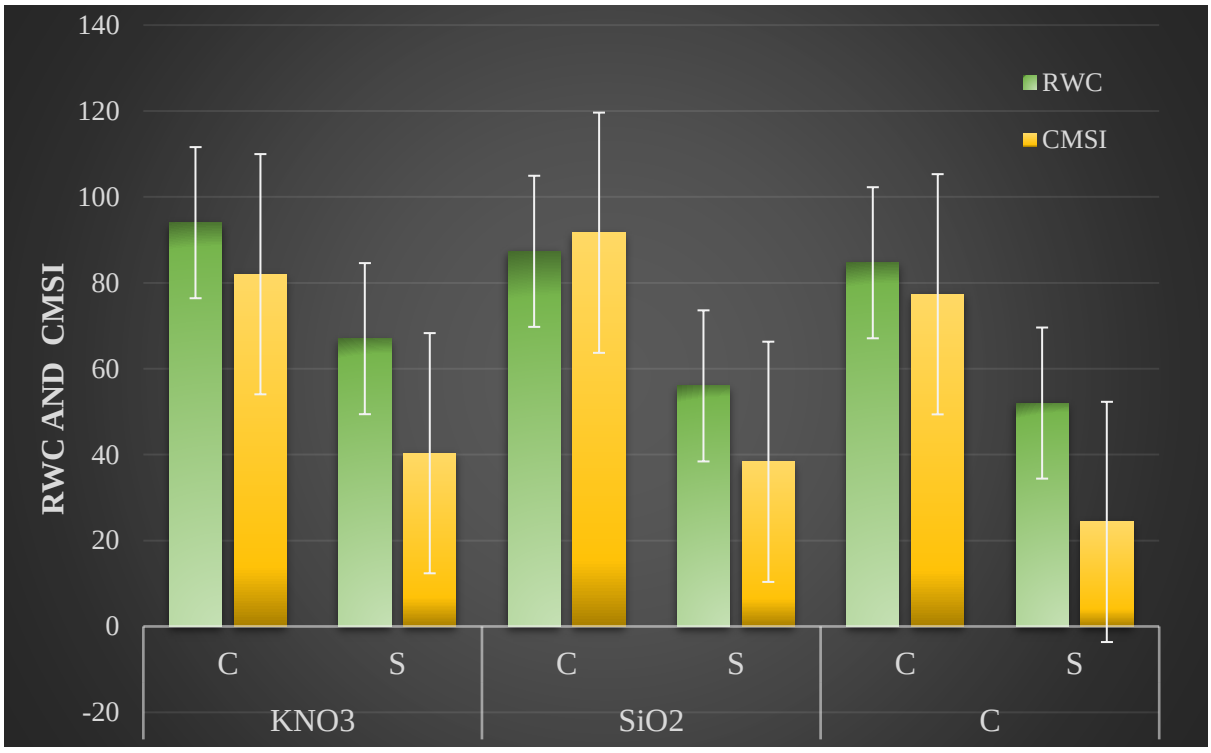

Figure 6. Effect of seed priming on relative water content (RWC) (%) and cell membrane stability Index (CMSI) (%) in chilli under water stress conditions.

5.2 EFFECT OF SELECTED BEST TREATMENT FOR WATER STRESS TOLERANCE AND ITS EFFECT ON PHYSIOLOGICAL AND YIELD TRAITS IN CHILLI.

5.2.1 Phenological traits

In the current study the control plants with 3% SiO_2 primed seeds showed early flowering *i.e.*, 28 DAT whereas seeds primed with 2.5% KNO_3 showed first flowering on 32 DAT and in case of unprimed seeds they showed first flowering at 34 DAT. Control plants with 3% SiO_2, 2.5% KNO_3 and unprimed seeds showed differences in the time of first fruiting which were 46 DAT, 47 DAT and 43 DAT, respectively. The plants exposed to water stress had significantly delayed fruiting for all the seed priming treatments. Minimum delay in fruiting was observed for seeds primed with 3% SiO_2 (52DAT) followed by unprimed seeds (55 DAT) and maximum delay in flowering was observed for seeds primed with 2.5% KNO_3 (56 DAT). Early emergence from primed seeds was sparked by their vigorous beginnings, which favoured the completion of subsequent phenological events and eventually resulted in early flowering (Rehman *et al.,* 2014). According to Ayyogari *et al. (*2014) low moisture and temperature variations led to early flowering and other phenotypic alterations that affect plant development and yield in chilli plants whereas, stress-related factors cause a steady decrease in the fruit size and fruit weight. In support of these present findings, Karim *et al.* (202) reported that yard-long bean cv. BARI Borboti-1 were hydro-primed at 12hrs, 2%KNO_3 at 12 hrs and unprimed seeds reached first flowering (41 days).

Some red chilli cultivars show signs of water stress during flowering if exposed to drought or high temperatures, it significantly reduced the number of flower buds, the percentage of flower fall and the percentage of fruit set (Abdulmalik *et al*., 2012).

5.2.2 Physiological traits

Under control condition, the RWC of seeds primed with 2.5% KNO_3, 3% SiO_2 and unprimed seeds were 94%, 87.33% and 84.67% respectively. Meanwhile, the plants grown under water stress conditions showed significant differences in the RWC in plant cells. RWC of seeds primed with 2.5% KNO_3 showed maximum value of 40.33%, followed by seeds primed with 3% SiO2 having 38.33% RWC and unprimed seeds showed a minimum RWC of 24.33% (Figure 6.). Reduced water availability under water stress in plants negatively affects metabolic processes like photosynthesis and evapotranspiration, which ultimately impair plant growth and development (Mardaninejad *et al.,* 2017). According to earlier studies, under water stress conditions, tolerant genotypes recorded higher RWC and were able to perform efficiently

different physio-biochemical processes than compared to susceptible genotypes (Moussa and Abdel-Aziz, 2008, Soltys-Kalina *et al*., 2016; Regibi *et al.,* 2019).

The CMSI under control conditions recorded by seeds primed with 2.5% KNO_3, 3% SiO_2 and unprimed seeds were 82%, 91.66% and 77.33%, respectively whereas, under stress conditions, CMSI in seeds soaked in 2.5% KNO_3, 3% SiO_2 and unprimed seeds were 40.33%, 38.33% and 24.33% respectively (Figure 6.). CMSI values get reduced when the plants are subjected to water stress conditions compared to control plants. Tolerant genotypes show a higher membrane stability index because of their high-water retention capacity under drought stress conditions in contrast to susceptible genotypes (Moussa and Abdel-Aziz, 2008).

Under control conditions, seeds primed with 2.5% KNO_3 (0.98 µmoles of proline g^{-1} of tissue), 3% SiO_2 (1.307 µmoles of proline g^{-1} of tissue) and unprimed seeds (0.593 µmoles of proline g^{-1} of tissue) exhibited lower proline content, which gradually increased during the stress condition when seeds were primed with 2.5% KNO_3 (2.977 µmoles of proline g^{-1} of tissue), 3% SiO_2 (3.133 µmoles of proline g^{-1} of tissue) and unprimed seeds (2.853 µmoles of proline g^{-1} of tissue) (Figure 8.). During water stress conditions, the proline content of primed seeds drastically increased when compared to unprimed seeds. The increased proline content in plant tissues might be due to enhanced free radical accumulation under water stress conditions as it is positively correlated with antioxidant levels (Zhang *et al*., 2000). In addition to this, proline is a multifunctional proteogenic amino acid which plays a major role under adverse environmental conditions. It also acts as an osmoprotectant, an antioxidant and a nutritional source by providing nitrogen and carbon to plants during unfavourable conditions. The catabolic regulation of proline was enhanced mainly due to the increased activity of PDH (a Pro degrading enzyme), which simultaneously reduces the membrane damage in plants under water stress conditions (Alamri *et al.,* 2020).

