ROLE OF PHYTOHORMONES IN MITIGATING WATERLOGGING STRESS IN SOYBEAN

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ROLE OF PHYTOHORMONES IN MITIGATING WATERLOGGING STRESS IN SOYBEAN

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CERTIFICATE

*This is to certify that the thesis entitled **"ROLE OF PHYTOHORMONES IN MITIGATING WATERLOGGING STRESS IN SOYBEAN"** submitted to the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) in AGRONOMY**, embodies the result of a piece of bonafide research work carried out by **TONUSREE SAHA,** Registration No. **14-05867** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

Dated:
Place: Dhaka, Bangladesh

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The Author

ROLE OF PHYTOHORMONES IN MITIGATING WATERLOGGING STRESS IN SOYBEAN

ABSTRACT

Waterlogging is a devastating environmental stress for soybean. A pot experiment was conducted at Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during kharif-II season from August to November, 2019 to find out the time-dependent responses of morpho-physiological, anatomical and biochemical attributes of soybean (*Glycine max*) to waterlogging stress and the role of foliar application of phytohormones in mitigating the adverse effects of waterlogging condition. The experiment was conducted following completely randomized design (CRD) with three replications consisted of 12 treatments as T_1: control, T_2: 0.5 mM salicylic acid (SA), T_3: 0.1 mM kinetin (KN), T_4: waterlogged for 3 days, T_5: waterlogged for 3 days + 0.5 mM SA, T_6: waterlogged for 3 days + 0.1 mM KN, T_7: waterlogged for 6 days, T_8: waterlogged for 6 days + 0.5 mM SA , T_9: waterlogged for 6 days + 0.1 mM KN, T_{10}: waterlogged for 9 days, T_{11}: waterlogged for 9 days + 0.5 mM SA, T_{12}: waterlogged for 9 days + 0.1 mM KN. Plants waterlogged for 3 days showed fewer damaging effects than 6 and 9 days of waterlogging. Leaf number, branch number, shoot length, root length, fresh weight and dry weight of 3 days waterlogged plants were higher than 6 and 9 days of waterlogging. Due to the exogenous application of SA and KN, the number of leaves, the number of branches, shoot length, root length, adventitious root were significantly increased. The number of pods per plant, seed yield and 1000-seed weight were higher due to exogenous application of salicylic acid and kinetin than waterlogging condition. Proline (Pro), malondealdehyde (MDA), H_2O_2 and electrolyte leakage (EL) increased under the waterlogging condition, which indicates higher oxidative stress. However, exogenous application of SA and KN significantly enhanced plant morpho-physiological and anatomical features and decreased Pro, MDA, H_2O_2 content than the waterlogged plants without phytohormone supplementation. Thus, the study indicates that the foliar spray of SA and KN is an effective approach in improving the growth and reproductive stage against waterlogged conditions.

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ABBREVIATIONS AND ACCRONYMS

ABA	Abscisic acid
ANOVA	Analysis of variance
BARI	Bangladesh Agricultural Research Institute
BBS	Bangladesh Bureau of Statistics
Chl	Chlorophyll
CV	Co-efficient of variance
Cd	Cadmium
DAS	Days after sowing
DW	Dry weight
DHAR	Dehydroascorbate reductase
EL	Electrolyte leakage
et al.	*et alibi* (and others)
FAO	Food and Agriculture Organization
FW	Fresh weight
FAOSTAT	Food and Agriculture Organization Statistics
Gly I	glyoxalase I
GPX	Glutathione peroxidase
H_2O_2	Hydrogen peroxide
i.e.	That is
J.	Journal

ABBREVIATIONS AND ACCRONYMS (Cont'd)

LSD	Least significance difference
MDA	Malondialdehyde
$^{1}O_2$	Singlet oxygen
Pro	Proline
PSII	Photosystem
RCBD	Randomize completely block design
ROS	Reactive oxygen species
RuBisCo	Ribulose -1, 5-bisphosphate caroxylase or oxygenase
RWC	Relative water content
WL	Waterlogging
%	Percentage
t	Ton
°C	Degree Celsius
µg	Microgram
cm	Centimeter
g	Gram
ha	Hectare
kg	Kilogram
m	Meter
mg	Milligram
mM	Millimole

Chapter I

INTRODUCTION

Waterlogging is a vital problem to crop production due to global climate change. Erratic pattern of rainfall, improper drainage practices, heavy soil texture and uneven land surface are also responsible for unwanted waterlogging. The consequences of waterlogging stress are disappointing like other environmental stresses as the degree of stress varies not only with plant species, but also with temperature, soil quality, plant age, humidity, stress period etc. If the stress becomes very high and/or persists for an extended period of time, it may result in an inpatient metabolic load on the cells, decrease growth and cause plant death in extreme conditions (Hasanuzzaman *et al.*, 2017). In many Asian countries, waterlogging stress is a common problem during the rainy season. Bangladesh is vulnerable to flood due to the presence of a monsoon season, which causes heavy rainfall. Our farmers mainly grow soybean during kharif season when a flash flood is common. Due to waterlogging, farmers face various problems during cultivation. Waterlogging consequences in various morpho-physiological and anatomical changes such as, leaf senescence, chlorosis, necrosis, stunted growth, nutritional imbalance, flower abortion and fruit abortion in plants that are very harmful to plant life cycle and the ultimate result is yield loss (Asharf, 2012; Hasanuzzaman *et al.*, 2016).

Waterlogging is the most hazardous abiotic stresses, which cause a significant reduction in the development and yield of crops. Waterlogging decreases plant available oxygen and lack of oxygen hampers plant root respiration. Oxygen deficit condition hampers root permeability and also causes root injury (Prasanna and Rao, 2014). In plant hypoxia condition (deficiency of oxygen) is created due to a short-term waterlogging of soil and consequently, when waterlogged for a longer time, plant roots are exposed to anoxic conditions (lack of oxygen). Waterlogging, as an abiotic stress, accumulate the excess amount of ROS in various forms and various subcellular compartments. Several researchers have reported the greater formation of H_2O_2 and increased MDA under waterlogging (Hasanuzzaman *et al.*, 2012; Sairam *et al.*, 2011; Kumutha *et al.*, 2009). The ROS generation disrupted membranes stability and reduced PSII output during waterlogged. The MDA and H_2O_2 levels were highest in the root of plants under anaerobic conditions. Plant develops different morphological (adventitious root development and elongation of the stem), anatomical (aerenchyma formation) (Wei *et al*. 2013). The most important physiological adaptive features are a well-balanced antioxidant protection mechanism that makes it easier to scavenge the damaging of ROS (increase antioxidative enzymes) under excess water.

Soybean *Glycine max* L. is considered as a versatile plant as because it can produce a plenty amount of protein and oil in both temperate and tropical environmental conditions. Soybean is a promising crop in Bangladesh, because climatic and soil conditions are both favorable for its cultivation. It can be grown both in rabi and kharif season. As soybean is moderately drought, tolerant it can be grown successfully in the summer season under rainfed condition. It is the second-largest source of edible vegetable oil and largest source of protein which is used for human nutrition, animals feed and biofertilizer for crops. Soybean seed contains 18-24% oil, 36-40% protein, 26-34% carbohydrates, 5% ash and 58% minerals. The yield of soybean in Bangladesh is found lower compared to that in other countries due to biotic and abiotic factors. Now-a-days, Soybean area and production are increased to 80 thousand hectares and 152 thousand metric tons (MT), respectively (USDA, 2018). Soybean plant cannot tolerate waterlogging condition and continuous heavy rainfall is also harmful for their growth. Many scientists showed the morpho-physiological, anatomical and biochemical responses of soybean under waterlogging stress. Waterlogging impacts on soybean seed germination, growth and development reduction of yield ultimately. Waterlogging stress takes a 2^{nd} position in abiotic stresses, which causes a significant loss in soybean production (Valliyodan *et al*., 2017). Waterlogging reduced plant height, stem diameter, Chl content in leaves, root FW and adventitious root number and the reduction of leaf number, leaf area and branch number during waterlogging stress as a result decrease photosynthetic activity and accumulation of starch granules (Mutava *et al*., 2015). It also showed that the N fixation also declines as a result of reduction in total biomass. The reduction of CO_2 assimilation photosynthesis rate significantly decreased (Miao *et al*., 2012). Due to waterlogging at the vegetative stage 17% to 43% and reproductive stage 50% to 56% yield decline was observed (Mustafa and Komatsu, 2014). Waterlogging influences the fermentation process, increases oxidative stress and also responsible for lowering

ATP level in the roots of soybean (Da-Silva and Do Amarante, 2020). Waterlogged plants receive nitrate nutrition, which partially restored ATP levels. As an increased accumulation of ATP and decreased production of ROS from fermentation and soybean tolerance to waterlogging stress. N supply and NO accumulation reduce the amount of % $O_2^{\bullet-}$, H_2O_2 and MDA in waterlogged roots of soybean (Da-Silva *et al.*, 2017; Duarte *et al.*, 2019; Souri *et al.*, 2020). Under the waterlogging condition, adventitious roots are formed, which is considered as one of the most important adaptation responses of plants. In soybean, adventitious roots under waterlogging showed high rupture of cortex cells, creating aerenchyma used as pores for O_2 transfer to roots, being a plant survival mechanism under these conditions (Beutler *et al.*, 2014). Adventitious roots are formed where aerenchyma cells are generated in the stem for getting available oxygen (Suralta and Yamauchi, 2008).