2.5% KNO_3 primed seeds showed high TCC under control conditions followed by 3% SiO_2 and unprimed seeds which were 4.577, 4.24 and 4.11 mg g^{-1}, respectively. For water stress conditions, there was a significant difference in TCC when the seeds were primed with 2.5% KNO_3 (1.807 mg g^{-1}), 3%SiO_2 (1.763 mg g^{-1}) and unprimed seeds (1.61mg g^{-1}) (Figure 8.). The decreased TCC may be due to the reduced leaf water potential which is directly related to abiotic stress response and this indicates a negative correlation with drought intensity. (Thomas and Howarth, 2000; Sahitya *et al.,* 2018). Even though the leaf water potential was reduced, plants could retain their ability to catch light and keep their vapour pressure deficit (VPD) and

radiant energy levels high (Goa *et al.*,2021). In agreement with the present study, Bukhari *et al.* (2021) found that spinach seeds primed with KNO_3 recorded decreased chlorophyll content, but was a less pronounced decline than unprimed seeds.

The catalase content increased under water stress conditions than the control plants. The seeds primed with 2.5% KNO_3 (510 n moles of H_2O_2 used min^{-1} g^{-1} weight of sample), 3% SiO_2 (488.33 n moles of H_2O_2 used min^{-1} g^{-1} weight of sample) and unprimed seeds (324.66 n moles of H_2O_2 used min^{-1} g^{-1} weight of sample) were recorded, with drastic reduction in the CAT content under control conditions for the seeds primed with 2.5% KNO_3 (200 n moles of H_2O_2 used min^{-1} g^{-1} weight of sample), 3% SiO_2 (488.33 n moles of H_2O_2 used min^{-1} g^{-1} weight of sample) and unprimed seeds (324.66 n moles of H_2O_2 used min^{-1} g^{-1} weight of sample).(Figure 7.). Increased catalase content under soil moisture stress leads to an increase in the internal energy associated with decreased CO_2 content, restricted stomatal opening by constrained usage of photosynthetic electron transport and ROS accumulation (Moosavi *et al.*, 2009). When ROS accumulation increases it accelerates processes like protein degradation, macromolecular damage, lipid peroxidation as well as DNA oxidation (Fallah *et al.*, 2017; Gill and Tuteja, 2010).

Under water stress conditions, the peroxidase activity of seeds was found to increase. Seeds primed with 2.5% KNO_3, 3% SiO_2 and unprimed seeds exhibited peroxidase activity of 0.135, 0.087 and 0.061 units min^{-1} mg^{-1} of protein, respectively. While in control conditions, the POD activity of seeds primed with 2.5% KNO_3 showed a maximum value of 0.135 units min^{-1} mg^{-1} of protein, followed by 3% SiO_2 primed seeds having 0.087 units min^{-1} mg^{-1} of protein and unprimed seeds having 0.061 units min^{-1} mg^{-1} of protein (Figure 7.). DNA synthesis in the embryo during priming lacks cell division and an increase in DNA synthesis rate in the embryo tissue leads to increased antioxidant enzyme activity under water stress conditions (Ahmadpour Dehkrodi and Bluchi, 2012).

The SOD activity under control conditions for seeds primed with 2.5% KNO_3, 3% SiO_2 and unprimed seeds were 400.6, 385 and 333.3 units mg^{-1} of protein respectively. For stress conditions, there was a significant difference between the seeds primed with 2.5% KNO_3 (492.6 units mg^{-1} of protein), 3% SiO_2 (482 units mg^{-1} of protein) and unprimed seeds (414.66 units mg^{-1} of protein) (Figure 7.). As SOD acts as the first line of defence mechanism which restricts the formation of superoxide radicals in cells by O_2 formation, other enzymes like CAT and POD become active which led to the formation of oxygen and water by converting H_2O_2

(Alamri *et al.,* 2020). Similar results were found in crops like tomato (Celik *et al.,* 2017), sunflower (Gunes *et al.,* 2008), poplar (Xiao *et al.,* 2008), cowpea (Manivannan *et al.,* 2007) and liquorice (Pan *et al.,* 2006) where there was an increase in the SOD content under stress condition. In a study conducted by Yan (2015), it was found that when Chinese cabbage seeds when primed with KNO_3 exhibited elevated CAT, POD and SOD in response to drought stress conditions. The results were in similarity with rice seeds primed with 2.5% KNO_3, and 3% SiO_2 showed an increase of CAT, POD and SOD activity in response to water stress conditions.

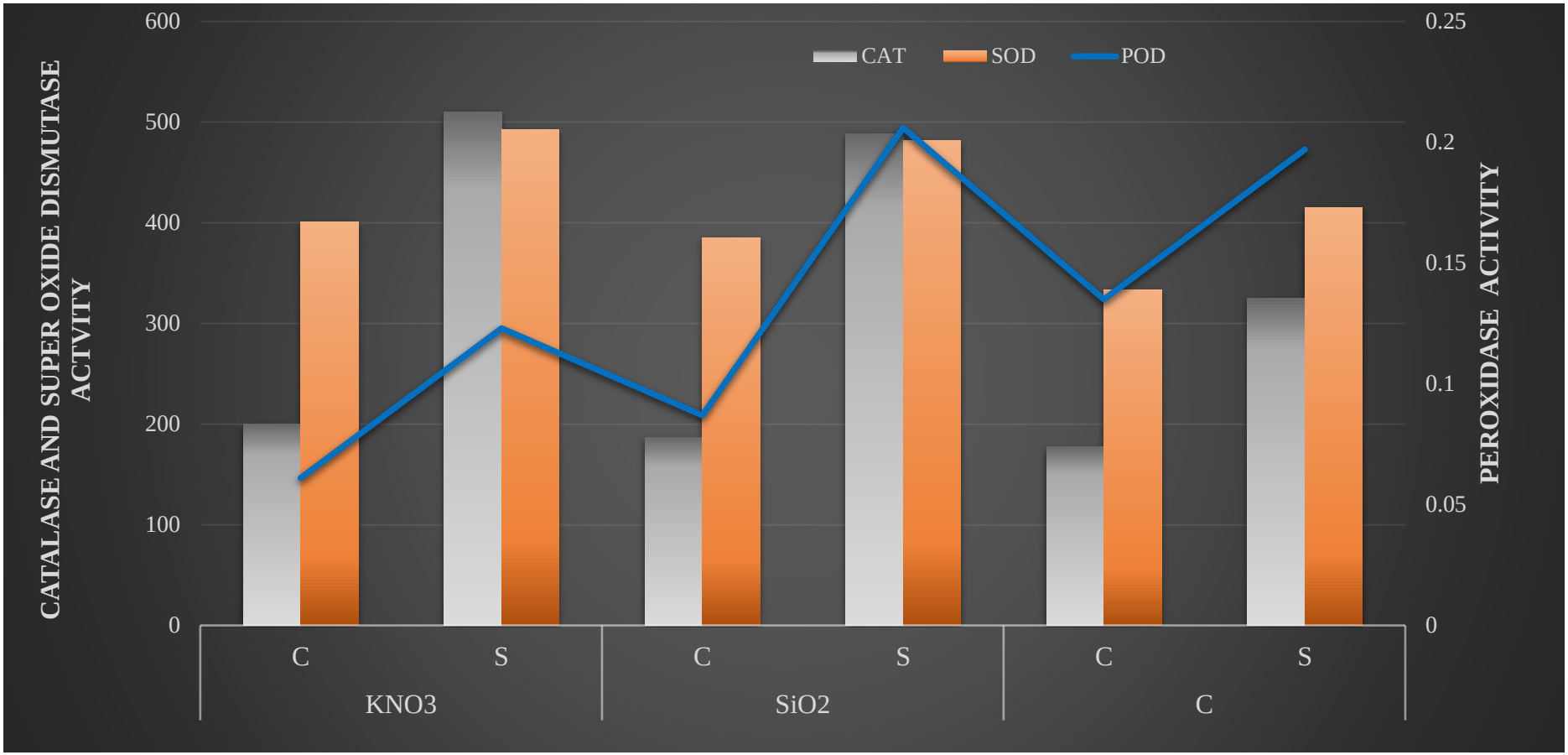

Figure 7. Effect of seed priming on catalase (n moles of H_2O_2 used min^{-1} g^{-1} weight of sample), peroxidase (units min^{-1} mg^{-1} of protein) and superoxide dismutase (units mg^{-1} of protein) in chilli under water stress conditions.

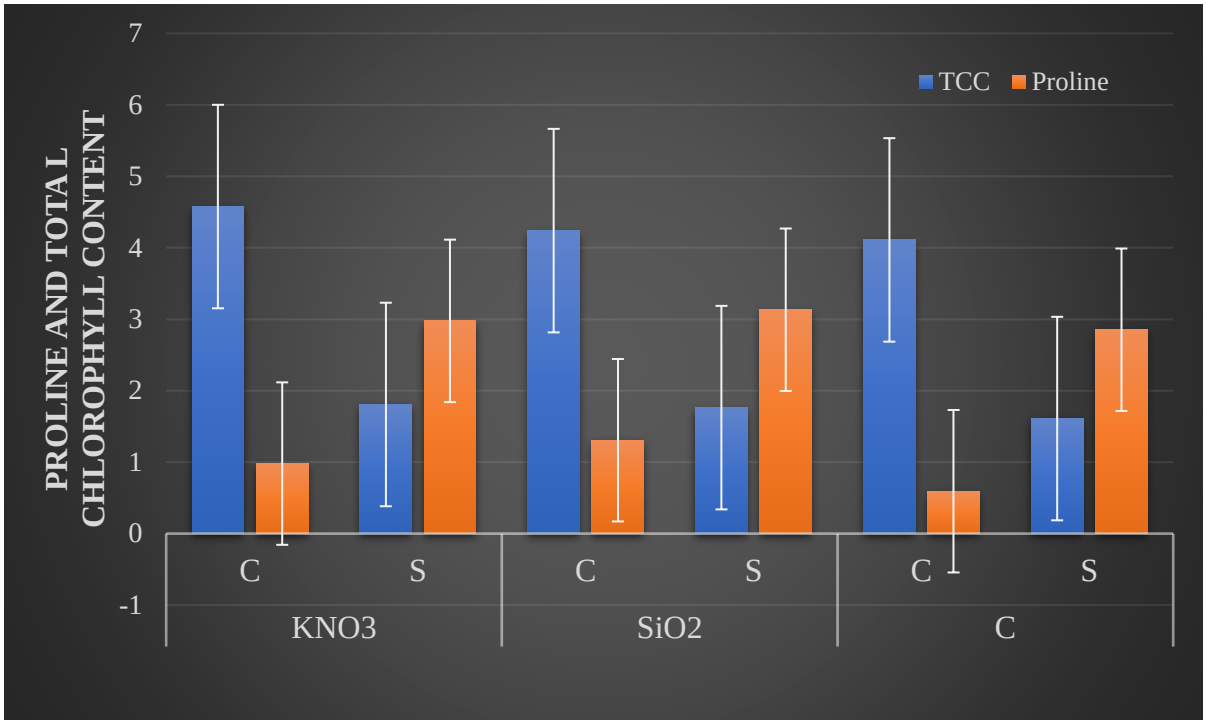

Figure 8. Effect of seed priming on proline content (µmoles of proline g^{-1} of tissue) and Total chlorophyll content (TCC) (mg g $^{-1}$) in chilli under water stress conditions

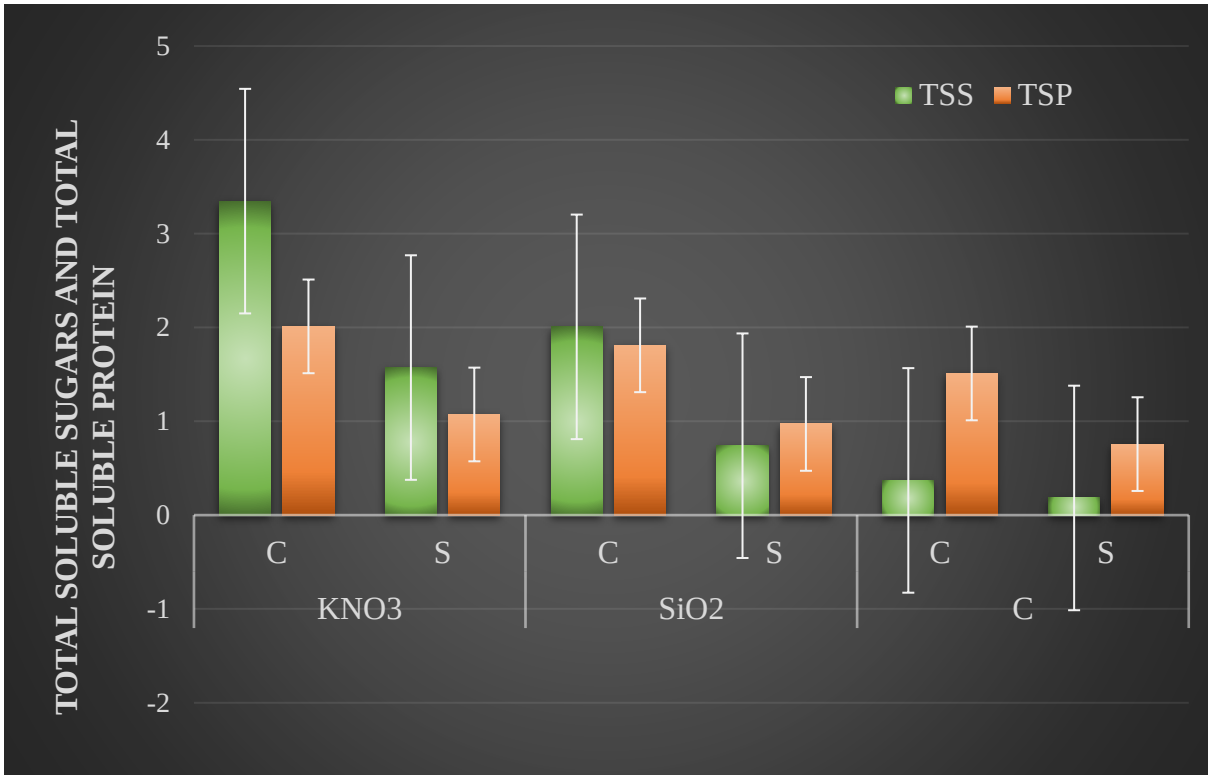

Figure 9. Effect of seed priming on Total Soluble sugars (TSS) (mg ml^{-1}) and Total soluble protein (TSP) (mg ml^{-1}) in chilli under water stress condition