Waterlogging stress threats for crop production, numerous investigations have made and attempts have taken to mitigate the hazardous effect of waterlogging stress by phytohormones. Phytohormones are a group of naturally occurring organic substance which influences physiological process at low concentrations. Plant growth hormones alleviate the harmful effects of stress. Phytohormones help plants to sustain under stress conditions by producing different types of signals. Phytohormones, especially auxin, kinetin and salicylic acid have long been implicated in the growth and development of plants. Salicylic acid is a phenolic compound which is naturally produced in plants. It is also an important signaling molecule by which regulates several physiological processes such as growth, photosynthesis, N metabolism, pro metabolism, the formation of antioxidant enzyme production are regulated in plants during stresses (Khan *et al.*, 2012; Miura and Tada, 2014). Several studies supported the vital role of SA in modulating the responses of plants to various naturally occurring stresses (Hayat *et al.*, 2010; Kadioglu *et al.*, 2011). In addition, it has been found that plants treated with SA showed better resistance to waterlogging stress (Mishra *et al.*, 2013). However, foliar application of SA increased adventitious root development and aerenchyma cells in soybean (Kim *et al.*, 2018; Yang *et al.*, 2013; Brodersen *et al.*, 2005). Salicylic acid increased antioxidative mechanism, which reduced cell death by declining MDA accumulation, stimulated seedling development, increased root activity, increased photosynthetic pigments in soybean plants under waterlogging. Salicylic acid application enhanced the rate of assimilation, net photosynthetic rate, internal CO_2 concentration and ultimate result increased yield (Zamaninejad *et al*., 2013). However, KN is a synthesized cytokinin that regulate cell division, apical dominance and reproductive development, the uptake of nutrients, assimilation into sink organs, delay senescence and also improving metabolism and transport facilities in plants (Brugière *et al*., 2008; Ahanger *et al*., 2020). Kinetin also increased pod number by increasing axillary branches, increased flower setting by delayed leaf and flower senescence and ultimately increased yield (Atkins *et al*., 2011). Previous studies showed that KN significantly increased seed yield by improving grain filling and maximum endosperm cell number and enhancing the sink capacity, resulting in more assimilate accumulation (Yang *et al*., 2016). After numerous investigation KN is a feasible one in which enhancing growth and inducing tolerance mechanism against waterlogging stress (Aldesuquy *et al*., 2014; Wang *et al*., 2015; Ahanger *et al*., 2018, 2020). From the above studies, we can see that several scientists work on responses of different crops to different plant hormones under waterlogging. Soybean is a major oil crop and is mainly cultivated during kharif season. Therefore, we think that soybean will perform well if we apply phytohormones under waterlogging.

Considering the circumstances stated above and based on the available literatures, this study was undertaken with the following objectives:

i. To investigate the waterlogging-induced morpho-physiological and yield damages in soybean plants.
ii. To study the oxidative stress under waterlogged condition.
iii. To investigate the role of phytohormones in mitigating waterlogging stress-induced damages.

Chapter II

REVIEW OF LITERATURE

2.1 Soybean

Soybean [*Glycine max* (L.)] is one of the most versatile, nutritional and economic leguminous plants in the world. It is the second-largest source of edible vegetable oil and the largest source of protein, which is used for human food, animal feed and biofertilizer for crops. Soybean seed contains 18-24% oil, 36-40% protein, 26-34% carbohydrates, 5% ash and 58% minerals. Soya seed accounted for 60% of the overall output of oil seed in the world. In South America soybean is a major crop and covered about 63% of the total cropped area (Hasanuzzaman *et al*., 2012; Sugiyama *et al*., 2015; Wingeyer *et al*., 2015). Soybean is the main cultivated crop of Brazil and the country is the first largest producer in the world. USA, Brazil, Argentina, China and India dominate the production of soybean worldwide. The United States and Brazil was leading global producer of soybean with production volume respectively 120.52 million MT and 124 million MT in 2019; China (4^{th}) and India (5^{th}) are the top soybean production country in Asia after the USA (STATISTA, 2019). In Soybean area and production are increased to 80 thousand hectares and 152 thousand metric tons (MT), respectively (USDA, 2018).

2.1.1 Plant characteristics

Soybeans are considered to be a self-pollinating legume, and as a result, insects are not required to pollinate. Soybean primary stem is erect, consists of thin parenchyma cells wall lacking chloroplasts with vascular bundles and epidermis. Vascular bundles of the stem are collateral type and aerenchyma is found during the waterlogging condition. Soybean root consists an outer layer of the epidermis and epidermal cells from root hair after 4 days of germination. Soybean roots form nodules as a result of mutualism between the plants and microbes present in the soil. During waterlogging condition adventitious root is found. Flower colors are white, pink, purple with a corolla which is 5 to 7 mm long (Giller and Dashiell, 2007). Fruits contain two or three small rounded seeds with yellow color and black hilum (Koivisto, 2006). The young pod's wall consists of epidermis with varying trichome density. According to BARI annual report, the range of plant height is 18-28 cm, flowering 42-47 days, the number of pod per plants 5-11, 100 seed weight 10-12 g, maturity 92-125 days.

Soybean plant is moderately salt-sensitive, intolerant to waterlogging, sensitive to low or high temperature as a result, germination decreased, growth and yield also decline. (Anto and Jayaram, 2010; Simaei *et al*., 2012). Ezin *et al*. (2010) seen adventitious root in tomato and Herzog *et al*. (2016) seen well-developed aerenchyma tissue formation in stem and roots in rice and wheat, which help to tolerate under waterlogging for a certain period. Liu *et al*. (2020) proposed ideal plant characteristics for soybean green revolution are appropriate plant height (resistant to lodging), short internode, more number of internode, no or a few branches, moderate pod number per node, higher podding rate, higher ratio of four seed per pod, moderate 100-seed weight, small petiole angle and short petal length.

2.1.2 Ecological requirements

Liu *et al*. (2008) reported that the optimum condition for germination of soybeans is 15–22°C. The germination percentage, moisture content, seedling vigor, pod number and total biomass reduced due to increase temperature gradually. Under cold stress its growth and symbiotic activities decreased. Youn *et al*. (2008) recorded that under cold stress in soybean reduced above DW 75-77%, below DW was up to 64-75%, decreasing the number of nodules about 68-73%, seed DW decreased up to 69% varies on genotype.

Pod setting is a critical process for the production of soybean. Temperature fluctuation (low or high temperature) is sensitive to pod setting process. Poor pod setting under low temperature stress due to insufficient pollination. Anomalous pollen grain causes tetrad like pod was observed at temperature stress (Ohnishi *et al*., 2010).

Nguyen *et al*. (2012) mentioned that the waterlogging caused yield reduction of soybean at the vegetative stage (17%) and reproductive stage (50%). Extension of waterlogging duration gradually reduced the above DW, below DW, root development, nodule number. USA, Brazil, Argentina, China dominate soybean production in the world. The leading producer of soybean in Brazil and Argentina practice intensive monoculture of soybean and also responsible for deforestation as a result negative effects on biodiversity and agro-ecosystem. The soil erosion rate increases for using mechanical weeding and intensive cultivation resulting in extreme soil fertility mining and disturbs microbial activity (Steinfeld *et al*., 2006; Wall and Nielsen, 2012).

No tillage causes an adverse impact on soil nitrogen, moisture, temperature, soybean nodulation, emergence, growth and development and ultimately decline production of soybean. Crop rotation also required to properly utilized the soil nutrient. Increased soybean yields are needed to investigate certain environmental factors, sufficient quantities of nutrients and water and better management of pests and prevent disease. Nitrogen fixation increased in soil by enhancing the microbial functions and increasing interactions between soybean and microorganism (Vanhie *et al*., 2015).

2.2 Abiotic stress in plants

Plants need the availability for high production of optimum environmental conditions and nutritional factors. When plants face any adverse environmental conditions during growing season and reduction of crops productivity is called as abiotic stress. Abiotic stress is the greatest limitation on crop production worldwide and the reduction of yields around 50 per cent (Rodríguez *et al*., 2005; Acquaah, 2007). The effect of stress depends on types of stressor, duration, genotype, environmental conditions, growing stage of plants. Air temperature became stressful within a few moments (Taiz and Zeiger, 2006).

There are a number of abiotic stresses such as salt stress, water stress and temperatures stress (high/low), heavy metal/metalloids, ozone, UV radiation, high/ low light, nutrient deficiency etc. which all assert significant indictment to plant development, metabolism, and yield capacity. Durations, occurrence and intensity is quietly unpredictable of the stress. Plant growth and physiological metabolism are negatively affected by abiotic stress conditions. If the stress becomes severe and/or ongoing for prolonged period, this can conduct to a toxic metabolic poise on cells, decreasing growth, and in acute condition, result in plant decease (Hasanuzzaman *et al*., 2014). Higher accumulation of ROS is a very common phenomenon under environmental stresses and results from wretched electron transport systems in the chloroplasts and mitochondria (Hasanuzzaman and Fujita, 2013).

Kooyers (2015) reported that plants can perceive even the lowest environmental stress signal and the most sensitive reproductive stage. Plants use different mechanisms to reduce the severity of abiotic stress but reproductive stress is more sensitive to stress. As a result, decrease the production and significant economic losses.