5.2.3 Quality traits

The capsaicin content under control conditions in seeds primed with 2.5% KNO3, SiO2 and unprimed seeds were 0.129,0.102 and 0.074% respectively whereas under water stress conditions 2.5% KNO_3, 3% SiO_2 and unprimed seeds were showing values of 0.022, 0.019 and 0.018% respectively (Figure10.). The enhanced capsaicin content might be due to the accumulation of reactive oxygen species (ROS) which directly affects the biological tissue under water stress conditions (Pene and Hughes, 2007; Kopta *et al.,* 2020). The shrinkage of fruit size is another morphological factor that supports the findings of a rise in capsaicin content under water stress conditions. The reduction in the placenta-to-pericarp ratio under drought stress during the reproductive phase might be primarily responsible for the smaller fruit size with a high content of capsaicin content (Plate 13.). When compared to plants grown in saturated conditions, the placenta may be smaller, but it has the highest concentration of capsaicin since the placenta's ability to synthesise capsaicin developed primarily during drought stress (Rathnayaka *et al.,* 2020). Along with the temperature and water availability, the age of the fruit and light conditions also play a major in the accumulation of capsaicin content in pepper fruits (Othman *et al.,* 2011). According to Sung *et al*. (2005), plants with higher levels of capsaicin concentration serve as a good indicator for assessing hot pepper cultivars. In support of the above findings, Kopta *et al.* (2020) reported that there was an increase in capsaicin content in chilli genotypes when subjected to water stress conditions.

Under control conditions, the ascorbic acid content of seeds primed with 2.5% KNO_3, 3%SiO_2 and unprimed seeds were 483.083, 474.3 and 397.133 mg $100g^{-1}$ of the sample, respectively. Under stress conditions, 2.5% KNO_3, 3% SiO_2 and unprimed seeds were 349.803, 393.567 and 363.733 mg $100g^{-1}$ of the sample, respectively(Figure 11.). Although the ascorbic content of chilli plants was good, the duration of the stress, genetic factors and cultivar performance play an important role in the accession of ascorbic acid in plants under water stress conditions (Kopta *et al.*,2020). The cultivars that are resilient to drought produce greater endogenous levels of ascorbic acid (Tuteja *et al.*,2012).

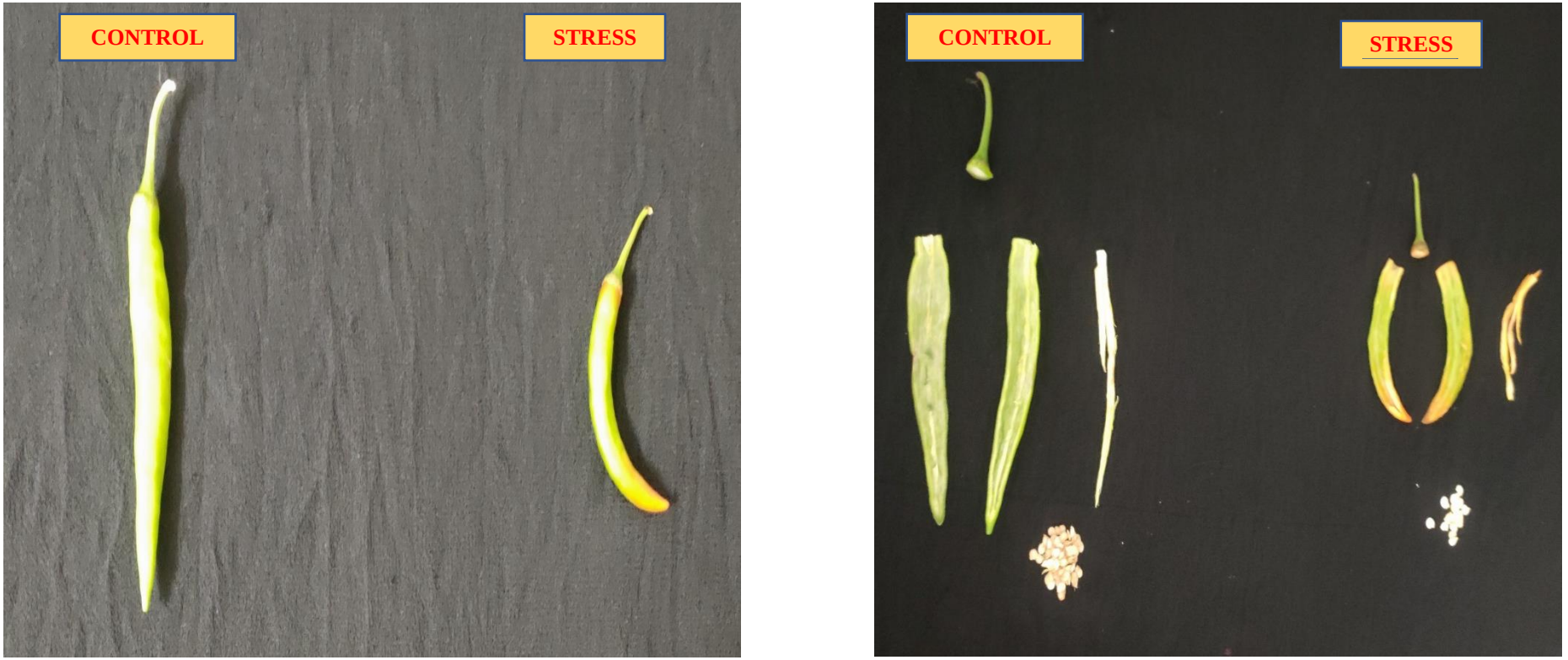

Plate 13. Capsaicin biosynthesis

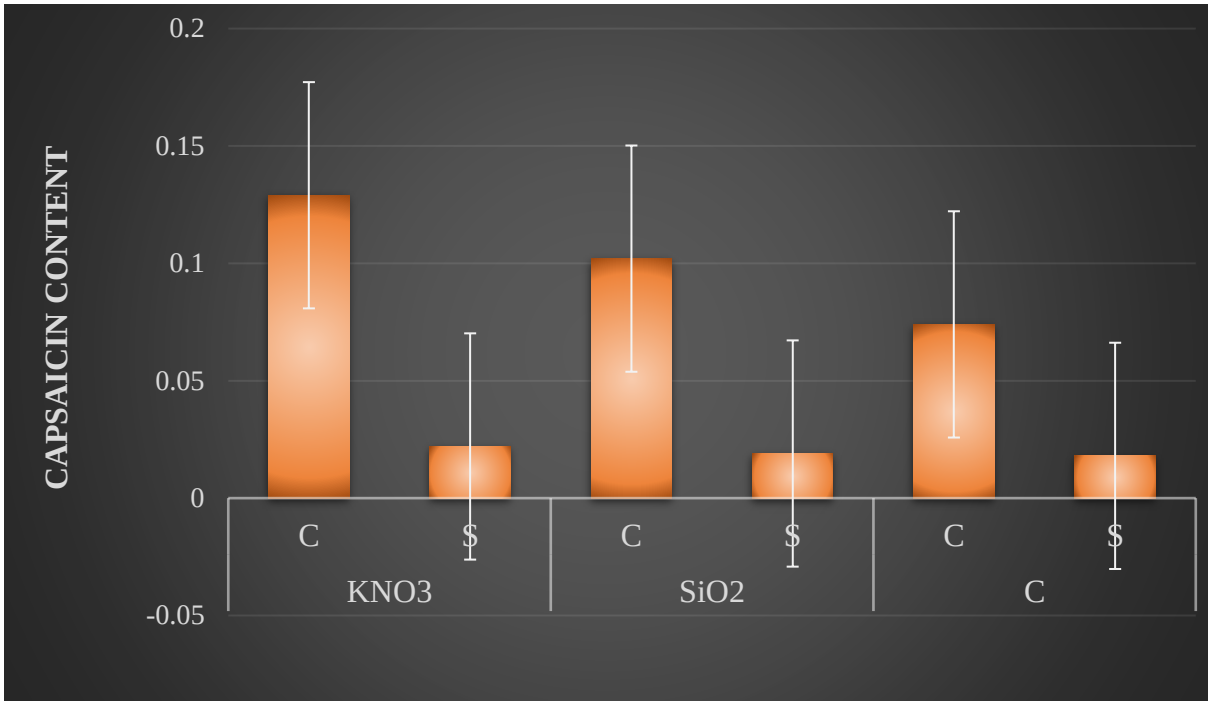

Figure 10: Effect of seed priming on capsaicin content (%) in chilli under water stress conditions.