2.3 Effect of waterlogging/flooding on crop plants

2.3.1 Effect on growth

The state of waterlogging significantly affects the plant growth, production and survival in different ways. Seedlings of *Muehlenbeckia florulenta* Meisn. during waterlogging causes senescence of the leaves, chlorosis, necrosis, wilting, stunted growth and disease-prone plants and pest infestation (Capon *et al*., 2009).

Waterlogging stress was reported to reduce specific leaf area and leaf areas by 26 and 36%, respectively and increased specific leaf weight by 22% compared to control in quinoa (*Chenopodium quinoa* Wild.) plants and also observed lower dry weight of root, stem and leaf in waterlogged plants (González *et al*., 2009).

Tolerant and sensitive type of mung bean (*Vigna radiata*) genotypes including T 44 and MH–96–1 (tolerant) and Pusa Baisakhi and MH–1K–24 (sensitive) were experimented by Kumar *et al.* (2013) for waterlogging induced changes. Thirty-day old plants were waterlogged for 3, 6 and 9 days. They observed that waterlogging reduced the leaf surface, the growth rate of crops, development of roots and nodulation capacity in all plants where tolerant plants showed lower reduction of these parameters. Different stages of summer maize were studied under waterlogging condition by Ren *et al*. (2014). Waterlogging conditions produced during the 3rd leaf stage, 6th leaf stage and 10th days after the maize tasseling stage for 3 and 6 days. The finding after 2 years of study showed that waterlogging significant impacted on the overall growth and production of summer maize.

Prasanna and Rao (2014) showed the remarkable variation on growth measuring factors of green gram as a result of waterlogging. Due to waterlogging during the life cycle shoot length, leaves number, flower number and total biomass were significantly decreased. The effect of waterlogging for 4 days was more serious compared to waterlogging treatment for 2 days over the unstress plant. In waterlogged plants, shoot length, leaves number and total biomass were reduced by 30–34% compared to control.

Luan *et al.* (2018) conducted an experiment on seven genotypes of barley to investigate morphological responses of sensitive and tolerant variety under waterlogging. Shoot length, SPAD value, tiller number in Naso Nijo and TF57 showed drastic decrease due to waterlogging, where the TX9425 and TF58 had less changes. Fresh weight of shoot substantially lowered in Naso Nijo, YYXT and TF57. The dry weight of root, all genotypes of the barley except for TF58, showed a significant decline.

Anee *et al*. (2019) conducted an experiment on sesame plants (*Sesamum indicum* L. cv. BARI Til-4) to investigated the effects of waterlogging where treatments were waterlogging for 2, 4, 6, and 8 days during the vegetative stage. The reduction of relative water content and photosynthetic pigment content were reduced as the waterlogging duration increased. The lower reduction of RWC was observed 2 days waterlogged and the highest reduction was observed 8 days waterlogged (75%) where control was 90% RWC. In stressed plants the content of chlorophyll (Chl) and carotenoid also decreased over time.

2.3.2 Effect on plant physiology and metabolism

Kumutha *et al*. (2009) recorded that the waterlogging stress on pigeon peas reduces the area of the leaves and accelerates the leaf senescence of the leaves by reducing the total content of Chl in leaves, thereby restricting the successful photosynthesis cycle and resulting in a major reduction in crop production. Under waterlogging, the phytology and catabolism of plants are disrupted. Restricted stomatal conductance, the transition of gases, metabolism of CO_2, and root hydraulic conductivity are some of the key results in waterlogged plants. Reduction of CO_2 entering the leaf which reduced transpiration leading to wilting of the leaves and decreased Chl content as a result lower dry matter accumulation (Ashraf, 2012).

Waterlogging results depends on the genotype, environmental factors, stage of growth and the period of waterlogging. Excessive waterlogging concequence is lack of oxygen which reduced root respiration, photosynthesis and CO_2 assimilation (Li *et al*., 2011; Prasanna and Rao, 2014). SPAD value, associated photosynthetic enzymes and photochemical proficiency of PSII reducing with expansion waterlogging time, resulting in a crucial reduction in output (Ren *et al*., 2014; Mano and Omori, 2015).

Anandana *et al*. (2015) reported certain damage to rice plant under prolonged waterlogging stress. *Oryza sativa* cv. Puzhuthiikar showed significant improvement in the length of the leaf blade, sheath length but the reduction of leaf blade area. As a result, photosynthesis rate, transpiration rate and intercellular CO_2 in rice plants have been increased. Tian *et al*. (2019a) also showed that the SPAD value significantly reduced with increasing the waterlogging duration. The highest SAPD value found at tasseling stage and the lowest SPAD value was at seedling stage for 9 days waterlogging.

Tian *et al*. (2019b) showed that the most important effects of photosynthetic enzymes occurred at the seedling, jointing and tasseling phase. The activity of the photosynthetic enzyme was significantly reduced with prolonged of waterlogging duration. KY16 and DMY1 RuBP carboxylase activities were reduced by 54.07% and 49.83% after waterlogging for 9 days and 52.92% and 51.06% after subsurface waterlogging for 15 days at the seedling stage in contrast to control.

2.3.3 Effect on plant anatomy

One of the main core stresses of waterlogged or flooded is oxygen deficiency in the root zone. Jiang and Wang, (2006) to investigate the anatomical change and showed that the development of aerenchyma was increased at waterlogged 15 cm and 5 cm. Within waterlogging, mitochondrial swelling occurred, especially at waterlogged 1 cm. Partial waterlogging at 15 cm and 5 cm may have a crucial impacts on the development of turf grass and physiological activities. Plant lenticels are thought to be engaged in the downward transfer of oxygen and various anaerobic metabolism out growth (ethanol, CO_2 and CH_4) within the plants. But, the concrete physiological function of lenticels is ambiguous; their existence is related to plants in waterlogging tolerance (Parelle *et al*., 2006). Plants under flooding/waterlogging conditions show many anatomical changes. Under waterlogging period, plants form adventitious root, lenticels hypertrophy and/or aerenchyma (Ashraf, 2012) adjustment to adverse conditions.

Ezin *et al*. (2010) seen adventitious root in tomato and Herzog *et al*. (2016) and seen well-developed aerenchyma tissues formation in stem and roots in rice and wheat, which help to tolerate under waterlogging for a certain period.

When plants are exposed to waterlogging, chloroplasts are easily damaged (Ren *et al*. 2016) and aerenchyma formation in shoots is a feasible anatomical method for screening waterlogging tolerance in maize and barley (Yamauchi *et al*., 2014; Zhang *et al*., 2016). Abd El-Aal and Rania, (2018) showed that the thickness of the upper epidermis, lower epidermis, palisade tissue, spongy tissue, blade thickness, upper collenchyma layers thickness, lower collenchyma layers thickness, phloem thickness, xylem tissue thickness, the number of xylem rows, thickness of widest xylem vessel, the length of midrib vascular bundle, the width of midrib vascular bundle and the thickness of leaf midrib were increased with the application of a different dose of lithovit (250 and 500 mg L^{-1}) and amino acids (2 and 4 ml L^{-1}) compared to untreated plants.

Luan *et al*. (2018) an experiment conducted on seven genotypes of barley to investigate anatomical responses sensitive and tolerance variety under waterlogging. Adventitious roots length, diameters, total surface area among seven barley genotypes is highest in TF58. In the tolerant genotypes, greater areas of aerenchyma were found under waterlogging stress compared to the control where were no major changes in aerenchyma. In control broad aerenchyma could be found in the middle of mesophyll cells and due to deterioration of lower mesophyll cells under waterlogging condition aerenchyma was more important.

2.3.4 Effect on nutrient availability

Flood water carries a lot of nutrients into the ecosystem and as a result high productivity of these area. During flooding soil physical and chemical properties changed. Due to waterlogging microbial populations and activities are inhibited. Waterlogging increases phosphate concentrations due to bacterial transformations (Lamers *et al*., 2006). Changes in phosphate levels and often lower nitrate levels occur simultaneously due to their denitrification loss or ammonium reduction. NO_3^- nitrification production is inhibited because there is insufficient oxygen availability. Potassium and iron availability is also affected by waterlogging. When iron concentrations may increase, potassium concentration decreases due to an exchange of soil particles (Antheunisse and Verhoeven, 2008). Waterlogging create adverse effects on numerous biochemical and morpho-physiological system of crops by inducing insufficiency of essential nutrients (Ashraf, 2012). Akhtar and Nazir, (2013) also reported flooding negatively affected plant growth and macro and micronutrients uptake. Stress can reduced nutrient intake under stress the growth of various plant parts including the roots and the aerial part is adversely affected as a result of less plant nutrient intake and ultimately reduces plant growth (Miransari, 2014; Li *et al*., 2015).

2.3.5 Effects on yield

The largest loss of biomass at maturity under waterlogging condition (Miralles and Slafer 2007; Arisnabarreta and Miralles 2008). The yield declines of barley due to reducing the number of grain, tiller contribution, and spike number per plant.

Specific evidence showed that the number of grain in barley was greater than in wheat because of the largest number of spikes (Alzueta *et al*., 2012).
Tolerant and sensitive type of mungbean genotypes including T44 and MH–96–1 (tolerant) and Pusa Baisakhi and MH–1K–24 (sensitive) were experimented by Kumar *et al.* (2013) to show the yield loss were 20.01%, 33.79% and 51.88%, respectively for 3, 6 and 9 days waterlogging. Tolerant cultivar could recover yield loss but sensitive cultivars 20% yield loss recorded at 3 days waterlogging. Lower yield loss in tolerant cultivars under 9 days waterlogged where sensitive cultivars showed 70% to 84.9% yield loss.

De San Celedonio *et al*. (2014) also showed that yield loss 34 to 92 % in wheat and 40 to 79 % in barley for 20 days waterlogging and 15 days waterlogging, respectively compared to control. Waterlogging stress also reduced total shoot biomass at immediately prior to anthesis. The greatest reductions of biomass at maturity from leaf 7 to leaf 10. The reduction rate from 24 to 66 % for wheat and from 34 to 60 % for barley. In waterlogging the maximum reductions yield was observed at anthesis to physiological maturity and from leaf 10 to anthesis (L10-At) in the waterlogging period was 20 days in early sowing and 15 days in late sowing.
In KPSI and CNXP-49, seed yield was reduced by 16 and 19%, respectively in vegetative stage of waterlogging and 23% and 30%, respectively in reproductive stage (Ahmed *et al*., 2015). Tian *et al*. (2019) investigated the effects of waterlogging on maize yield. The lowest dry weight of stem, leaf and ear number was recorded at the seedling stage, followed by the jointing stage and tasseling stage. In contrast, to control plants maximum seed yield was reduced in DMY1 and KY16 (around 64.8% and 80.2%) for 9 days under waterlogging stress at seedling stage. Similarly, under subsurface waterlogging for 15 days the grain yield of DMY1 and KY16 decreased by 61.5% and 71.9%, respectively at the seedling stage. Waterlogging and subsurface waterlogging had the least impact on the grain yield at the tasseling stage. The grain filling greatest reduced at the seedling stage for 9 days waterlogging and subsurface waterlogging for 15 days followed by the jointing and tasseling stage.

2.4 Waterlogging induced oxidative stress and antioxidant defense system in plants

2.4.1 Oxidative stress under waterlogged condition

Waterlogging, as an abiotic stress accumulate excess amount of Reactive oxygen species (ROS) in different forms and different subcellular compartments. These ROS include both free radicals ($O_2^{\bullet -}$ and $OH^{\bullet}$) and molecules such as H_2O_2 and $^1O_2$ (Jaspers and Kangasjärvi, 2010). Several researchers have found that the accumulation of H_2O_2 and MDA increased under anaerobic conditions (Kumutha *et al.*, 2009; Hasanuzzaman *et al.*, 2012; Sairam *et al.*, 2011). Kumutha *et al.* (2009) conducted an experiment on pigeonpea genotypes ICP 301 (tolerant) and Pusa 207 (susceptible) in waterlogging for 4 days and 6 days. $O_2^{\bullet -}$, H_2O_2 and thiobarbituric acid reactive substances (TBARS) increased in 4 days and 6 days waterlogging compared to control plants. Antioxidant enzymes increased under waterlogging. Due to higher antioxidant activities, ICP 301 was more tolerance than Pusa 207 in waterlogging.

Reactive oxygen species generation disrupted membranes stability and reduced PSII output during waterlogged. The amount of MDA and H_2O_2 were highest in *Vigna sinensis* root under waterlogging stress. At 30 days in *V. unguiculata* 30 days MDA content increased by 32% and H_2O_2 by 43% at the root (El-Enany *et al*. 2013).

Higher content of $O_2^{\bullet -}$ and H_2O_2 and MDA were reported with increasing waterlogging and subsurface waterlogging period at seedling stage, jointing stage and tasseling stage of maize DMY1 and KY16 varieties. Among the stages significantly increased at the seedling stage for 9 days waterlogging and 15 days subsurface waterlogging in both varieties. In DMY1 $O_2^{\bullet -}$ content increased (170.60% and 157.92%), H_2O_2 content increased (117.68% and 102.16%), MDA increased (72.59% and 31.65%) and KY1 $O_2^{\bullet -}$ content increased (191.96% and 163.19%), H_2O_2 content increased (134.27% and 126.24%), MDA increased (104.47% and 72.62%), respectively waterlogging for 9 days and subsurface waterlogging for 15 days. Antioxidant enzyme activities increased in plant cells under waterlogging and subsurface waterlogging stress which reduced the injury of plants caused by ROS (Tian *et al*., 2019).

Anee *et al*. (2019) conducted an experiment on sesame plants, and treatments were waterlogging for 2, 4, 6, and 8 days during the vegetative stage where the highest MDA and H_2O_2 contents were recorded at 8 days waterlogging. MDA level was 39% higher than control and similar results were recorded at 6 days of waterlogging.

2.5 Soybean crop responses to walerlogging/flooding

2.5.1 Effect on growth

Cho and Yamakawa (2006) showed the number of leaves, branch number, nodulation significantly reduced due to waterlogging. It also showed that the N fixation also declines as a result of reduction in total biomass. Reduction of CO_2 assimilation photosynthesis rate significantly decrease, ultimately decrease dry weight. During reproductive stages (beginning flowering, pod formation) waterlogging decreased shoot, dry matter, grain filling and early reproductive stage 67% yield loss observed (Youn *et al*., 2008).

Miao *et al*. (2012) recorded that nodule number significantly decline in Hefeng 50 and Kenfeng were 84% and 64% respectively, compared to control. Waterlogging also decline the weight of nodule as 73%, 77% for Hefeng 50 and 71%, 49% for Kenfeng 16 at pod bearing and grain filling stage, respectively. As a result, reduction of N fixation and less accumulation of N in cells which was reduced the shoot weight and total biomass.

Beutler *et al*. (2014) conducted an experiment on soybean under waterlogging for 2, 4, 8, 16 and 32 days at flowering stage (R_2) and grain filling stage (R_5) where found shoot length, shoot diameter, leaf surface, branch number significantly reduced with an extension of waterlogging duration and the highest reduction occurred 32 days waterlogging at R_2 and R_5 stages. The number of nodule decrease with increasing waterlogging duration as a result N accumulation significantly decline ultimate result reduction of shoot biomass and dry weight. Waterlogging causes 34% dry matter reduction was observed.

The soybean plant height, stem diameter, Chl content in leaves, below ground weight and adventitious root number and weight were significantly reduced with increased the duration of waterlogging. The reduction of leaf number, leaf area and branch number during waterlogging stress as a result decrease photosynthetic activity and accumulation of starch granules (Mutava *et al.*, 2015).

The global climate changes, insufficient drainage system and low infiltrationof soils could cause the waterlogging that impacts on reduction in soybean seed germination, growth and development thereby ultimately reduced yield (Valliyodan *et al.*, 2017). Wu *et al*. (2017) recorded the higher germination rate in waterlogging stress were fungicide-treated seeds rather than untreated seeds.

Kim *et al*. (2019) conducted an experiment on soybean to select resistance (PI408105A, GCS2368, GCS0017), susceptible (S99-2281, GCS3170, GCS2309) cultivars under waterlogging for 14 days. The Chl content, adventitious root number and weight in the first trifoliate leaf showed a significant difference between the resistant and susceptible varieties after 9 days of treatment. Waterlogging stress in vegetative stage decreased yield by 43%.

2.5.2 Effect on plant physiology and metabolism

Waterlogged condition responsible for decrease N fixation which affects soybean growth, physiology and metabolism as a result decline photo assimilation, photosynthesis and hamper nutrient uptake by plants (Davanso *et al.*, 2002). Transpiration, Ribilose-1 and 5-bisphosphate (RuBP) carboxylase activities decline under waterlogging observed by (Mutava *et al.*, 2015).

Borella *et al.* (2017) showed that under waterlogging stress, respiration metabolism changes and form toxic substances such as lactic acid, ethanol. Da-Silva and Do Amarante, (2020) showed that waterlogging increased fermentation and oxidative stress and also lowered ATP levels in the roots of soybean. Waterlogged plants receiving nitrate nutrition and as a result increased NO that stored ATP levels. As a increased accumulation of ATP and decreased production of ROS from fermentation and soybean tolerance to waterlogging stress. N supply and NO accumulation decreased levels of % $O_2^{\bullet-}$, H_2O_2 and MDA in waterlogged roots of soybean (Da-Silva *et al*., 2017; Duarte *et al*., 2019; Souri *et al*., 2020).

2.5.3 Effect on yield

Waterelogging is the responsible for significant yield reduction in world wide. It has been recorded about 25% of soybean yield loss and waterlogging at reproductive stage is mainly responsible for yield reduction in soybean. At the vegetative stage 17% to 43% and reproductive stage 50% to 56% yield decline was observed (Mustafa and Komatsu, 2014).

Rhine *et al*. (2010) conducted an experiment on clay and silt loam soils to investigate the pod formation and nutritional reactions of soybean five varieties under waterlogging (Manokin, P94B73, Mersch Denver, Delsoy 4710, DK4868) and waterlogged for 0, 2, 4, 6, 8 days at vegetative stage (V_5), flowering stage (R_2) or reproductive stage (R_5). The V_5 growth stage suffered less amount of yield losses and the largest yield losses recorded at the R_5 growth stage. In R_5 yield reduced by 20%-39% compared to control. P94B73 cultivar had no major yield improvement in the three stages for 8 days of waterlogging compared to control. N, P and K tissue concentrations decreased with the extension of waterlogging duration and significantly reduced N, P, K recorded for 8 days waterlogging in clay soil rather than silt loam soil. P94B73 cultivar produced the highest N in the leaves under waterlogged conditions, about 18–40% higher than other cultivars.