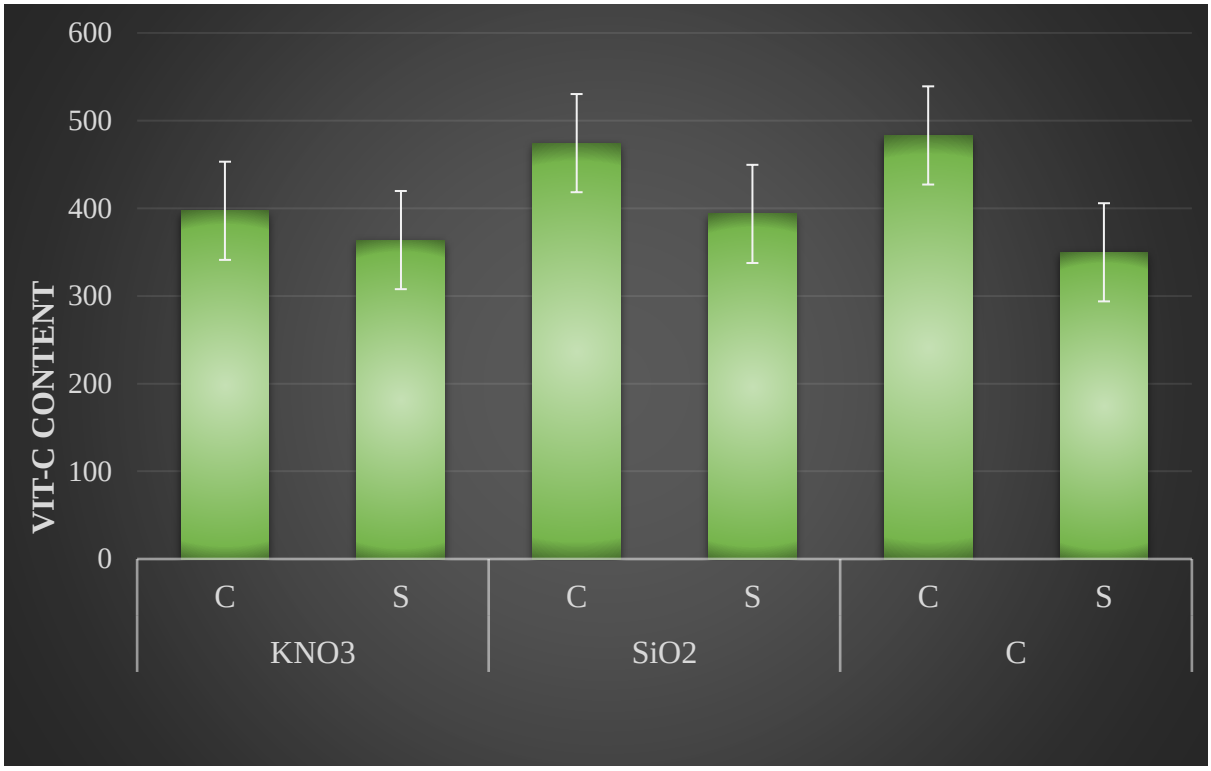

Figure 11: Effect of seed priming on Vit-C content (mg $100g^{-1}$ of sample) in chilli under water stress conditions.

5.2.4 Yield traits

Under control conditions, the plant height of seeds primed with 2.5% KNO_3, 3%SiO_2 and unprimed seeds were 58.667, 53.33 and 48.33 cm respectively. Whereas in stress conditions 2.5% KNO_3, 3% SiO_2 primed and unprimed seeds are 57, 56 and 43 cm respectively. Abiotic stress has a negative impact on the cropping period of chillies which led to reduction of growth and yield loss (Sarada *et al.,* 2015; Suwingo *et al.,* 2017).

The number of flowers plant^{-1} under stress conditions for seeds primed with 2.5% KNO_3, 3% SiO_2 and unprimed seeds were 30, 33 and 27 number respectively. While under stress conditions, 2.5% KNO_3 (21), 3%SiO_2 (19) primed and unprimed seeds (22) recorded a reduction in the number of flowers plant^{-1}. In concurrence to it, a delay in the occurrence of flowering and a reduction in flower production due to water stress can be observed in C. *chinense* (Jaimeza *et al.*,1999).

Under stress conditions, the number of fruits plant^{-1} of seeds primed with 2.5% KNO_3, 3%SiO_2 and unprimed seeds were 16,15 and 11.667 respectively. There was a significant difference in the number of fruits plant^{-1} under water stress conditions. Meanwhile, under control conditions, the number of fruits plant^{-1} by seeds primed with 2.5% KNO_3 (27), 3%SiO_2 (27) and unprimed seeds (19) were recorded high. In support of this research, Mohammad *et al.* (2021) noted that morphological features including flower survival percentage, fruit production, and fruit shape steadily declined at the flowering stage when chilly genotypes were subjected to water stress conditions.

The fruit yield plant^{-1} under water stress conditions in seeds primed with 2.5% KNO_3, 3% SiO_2 and unprimed seeds were 44.293, 38.723 and 34.747 grams respectively. A significant difference was observed under control conditions for fruit yield plant^{-1} when seeds are primed with 2.5% KNO_3, 3% SiO_2 and unprimed seeds were 86.723, 76.381 and 68.523, respectively (Figure 12.). The reduced fruit yield under water stress conditions is positively correlated to water supply during the crop growth period (Ratnayaka *et al.,* 2020). Because of water stress conditions, plant tissues led to osmotic adjustment along with a reduction of leaf area due to wilting of leaves which affects the photosynthetic rates and is directly related to fruit diameter in pears (Ismail *et al.*,1997).

Under control conditions, seeds primed with 2.5% KNO_3 (69 cm^3), 3% SiO_2(67.33 cm^3) and unprimed seeds (53.667 cm^3) recorded maximum root volume when compared to stress conditions with the same priming methods (42,39.667 and 36 cm^3 respectively) (Plate 14.).

Similar results were found by Riduan *et al. (*2005) in chilli where root volume extended up to 30 cm and was able to withstand water stress conditions.

Under control conditions, the number of seeds fruit^{-1} in seeds primed with 2.5% KNO_3, 3% SiO_2 and unprimed seeds were 73.33, 74.667 and 74.667, respectively. For stress conditions, seeds primed with 2.5% KNO_3 (40 g), 3%SiO_2 (41g) and unprimed seeds (36) recorded a lower number of seeds in fruits (Figure 12.). Similar results were also found by Das and Jana (2015) when lentil seeds (*Lens culinaris* medikus) were hydro-primed and two urea sprays at the branching and pod initiation stages recorded a higher number of pods plant $^{-1}$ and number of seeds pod $^{-1}$ when compared to non-primed seeds without urea sprays and Samarah *et al.* (2006) that flowering during water stress conditions had a greater impact on the number of fruits plant $^{-1}$ and seed weight.