Miao *et al*. (2012) showed that waterlogging affects more at flowering and grain filling stages in soybean (Hefeng 50 and Kenfeng 16 variety) and as a result pod, filled-grain number reduced ultimate affected in significant yield loss. The grain yield loss 30% to 41% for Hefeng 50 and 15% to 44% for Kenfeng 16 compared to control.

Beutler *et al*. (2014) observed significant decline of soybean yield with extending waterlogging duration and they conducted an experiment with waterlogging for, 4, 8, 16 and 32 days at flowering stage (R_2) and grain filling stage (R_5). The reduction of yield at flowering and grain filling stages was 17% and 29% for 16 days, 41% and 36% for 32 days waterlogging respectively. It indicated that the highest yield loss was observed at grain filling stage.

2.6 Phytohormones

2.6.1 Role of different phytohormones on abiotic stress tolerance

Environmental stress has negative impacts on plant development and potential yield. External supplementation of phytohormones such as auxin, abscisic acid, ethylene, cytokinins, SA, brassinosteroids, and NO are important to resist the plant under stress and reduce the yield loss (Farooq *et al*., 2010; Hasanuzzaman *et al*., 2013). Sheteawi, (2007) recorded the supplementation of jasmonic acid of soybean at 1µM foliar spray, improved photosynthetic pigments, physiology, and yield under salt stress (50 and 100 mM NaCl) for 76 days. The effective result showed 100 mM NaCl and JA eliminated the harmful impacts of salinity. The application of methyl jasmonate at 20 and 30µM on soybean alleviation of salinity (60mM NaCl) for 14 days. It improved the growth and photosynthesis rate (Yoon *et al*., 2009).

The functions of gibberellins are germination, cell elongation and nodulation. Gibberellins enhanced germination, development and reduced yield loss under salt stress. Hamayun *et al*. (2010) showed that the application of Gibberellic acid GA3 (0.5, 1, 5 µM) increased Chl content and growth of soybean under salt stress (100 mM NaCl) for 14 days. It has been suggested that the external application of gibberellins can mitigate the harmful impacts of salt stress on the growth and yield of soybeans with balance other hormones level.

Abscisic acid helped to survive under drought stress of maize (Wang *et al*., 2008), wheat (Guoth *et al*., 2009) and rice (Tian *et al*., 2015). ABA can diminish salt stress in common bean and potato (Etehadnia *et al*., 2008). Under stresses like drought or salinity, ABA significantly increased and as a result, stimulates stomatal closure and adaptive physiological reactions (Cutler *et al*., 2010; Kim *et al*., 2010).

Plant hormones are the primary moderator of plant development and provide adaptive repercussion under abiotic stresses (Sreenivasulu *et al*., 2012). Anjum *et al*. (2013) also showed the external use of 50µM methyl jasmonate improved yield and the yield-contributing factor of soybeans under drought.

Alam *et al*. (2014) examined the function of jasmonic acid on the different mustard species to tolerance under drought stress. Foliar sprayed of JA on seedling increased glutathione reductase and glyoxalase I (Gly I) activities in *B. napus*; risen monodehydroascorbate reductase activity in *B. campestris;* and rise DHAR, GR, GPX, Gly I and Gly II functions in *B. juncea*. Jasmonic acid enhanced total biomass, Chl content, Relative water content in all species and dry weight high only in *B. juncea*. *Brassica juncea* had little oxidative stress under drought.

Abscisic acid is an isoprenoid phytohormone and acts as signaling mediator which controls stomatal opening, protein storage and helps plant to survive under different stress (Ng *et al*., 2014). The hormone can reduce of the hydraulic conductivity of leaves and roots in Arabidopsis by the downregulating of aquaporins in the sheath cells of the bundle (Li *et al*., 2014). In drought stress ABA regulate stomatal closure, reduces gas exchange resulting in reduction of photosynthesis (Mittler and Blumwald, 2015).

Brassinosteroid (BR) is able to alleviate the stresses by improving seed germination, growth and increased production of proteins, biomass yield. by producing proteins, BR was able to alleviate the rice seed germination under salt stress. Under salt stress in wheat, BR increased plant Chl content, shoot biomass and yield, but can not help to increase nutrients uptake (Eleiwa *et al*., 2011). Brassinosteroid helps maize seedlings to alleviate salinity stress by increased the activity of antioxidant enzymes and reduced ROS accumulation by decreasing MDA production (Zhang *et al*., 2016; Yang *et al*., 2016). Upreti and Murti, (2004) suggested that pretreatment of brassinosteroid (1 and 5 µM) under drought stress root nodulation of beans increased and pea plants (Ferguson and Mathesius, 2014).

Moumita *et al*. (2019) showed that the foliar application of gibberellic acid alleviates drought stress impair in *Triticum aestivum* 'BARI Gom-21' by reducing oxidative stress ($O_2^{\bullet-}$ and H_2O_2 content) and increasing antioxidant enzyme activities (catalase and ascorbate peroxidase).

2.6.2 Role of salicylic acid (SA) and kinetin on plants growth and development

Khan *et al*. (2003) observed the role of SA on the photosynthesis and growth of soybeans (C3) and corn (C4). The application of SA increased the photosynthesis but did not show any effects on Chl content.

The role of KN were delaying leaf senescence by inhibiting protein synthesis and rising photosynthesis rate, number of flowering and rising the pod setting (Yashima *et al*., 2005). It also increased the seed dry weight of soybeans (Liu *et al*., 2008) and increased the development of nodules (Ding and Oldroyd, 2009). Foo and Davies (2011) showed that KN increase nodulation and Sato *et al.* (2002) mentioned SA was not responsible for the formation of nodule. Nazar *et al.* (2011) showed that 0.1 mM and 0.5 mM concentration SA increased photosynthesis rate and development in mung bean but the concentration 1.0 mM prohibited growth and development of mung bean.

Salicylic acid regulates different roles in plant life cycle including germination, flowering, leaf senescence and induced plant resistance under abiotic stress conditions (Jibran *et al*., 2013).

Salicylic acid was occupied with maintaining plant photosynthesis rate, N metabolism, pro metabolism, formation of glycinebetaine and antioxidant enzyme production during stresses (Khan *et al.*, 2012; Miura and Tada, 2014). In rice (*Oryza sativa*) root system consist crown root which play a key role in rice growing. The application of cytokinin and auxin increased the grown of crown root in rice (Gao *et al*., 2014).

2.6.3 Role of salicylic acid, kinetin and other plant hormones on abiotic stress tolerance

External application of SA increase antioxidant enzymes activities of plants and increased resistance to different stresses by decling accumulation of ROS. Salicylic acid has different effects on stress adaptation, it depends on cultivars, concentration, method of application and duration (Metwally *et al*., 2003).

Salicylic acid is a signaling particle that induces tolerance of plants to various stresses (Horvath *et al*., 2007). Exogenous SA decreased the effects of drought stress in wheat (Waseem *et al*., 2006) and salinity stress in maize and wheat (Arfan *et al*., 2007).

Simaei *et al*. (2012) found the external insertion of SA (100μM) risen flavonoid content, decline Na uptake, Na/K ratio, lipoxygenase activity under salinity stress (100 mM NaCl) for 7 days. Bîrsan *et al*. (2014) recorded the SA spraying at small amount (0.01%) increased the adaptation by adjusting Pro level in soybean plants under drought stress for 14 days.

Alam *et al*. (2013) showed the responses of SA on mustard (BARI Sharisha 11) seedlings under drought stress. 2 set 10 days old seedlings kept in drought (10% and 20% PEG, 48h), where one set of seedlings applied 50 μM SA. Under drought stress increased Pro, MDA and H_2O_2 content was observed. But external supplementation of SA gave support plants to become more resistant to drought by reducing oxidative damage and enhancing their antioxidant mechanism.

Singh and Prasad (2014) found exogenous spray of KN to mitigate Cd toxicity in eggplant seedlings. Cd_1 (3 mg Cd kg^{-1} soil) and Cd_2 (9 mg Cd kg^{-1} soil) doses of Cd suppressed the growth of *eggplant* seedlings because Cd was reserved in roots and shoots. Kinetin at 10 μM diminished Cd toxicity by reducing Cd accumulation in plants. Kinetin increased Chl a, Chl b, carotenoids contents and decreased the oxidative stress by reducing $O_2^{\bullet-}$, H_2O_2 and MDA contents and increase antioxidant enzyme. So, the external supplementation of KN increased physiological and metabolic activity of eggplant even in presence of toxic level of Cd by increasing the antioxidant mechanism and photosynthesis rate.

Hamayun *et al*. (2015) showed that Soybean growth was significantly increased and alleviating the negative effect under salinity by using KN. Shoot fresh weight (7.57 gm), shoot dry weight (1.66 gm), root fresh weight (10.53 gm), and root dry weight (1.13 gm) were recorded in KN (1 µM) and increased Chl content in soybean plants. Ahanger *et al*. (2018) observed the protective function of kinetin (10 µM KN) against the effects of salinity (150 mM NaCl) stress in tomato. The use of KN significantly enhanced growth and biomass by declining the adversed impacts of salt on plants. Chlorophyll and carotenoid contents, photosynthesis, membrane stability and antioxidant mechanism (both enzymatic and non-enzymatic) and osmotic accumulation significantly rise as the supplementation of KN. Kinetin reduced the accumulation of ROS by reducing ($O_2^{\bullet-}$, H_2O_2 and MDA content).

2.7 Role of salicylic acid, kinetin and other plant hormones in mitigating waterlogging stress

Lian *et al*. (2000) found the consequence of SA (5, 1, 0.5, 0.1, 0 mM) concentration on the development and increased nodule number of soybean under waterlogging stress. Application of 5 mM SA had no adverse impacts on the development of plants. Application of 5 mM SA reduced the process of N fixation and seedling grown sterile soil.

Younis *et al*. (2002) showed that the plant height, root length and total biomass of 14 days old cowpea and maize were significantly inhibited in waterlogging but application of KN increased growth parameters and IAA, GA33, zeatin and reduced ABA. Foliar application of KN increased Pro, anthocyanin, phenolic compounds in plants. So, the foliar application of kinetin alleviated the flooding and salt stress in cowpea and maize by reducing injury and reclamation of normal conditions.

Waterlogging stress damaged cell and antioxidant defense system that reduced the development and production of plants. The application of SA (2 mM) stimulated growth and development, increased root activity, increased photosynthethic pigments, and pro content and also reduced MDA content and cell permeability in soybean plants under waterlogging stress (Jianguo *et al*., 2006).

Mishra *et al*. (2013) showed the role of SA in waterlogging on soybean. The application of SA 100, 200, and 400 ppm significantly rised total protein content and reduced ROS ($O_2^{\bullet-}$ and H_2O_2 content) in soybean leaves. Salicylic acid also increased enzymatic activities and non-enzymatic activities (carotenoids, ascorbic acid, nonprotein thiol and pro).

Ren *et al*. (2017) recorded the result of external supplementation of 6-benzyladenine (100 mg L^{-1}) after waterlogged for 6 days in maize increased the photosynthetic pigments, leaf area and gaseous exchange and declined MDA content, as a result, lower ROS accumulation. Due to foliar application of 6-BA risen photosynthesis (9-37%). So, 6-benzyladenine significantly mitigated the negative impacts of waterlogging.

Zhang *et al*. (2019) observed that the exogenous application of melatonin (MT) (100 μM) at one day before of waterlogging for 10 days in 60 days old *Medicago sativa* seedlings to increase plant height, photosynthetic pigments, rate of net photosynthesis (11%), photochemical efficiency (28%) by reducing leaves senescence, necrosis and also risen polyamines content in leaves, gene expression. Waterlogging increased MDA, electrolyte leakage (EL) and ethylene but using MT significantly decline MDA, EL and ethylene and improve physiological features, metabolic enzymes and gene expressions. Melatonin plays a key role to mitigate the negative effects of waterlogging on the growth system of plants.

Chapter III

MATERIALS AND METHODS

This chapter represents a concise description of the experiment.

3.1 Location

The experiment was conducted at the experimental shed of the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka (90º 77´ E longitude and 23º 77´ N latitude), Bangladesh, during the period from August 2019 to November 2019. The biochemical attributes were carried out at the Crop Science Laboratory at Sher-e-Bangla Agricultural University, Dhaka. It has been shown Appendex-I.

3.2 Characteristics of Soil

The soil of the experiment belonged to the Modhupur tract (AEZ No. 28). It was a medium high land with non-calcarious dark grey soil. The pH value of the soil was 5.6. The characteristics of the experimental soil have been analyzed from the Soil Testing Laboratory, Soil Research and Development Institue (SRDI), Khamarbari, Dhaka.

3.3 Weather condition of the experimental site

The area of the experimental site was under the subtropical climate and was characterized by high temperature, high humidity and heavy precipitation with occasional gusty winds during Kharif- II season. The detailed meteorological data has been collected from the Bangladesh Meteorological Department, Agargoan, Dhaka, Bangladesh.

3.4 Materials

3.4.1 Planting materials

Only one soybean variety Sohag was used as planting material in the experiment. This variety was developed by Bangladesh Agriculture Research Institute (BARI), Gazipur, Bangladesh in 1991. Plant height is 36-42 cm, capsule/plant 25-30, seeds/capsule 1-2, 100 seed weight 11-12 g, seed color bright yellow and comparatively large size, vaiable conservation, capacity is good, crop duration in rabi season 100-110 days, kharif season 90-100 day. In rabi season sowing mid-December to mid-January, sowing in kharif season mid-July to August. Seed content protein 40-45% and oil content 21-22%. Yield 1.5-2 t ha^{-1}. This variety is tolerant to Yellow mosaic virus.

3.4.2 Soil preparation for pot experiment

Empty plastic pots with 18-inch depth and 14-inch diameter were used for both experiments. Twelve kilograms of sun-dried soils along with organic manures and fertilizers were put in each pot. After that, pots were prepared for seed sowing.

3.5 Treatments

The experiment was consisted 12 treatments:

1. Waterlogging for 0 days (W_0)
2. 0.5 mM Salicylic acid foliar spray (SA)
3. 0.1 mM Kinetin foliar spray (KN)
4. Waterlogged for 3 days (W_3)
5. W_3 + SA
6. W_3 + KN
7. Waterlogged for 6 days (W_6)
8. W_6 + SA
9. W_6 + KN

10. Waterlogged for 9 days (W_9)
11. W_9 + SA
12. W_9 + KN

Waterlogging treatments were imposed at 19 DAS and foliar spray were started at 16 DAS.

3.6 Design and layout of the experiment

The experiments were laid out in a Completely randomized design (CRD) with three replications.

In the experiment there were two sets of pot- 1st set with 36 pots for measuring growth parameters and taking biochemical data; 2nd set with 36 pots for measuring yield parameters.

3.7 Seed collection

The seed of Sohag variety was collected from Oil Crop Research Centre, Bangladesh Agriculture Research Institute (BARI), Joydebpur, Gazipur, Bangladesh

3.8 Pot preparation

The collected soil was sun-dried, crushed and sieved. The recommended amount of cowdung and fertilizers were mixed with soil before filled up the pots. Each pot was filled up with 12 kg soil. Then the Pots were arranged at experimental shed house of Sher-e-Bangla Agricultural University. Finally, water was added to bring the soil water level to field capacity.

3.9 Fertilizer application

Organic manure, urea, triple super phosphate, muriate of potash and gypsum were used at the recommended dose in experimental pots. The total amount of fertilizers was mixed with soil at soil preparation.

3.10 Treatments application

3.10.1 Maintaining waterlogged condition

Water supply in the pot until created anoxia condition. Everyday checked the water level 2-3 cm above the soil surface and maintained. Water removed from the pots 21 DAS (after 3 days), 24 DAS (after 6 days), 27 DAS (after 9 days) and allowed to recovery.

3.10.2 Foliar spray

3.10.2.1 Salicylic acid preparation

The molecular weight of SA is 138.12 g mol^{-1}. 69.1 mg of SA dissolved in 1 L distilled water to make 0.5 mM solution. Tween-20 was added in the dissolved solution.

Salicylic acid was sprayed 7 days interval from 16 DAS to till pod formation.

3.10.2.2 Kinetin preparation

The molecular weight of KN is 215.22 g mol^{-1}. Therefore, 21.5 mg of KN dissolved in 1 L distilled water to make 0.1 mM solution. Tween-20 was added in the dissolved solution.

Kinetin was sprayed 7 days interval from 16 DAS to till pod formation.

3.11 Intercultural operations

3.11.1 Gap filling and thinning

After sowing seeds continuous observation was kept. It was observed that some seeds failed to germinate. So, there was need of gap filling to maintain uniform plant population in every pot. Gap filling was done 12 DAS. Keen observation was made for thinning to maintain 7 seedlings. Thinning was done to maintain spacing of the plants.

3.11.2 Weeding and mulching

Sometimes there were some weeds observed in pots which were uprooted manually. Several times mulching was done to conserve moisture.

3.11.3 Irrigation

Irrigation was given to maintain field capacity moisture level.

3.11.4 Plant protection measure

Furadan @ 5 G was mixed with the soil before seed sowing to protect the plants from fungus, nematodes, and insects. Plants were infested by caterpillers and mites at 18 DAS. So, Ripcord @ 10 EC insecticide was applied to control infestation at 7 days interval.

3.12 Collection of data

Growth, physiological and biochemical parameters related data were collected after the completion of treatment duration at every stage. Yield parameters were taken after harvest.

Data were taken on the following parameters:

3.12.1 Crop growth parameters

- Plant height
- Number of leaves plant^{-1}
- Number of branches plant^{-1}

- Leaf area
- Shoot fresh weight plant^{-1}
- Shoot dry matter weight plant^{-1}
- Root length plant^{-1}
- Root fresh weight plant^{-1}
- Root dry weight plant^{-1}
- Root phenotypes

3.12.2 Physiological parameters

- Electrolyte leakage
- Relative water content
- SPAD value of leaf

3.12.3 Anatomical observation

Pictures of stem, leaf and roots transverse sections were taken with the help of digital microscope.