Seed yield plant $^{-1}$ under control conditions in seeds primed with 2.5% KNO_3, 3% SiO_2 and unprimed seeds were 3.767, 4.117 and 3.25 grams respectively. While under stress conditions, the seed yield plant^{-1} was gradually reduced in seeds primed with 2.5% KNO_3, 3% SiO_2 and unprimed seeds are 2.857, 3.067 and 2.61 grams respectively (Figure 12.). With support to the present findings, soybean cultivars were subjected to water stress during flowering and seed-filling stages causing decline in seed yield plant^{-1} and 1000 seed weight (Makeli *et al.*,2013). In agreement to study, Afzal *et al*. (2013) explained that the Maize hybrids like SP 13 were primed with 0.5%$ZNSO_4$ and 1.5% $ZNSO_4$, priming with 1.5% $ZNSO_4$ recorded higher grain yield when compared to 0.5% $ZNSO_4$ and unprimed seeds.

The dry matter content has a significant difference under control conditions when seeds are primed with 2.5% KNO_3, 3% SiO_2 and unprimed seeds were 46.22, 37.687 and 33.517 grams respectively. While under stress conditions dry matter of seeds primed with 2.5% KNO_3, 3% SiO_2 and unprimed seeds were 31.6, 26.573 and 24.573 grams respectively. In agreement with these findings, when cotton plants were exposed to drought conditions they indicated a reduced no of flowers with a decrease of dry matter in both shoot and root (Shafiq *et al.*,2015). Increased moisture loss or transpiration rates due to water scarcity may result in leaf and cell turgor, reduced water potential, stomata closure, decreased water content, slower cell growth, and reduced cell expansion all of which impact the accumulation of dry matter and growth (Jaleel *et al.*, 2009, Delfine *et al.*, 2002, Anjum *et al.*, 2011). The soybean variety JS-9305 were primed with 1000ppm GA_3 (12 hrs) revealed an increase in dry matter content of seedling

which drastically enhanced plant height, number of pods plant, number of seeds plant and seed yield in contrast to unprimed seeds (Agawane and Parhe,2015).

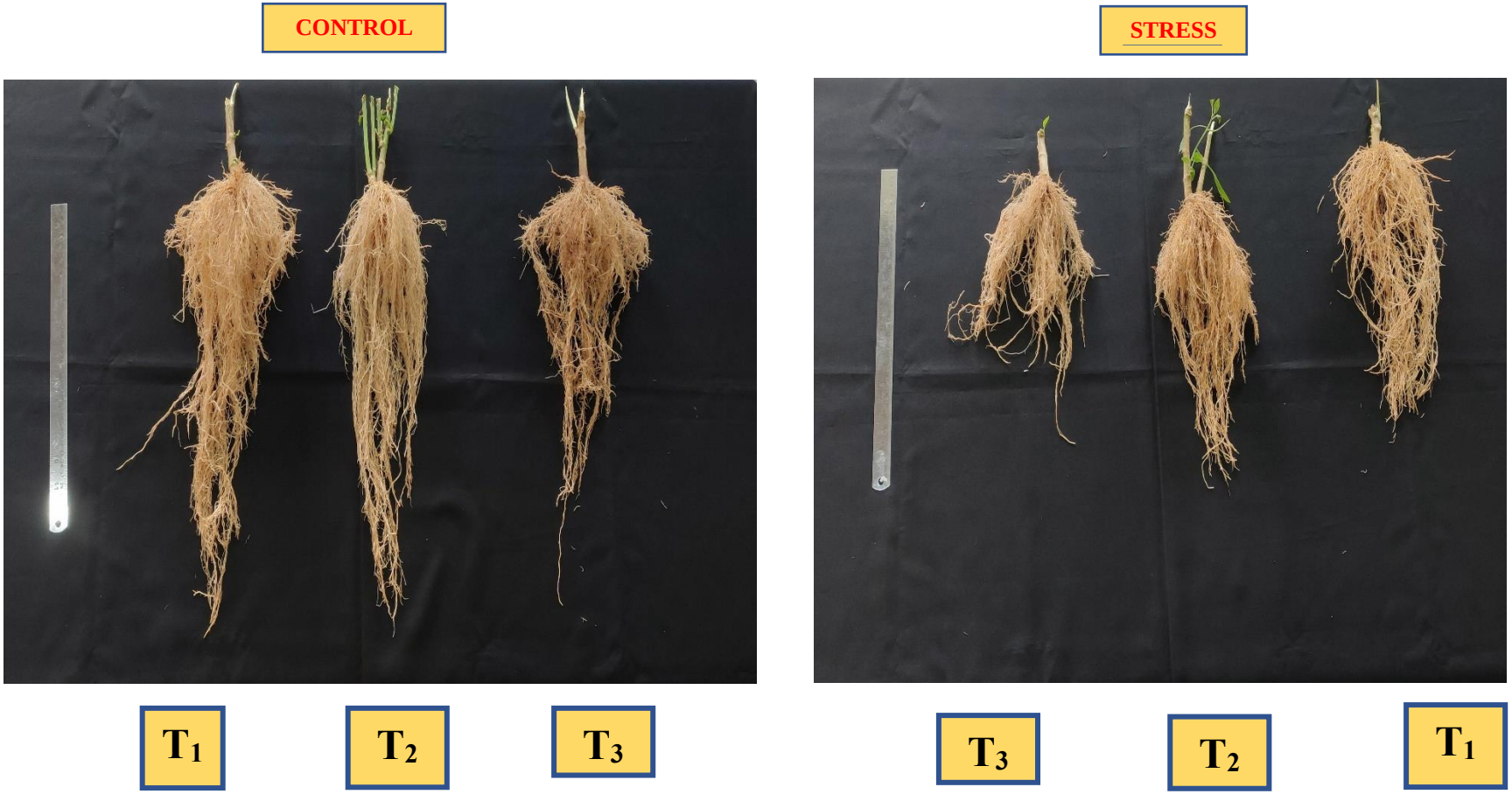

Plate 14. Effect of seed priming methods on Root volume under water stress conditions in chilli

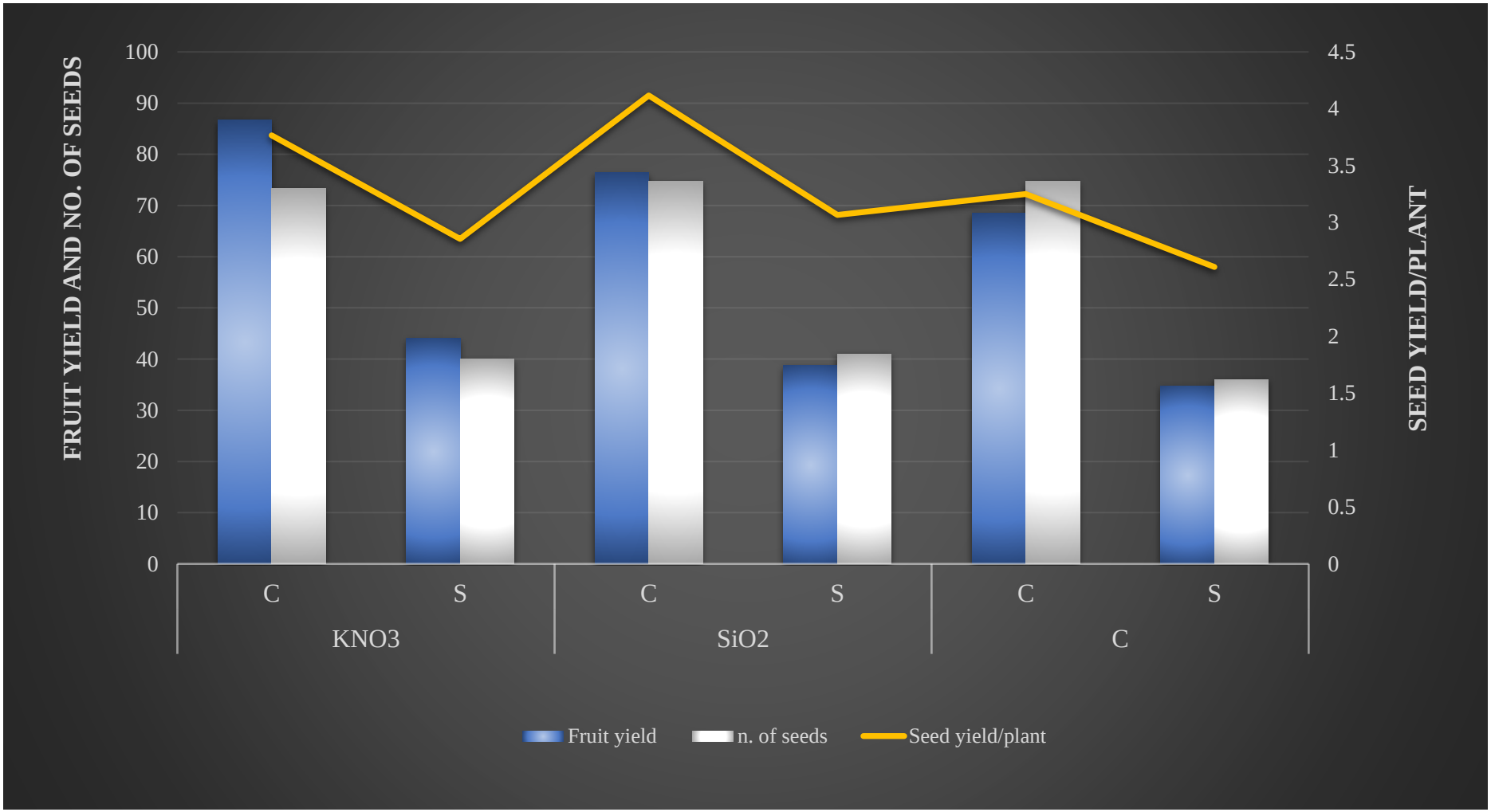

Fig 12: Effect of seed priming on Fruit yield (grams), Number of seeds plant^{-1} (grams) and seed yield plant^{-1} (grams) in chilli under water stress conditions.

Summary

Summary

Chilli is one of the most important spice crop that belongs to the Solanaceae family widely used by the human population in their meal and also for medicinal purposes. Chilli is mainly a tropical horticultural crop which requires a hot climate during the flowering and pod formation stages. In India, mainly southern states with light loamy soil rich in lime are suitable for chilli cultivation. Due to recent climatic changes, production, distribution, attack of pests and diseases and improper value chains have affected chilli production gravely.

Water stress condition is one of the major impacts of climate change on Agri-ecosystems. Water stress condition caused by less rainfall or dry weather during cropping seasons leads to infestation by thrips and whitefly that causes leaf curling, stunted growth and wilting of the plant. To reduce the effect of drought on plants, priming can be done with water, low-potential chemicals, salt solutions, nutritive solutions, biocontrol agents inoculum and foliar spray before germination. Seed priming helps the plants to overcome adverse conditions during crop establishment and helps to maintain defence mechanisms and yield.

A study entitled "Efficacy of seed priming for inducing stress tolerance in chilli (*Capsicum annum* L.) under water stress condition" was undertaken with the objective to evaluate the various seed priming treatments for water stress tolerance in chilli at the seedling stage and selected best treatments were evaluated for physiological and yield traits under pot culture study. Seeds of the chilli variety 'Vellayani Athulya' were primed with 2.5% potassium nitrate $(KNO)_3$, 3%silicon dioxide (SiO_2), 2.5Mm salicylic acid (SA) and distilled water for 12 hours and subjected to three different stress levels *i.e.*, control, mild stress (5% - PEG 6000) and moderate stress (10% - PEG 6000) during seedling stage. Seed priming experiment was carried out in a factorial completely randomized design (CRD) with the first factor being priming using different solutions and the second factor being the stress levels which were replicated thrice.

The results of the first experiment noted that chilli seedlings responded distinctively to the different treatments. 2.5% KNO_3 under moderate stress recorded significantly higher proline content (0.24 µM) and enhanced seedling vigour index-I (SVI 1) compared to 3% SiO_2 and untreated treatments. The hydrogen peroxide (H_2O_2) concentration were significantly higher in mild stress with unprimed seeds (0.93 µM), wherein lower content was recorded in 3%SiO2 primed seeds (0.38 µM). Further, reduced malonaldehyde (MDA) content and

increased seedling vigor index-II (SVI 2) performance were noted in both 2.5% KNO_3 and 3% SiO_2 treatments. Seeds failed to germinate under 2.5mM salicylic acid.

From the first experiment, 2.5% KNO_3 and 3% SiO_2 were selected for further study as both were on par with each other. Then the seeds primed with both treatments were sown in portrays. Seedlings were transplanted to pots 30 days after sowing (DAS) and irrigation and other cultural practices were followed according to KAU POP. Water stress was induced by withholding irrigation for four days where relative water content (RWC) of leaves reaches 70% during flower initiation stage. During this period, physiological parameters were taken from both the stress and control plants. The results of second experiment revealed that seeds primed with 3% SiO_2 recorded early flowering whereas, seeds primed with 2.5% KNO_3 recorded the first fruiting stage.

Under water stress conditions, seeds primed with 2.5% KNO_3 recorded significantly high values for relative water content (RWC) (67%), cell membrane stability Index (CMSI) (40.33%), total chlorophyll content (TCC) (1.807mg/g), catalase activity (CAT) (510 nmoles of H_2O_2 used min/g weight of sample), superoxide dismutase (SOD) (492.6 Units/mg of protein), total soluble sugars (TSS) (1.573mg/ml) and total soluble protein (TSP) (1.073mg/ml) where 3% SiO_2 primed seeds recorded significantly higher values for total proline content (2.977µmoles of proline/g of tissue). For control conditions, 2.5%KNO_3 primed seeds recorded significantly high values for relative water content (RWC) (94%), total chlorophyll content (TCC) (4.577mg/g), catalase activity (CAT) (200 units/min/g weight of sample), peroxidase activity (POD) (0.135 units/min/mg of protein), superoxide dismutase(SOD)(400 units /mg of protein) total soluble sugars (TSS) (3.347 mg/ml), total soluble protein (TSP) (2.012 mg/ml) and 3% primed seeds recorded significantly higher cell membrane stability index (CMSI) (91.66%), total proline content (1.307 µmoles of proline/ g of tissue).

Under stress condition, capsaicin content was recorded high in seeds primed with 2.5% KNO_3(0.022%) and ascorbic acid was recorded highest in seeds primed with 3% SiO_2(393.567mg) Whereas, in control both capsaicin content (0.129%) and ascorbic acid content (483.083mg) were recorded higher in seeds primed with 2.5% KNO_3.