3.12.4 Oxidative stress indicators

- Lipid peroxidation
- H_2O_2 content
- Proline content

3.12.5 Yield contributing parameters

- Number of pods plant^{-1}
- Number of seeds plant^{-1}
- Weight of seeds pot^{-1}
- Pod length
- 1000-seed weight
- Seed yield plant^{-1}
- Stover yield plant^{-1}
- Biological yield plant^{-1}

3.13 Procedure of measuring crop growth parameters

3.13.1 Plant height

The length of the plants was recorded at 20 DAS, 30 DAS and 40 DAS. The data was measured from ground level to tip of the leaf was counted. Then the average height of 3 plants was considered as the length of the plant for each pot and expressed in cm.

3.13.2 Number of leaves plant^{-1}

The leaves of each plant were counted after the treatment 30 DAS and 40 DAS. The average value of leaves of 3 plants was considered as the total leaves plant^{-1}.

3.13.3 Number of branches plant^{-1}

Number of branches each plant was counted 30 DAS and 40 DAS. The average value of 3 plants was considered as the total branch plant^{-1}.

3.13.4 Leaf area

For measuring leaf area, firstly leaf images were taken by a digital camera and then the area was calculated using Image-J software (Ahmad *et al*., 2015).

3.13.5 Fresh weight plant^{-1}

One sample plants uprooted from each pot randomly and washed them in water. Then the plants were weighed in a balance and averaged them to have fresh weight plant^{-1} and taken after completion of treatment duration.

3.13.6 Dry weight plant^{-1}

Sample plants was dried after taken fresh weight in an electric oven maintaining 80 °C for 48 hours. Then samples were weighed in an electric balance and averaged them to have dry weight plant^{-1}. The data were collected after completion of treatment duration.

3.13.7 Root length

Sample plants uprooted from each pot and then washed in water and clean tissue paper and then taken length in cm.

3.13.8 Root fresh weight plant^{-1}

One sample plants uprooted from each pot randomly and washed them in water. Then the plants were weighed in a balance and averaged them to have fresh weight plant^{-1} and taken after the completion of treatment duration.

3.13.9 Root dry weight plant^{-1}

One sample plants after weighing for fresh weight was dried them in an electric oven maintaining 80 °C for 48 hours. Then the plants were weighed in an electric balance and averaged them to have dry weight plant^{-1}. The data were collected after the completion of treatment duration.

3.13.10 Root phenotypes

After the WL period root samples collected from the pots and the samples washed twice with water. Images of the clean root samples were captured with a digital camera.

3.14 Procedure of measuring physiological parameters

3.14.1 SPAD value

Five leaves have been chosen from every pot at random. The upper, middle and bottom of each leaflet were calculated with at LEAF (FT Green LLC, USA) as SPAD value. The averaged chlorophyll content was calculated by converting at LEAF value into SPAD units and the total Chl content was calculated. SPAD value of leaves were taken 20 DAS and 40 DAS.

3.14.2 Relative water content

Relative water content was measured according to Barrs and Weatherly (1962). Three leaves were weighed as FW and then sink in Petri dishes with distilled water for 24h. After 24 h, the leaves were weighed again after removing excess surface water, and considered as turgid weight (TW). Then DW was measured after drying at 80 °C for 48 h. Leaf RWC was calculated using the following formula:

$$\mathrm{RWC}\ (\%) = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \times 100$$

3.14.3 Electrolyte leakage

Electrolyte leakage was recorded after recovery. For measuring EL, 0.5 g leaf samples were collected from each pot and put into the Falcon tube and added 15 ml distilled water. The Falcon tubes then incubated in a water bath at 40 ^{0}C for 1 hour. After cooling, electrical conductivity (EC_1) was recorded with an electrical conductivity meter. Samples were again incubated in an autoclave machine for about 1 hour. Cooling all samples at room temperature and recorded (EC_2). Electrolyte leakage was calculated using the following formula:

$$\mathrm{EL}\ (\%) = \frac{\mathrm{EC1}}{\mathrm{EC2}} \times 100$$

3.15 Procedure for observing anatomical responses

After waterlogging treatment, samples were taken from the stem, leaf and root. Stem and root transversely sectioned into thin segments and scraping of leaves were double-stained with Safranine mixture, mounted in glycerin and photographed with a tetra view LCD digital microscope (Celestron, USA).

3.16 Procedure for measuring oxidative stress indicators

3.16.1 Measurement of lipid peroxidation

Level of lipid peroxidation was measured by estimating MDA content according to Heath and Packer (1968) with slight modification by Hasanuzzaman *et al*. (2012). At first, leaf samples (0.5 g) were colledted from the plants and then these were homogenized by adding 3 mL 5% (w/v) trichloroacetic acid (TCA). Then the homogenate was centrifuged at 11,500 × g for 15 min. The supernatant (1 mL) was mixed with 4 mL of thiobarbituric acid (TBA) reagent (0.5% of TBA in 20% TCA). This solution was heated at 95 °C for 30 min in a water bath and then quickly cooled in an ice bath and centrifuged again at 11,500 × g for 10 min. The absorbance of the colored supernatant was measured at 532 nm and was corrected for non-specific absorbance at 600 nm. MDA content was calculated by using the extinction coefficient 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} FW.

3.16.2 Determination of hydrogen peroxide content

Hydrogen peroxide was determined according to the method of Yu *et al*. (2003). Leaf tissue (0.5 g) was collected and homogenized by adding 3 mL of 50 mM potassium–phosphate (K–P) buffer (pH 6.5) at 4 °C. Then the homogenate was centrifuged at 11,500 × g for 15 min. The supernatant (2 mL) was mixed with 666.4 µL of 0.1% $TiCl_4$ in 20% H_2SO_4 (v/v) and was kept at room temperature for 10 min. After that, the mixture was again centrifuged at 11,500 × g for 12 min. Then the supernatant was measured spectrophotometrically at 410 nm to determine H_2O_2 content using the extinction coefficient 0.28 μM^{-1} cm^{-1} and was expressed as nmol g^{-1} FW.

3.16. 3 Measurement of proline content

Proline content was measured according to Bates *et al*. (1973). Fresh leaf tissue (0.5 g) was collected and homogenized by adding 5 mL of 3% sulfosalicylic acid in an ice-cold condition, and the homogenate was centrifuged at 11,500 ×g for 15 min. The supernatant (1 mL) was mixed with 1 mL of acid ninhydrin and 1 mL of glacial acetic acid, and the mixture was placed in a water bath (100 °C) for 1 h. The mixture was then transferred to a test tube and kept on ice for cooling. Toluene (2 mL) was added to the cooled mixture and mixed thoroughly using a vortex machine. After few minutes, chromophorecontaining toluene was read spectrophotometrically at 520 nm. The Pro content of the sample was determined by comparing with a standard curve of known concentration of Pro.

3.17 Procedure of measuring yield and yield contributing parameter

3.17.1 Number of pods plant^{-1}

Pod numbers were counted from the four plants and then averaged.

3.17.2 Number of seeds plant^{-1}

Ten pods were selected randomly from each pot and counted the number of seed in each pod and averaged.

3.17.3 Weight of seeds plant^{-1}

The seeds were separated from stover and then sun dried and weighed and averaged.

3.17.4 Pod length

Ten pods were selected randomly from each pot and measuring by a measuring scale and it was expressed in cm.

3.17.5 1000- seed weight

Thousand sun-dried clean seeds were counted from the 3 replication and then weighed.

3.17.6 Seed yield plant^{-1}

Seeds were separated by threshing and sun-dried and then weighed and to averaged to have grain yield plant^{-1}.

3.17.7 Stover yield plant^{-1}

After separation of grains from plants, then sun-dried and weighed the shell.

3.17.8 Biological yield plant^{-1}

The biological yield was calculated by using the following formula:
Biological yield= Grain yield + straw yield

3.18 Statistical analysis

The data obtained for different parameters were statistically analyzed following computer-based software CoStat v.6.400 (CoStat 2008) and mean separation was done by Fisher's least significant difference (LSD) at 5% level of significance. Correlation analysis was done by using SPSS v.27 (SPSS 2020) at both 1% and 5% level of significance.

Chapter IV

RESULTS AND DISCUSSION

4.1 Crop growth parameters

4.1.1 Plant height

Waterlogging greatly affects growth parameters. Waterlogged *G. max* plants showed remarkably decreased plant height compared to control plants and the increment happened in a duration-dependent manner. The lowest plant height was recorded in longer duration waterlogged plants (9 days) at any measurement (Figure 1A, 2A and 3A). It was 24% lower at 20 DAS, 29% lower at 30 DAS and 35% lower at 40 DAS compared to control (Figure 1A, 2A and 3A). On the other hand, plant height was increased in foliar SA and KN applied plants at all growth stages. Maximum plant height recorded KN applied plants (Figure 1B, 2B and 3B). A significant variation in shoot length was observed among stressed and non stressed plants. However, the highest plant height was recorded KN supplemented control plant at every growing period. Compared to control, plant height was decreased 25%, 35% and 38% for longer duration (9 days) at 20, 30 and 40 DAS, respectively (Fig. 1C, 2C and 3C). But Supplementation of SA and KN with waterlogging, a crucial increase in plant height was observed. At 30 DAS, the significant result showed which was 9%, 14% and 20% higher for SA and 10%, 16% and 28% higher for KN at 3, 6 and 9 days of waterlogging, respectively in contrast to the same duration of waterlogging only (Figure 2C).