Under stress condition, the yield components like plant height(57cm), number of fruits/plant (16 no), fruit yield (44.293g), dry matter (31.6g) and root volume(42cm^3) were recorded as significantly higher in seeds primed with 2.5% KNO_3. Further, the number of seeds/plant (41 no) and seed yield (3.067g) were recorded as significantly higher in seeds

primed with 3% SiO_2 and the number of flowers/plant (22 no) were found highest in unprimed seeds whereas, in control condition, 2.5% KNO3 primed seeds recorded significantly higher plant height(58.66cm), fruit yield (86.723g), root volume (69 cm3) and dry matter (46.22g) and 3%SiO2 primed seeds recorded significantly higher number of flowers/plant (33) and seed yield (4.117g). Both 2.5% KNO_3 and 3% SiO_2 are on par with each other for number of fruits/plant (27), while SiO2 and unprimed seeds are on par to each other for no. of seeds/plant (74.667).

From the seedling stage study, it has been concluded that seeds primed with 2.5% KNO_3 recorded high proline content and SVI II with reduced MDA content, while 3 % SiO_2 primed seeds recorded low H_2O_2 content under moderate stress conditions. Whereas, in the pot study, 2.5% KNO_3 primed seeds were found to be more effective under water stress conditions as they showed enhanced antioxidants levels, with high water absorption capacity, increased chlorophyll content, soluble sugar and protein content along with the high capsaicin, ascorbic acid contents with increased plant height, number of fruits, fruit yield, root volume and dry matter. Further, 3% SiO_2 primed seeds recorded high proline, peroxidase activity, ascorbic acid content, no: of seeds/plant and seeds yield/plant under water stress conditions. Seed production was best under 3% SiO_2 priming and can be suggested for chilli. Therefore, seed priming with 2.5% KNO_3 and 3% SiO_2 can be suggested for water stress tolerance in chilli.

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Abstract

EFFICACY OF SEED PRIMING FOR INDUCING STRESS TOLERANCE IN CHIILI (*Capsicum annum* L.) UNDER WATER STRESS CONDITION

By

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(2020-11-37)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF SEED SCIENCE AND TECHNOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2023

ABSTRACT

A study entitled "Efficacy of seed priming for inducing stress tolerance in chilli (*Capsicum annum* L.) under water stress condition" was undertaken with the objective to evaluate the various seed priming treatments for water stress tolerance in chilli at the seedling stage and selected best treatments were evaluated for physiological and yield traits under pot culture study. Seeds of the chilli variety 'Vellayani Athulya' were primed with 2.5% potassium nitrate $(KNO)_3$, 3%silicon dioxide (SiO_2), 2.5mM salicylic acid (SA) and distilled water for 12 hours and subjected to three different stress levels *i.e.*, control, mild stress (5% - PEG 6000) and moderate stress (10% - PEG 6000) during seedling stage. Seed priming experiment was carried out in a factorial completely randomized design (CRD) with the first factor being priming using different solutions and the second factor being the stress levels which were replicated thrice.

The results of the first experiment noted that chilli seedlings responded distinctively to the different treatments. 2.5% KNO_3 under moderate stress recorded significantly higher proline content (0.24 µM) and enhanced seedling vigour index-I (SVI 1) compared to 3% SiO_2 and untreated treatments. The hydrogen peroxide (H_2O_2) concentration were significantly higher in mild stress with unprimed seeds (0.93 µM), wherein lower content was recorded in 3%SiO2 primed seeds (0.38 µM). Further, reduced malonaldehyde (MDA) content and increased seedling vigor index-II (SVI 2) performance were noted in both 2.5% KNO_3 and 3% SiO_2 treatments. Seeds failed to germinate under 2.5mM salicylic acid.

From the first experiment, 2.5% KNO_3 and 3% SiO_2 were selected for further study as both were on par with each other. Then the seeds primed with both treatments were sown in portrays. Seedlings were transplanted to pots 30 days after sowing (DAS) and irrigation and other cultural practices were followed according to KAU POP. Water stress was induced by withholding irrigation for four days where relative water content (RWC) of leaves reaches 70% during flower initiation stage. During this period, physiological parameters were taken from both the stress and control plants. The results of second experiment revealed that seeds primed with 3% SiO_2 recorded early flowering whereas, seeds primed with 2.5% KNO_3 recorded the first fruiting stage.

Under water stress conditions, seeds primed with 2.5% KNO_3 recorded significantly high values for relative water content (RWC) (67%), cell membrane stability Index (CMSI) (40.33%), total chlorophyll content (TCC) (1.807mg/g), catalase activity (CAT) (510 nmoles

of H_2O_2 used min/g weight of sample), superoxide dismutase (SOD) (492.6 Units/mg of protein), total soluble sugars (TSS) (1.573mg/ml) and total soluble protein (TSP) (1.073mg/ml) where 3% SiO_2 primed seeds recorded significantly higher values for total proline content (2.977µmoles of proline/g of tissue). For control conditions, 2.5%KNO_3 primed seeds recorded significantly high values for relative water content (RWC) (94%), total chlorophyll content (TCC) (4.577mg/g), catalase activity (CAT) (200 units/min/g weight of sample), peroxidase activity (POD) (0.135 units/min/mg of protein), superoxide dismutase(SOD)(400 units /mg of protein) total soluble sugars (TSS) (3.347 mg/ml), total soluble protein (TSP) (2.012 mg/ml) and 3% SiO_2 primed seeds recorded significantly higher cell membrane stability index (CMSI) (91.66%), total proline content (1.307 µmoles of proline/ g of tissue).

Under stress condition, capsaicin content was recorded high in seeds primed with 2.5% KNO_3(0.022%) and ascorbic acid was recorded highest in seeds primed with 3% SiO_2(393.567mg) Whereas, in control both capsaicin content (0.129%) and ascorbic acid content (483.083mg) were recorded higher in seeds primed with 2.5% KNO_3.

Under stress condition, the yield components like plant height(57cm), number of fruits/plant (16 no), fruit yield (44.293g), dry matter (31.6g) and root volume(42cm^3) were recorded as significantly higher in seeds primed with 2.5% KNO_3. Further, the number of seeds/fruit (41 no) and seed yield (3.067g) were recorded as significantly higher in seeds primed with 3% SiO_2 and the number of flowers/plant (22 no) were found highest in unprimed seeds whereas, in control condition, 2.5% KNO3 primed seeds recorded significantly higher plant height(58.66cm), fruit yield (86.723g), root volume (69 cm3) and dry matter (46.22g) and 3%SiO2 primed seeds recorded significantly higher number of flowers/plant (33) and seed yield (4.117g). Both 2.5% KNO_3 and 3% SiO_2 are on par with each other for number of fruits/plant (27), while SiO2 and unprimed seeds are on par to each other for no. of seeds/fruit (74.667).

Among the seed priming treatments, 2.5% KNO_3 was found to be an effective treatment under water stress conditions as it shows enhanced antioxidants levels, with high water absorption capacity, increased chlorophyll content, soluble sugar and protein content along with the high capsaicin and ascorbic acid content with increased plant height, number of fruits, fruit yield, root volume and dry matter where 3%SiO_2 primed seeds recorded high proline, peroxidase activity, ascorbic acid content, no of seeds/plant and seeds yield/plant under water stress conditions. For seed production, 3% SiO_2 priming method can be suggested for chilli.

Therefore, seed priming with 2.5% KNO_3 and 3% SiO_2 can be suggested for water stress tolerance in chilli.