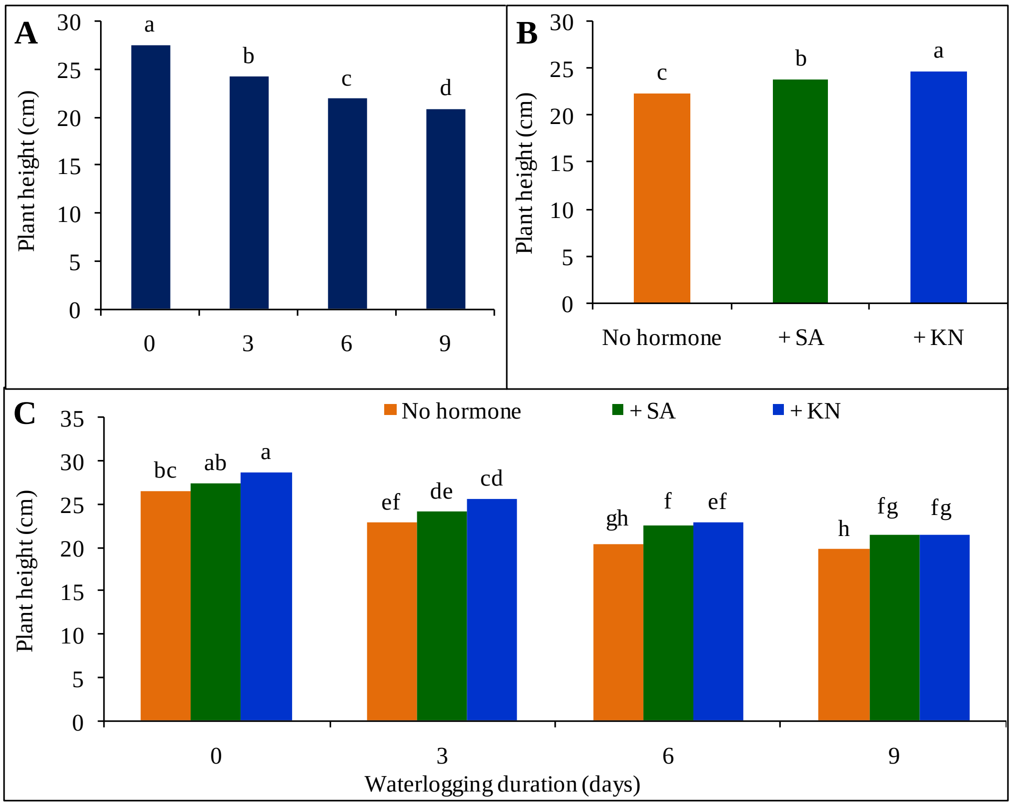

Figure 1. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on plant height of *G. max* at 20 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

In this study, shoot length was decreased due to waterlogging in a duration-dependent manner at any ages of plants (Figure 1, 2 and 3). Waterlogging-induced decreasing plant height was observed in soybean in several plant studies (Kim *et al.* 2018). Waterlogging hampers the growth of meristematic tissues, reducing carbohydrate supply to the growing cells. Several studies on the waterlogging in soybeans showed that many proteins regulating glucose degradation occured under waterlogging. However, proteins related to energy production increased and were involved in the maintenance of the structure of cells under this condition (Nanjo *et al.*, 2013). These might result in the decrease in plant height in this study. Plant can not uptake the proper amount of nutrient N, P and K, which result in nutrient deficiency symptoms and finally reduction of shoot length (Rhine *et al.*, 2010). Different plants reduced plant height under waterlogging stress was also reported by several researchers (Anee *et al.*, 2019; Wei *et al.*, 2013; Saha *et al.*, 2016). On the other hand, exogenous SA and KN retired the harmful impacts of waterlogging on soybean in the case of plant height. Salicylic acid plays different plant activities, including plant growth and enhanced plant tolerance under different types of stresses. Salicylic acid improved nutrient uptake and reduced ethylene production. Several researchers found that SA increased plant height under abiotic stress by reducing oxidative stress (Bai *et al.*, 2009; Khan *et al.*, 2003). The functions of KN is shoot differentiation, cell division and chloroplast development (Fahad *et al.*, 2015). As a result, increase photosynthesis and energy production, which helps to tolerance against stress. The foliar application of KN increased plant height under stress supported by (Ahanger *et al.*, 2020; Wang *et al.*, 2015).

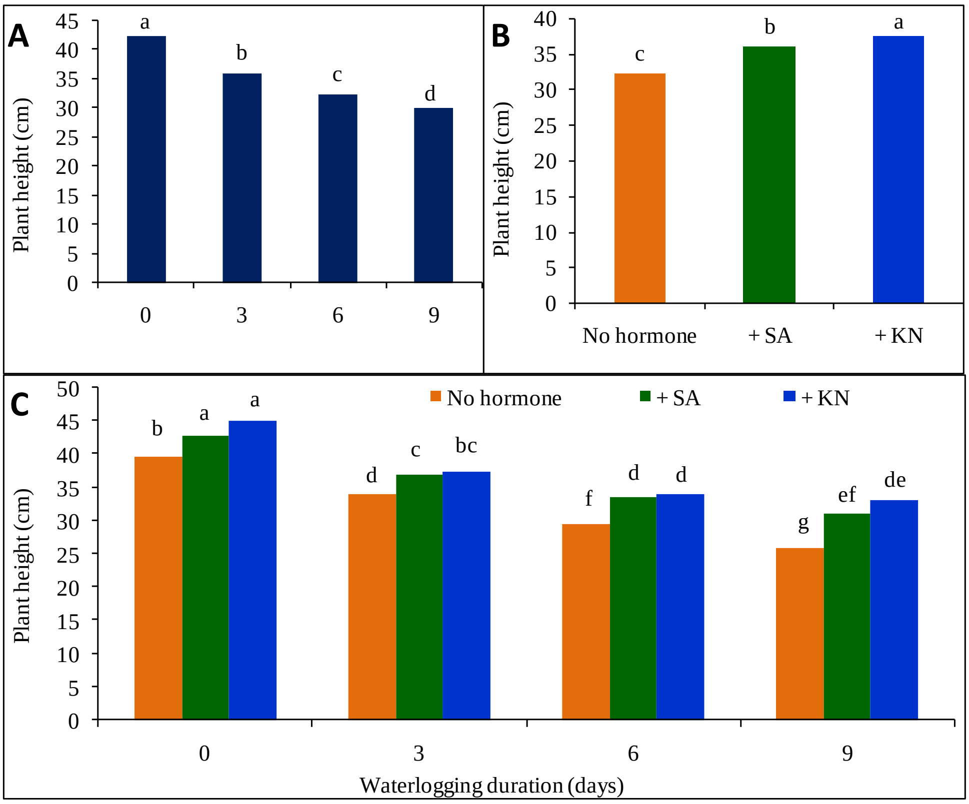

Figure 2. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on plant height of *G. max* at 30 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

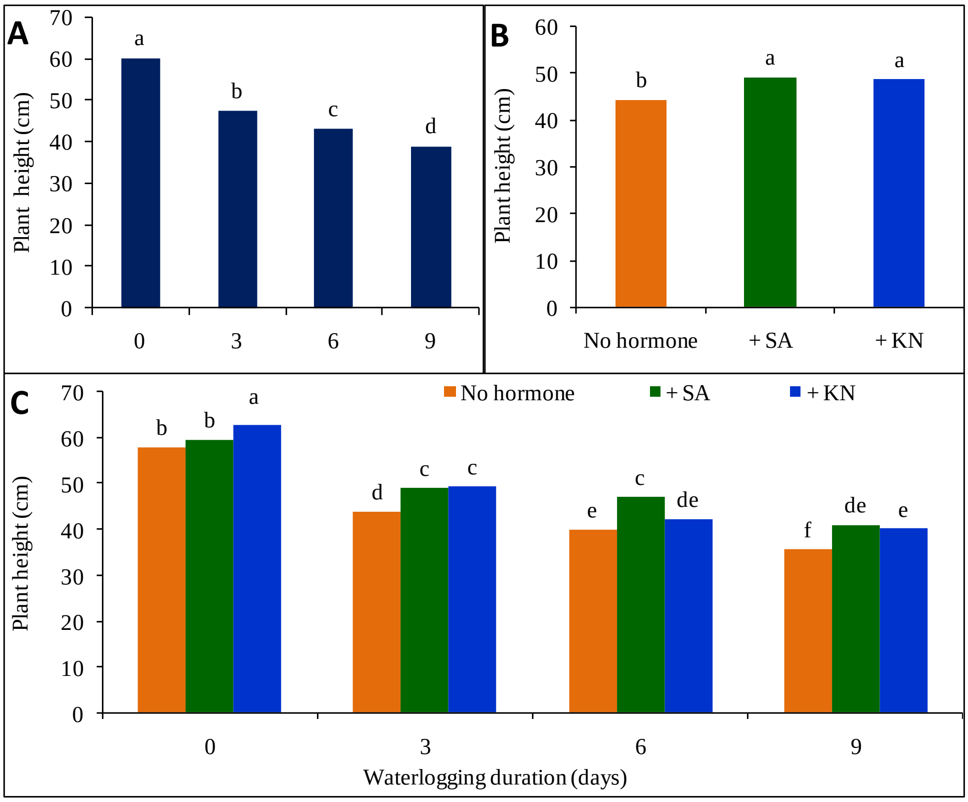

Figure 3. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on plant height of *G. max* at 40 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.2 Number of leaves plant^{-1}

Leaves number was drasticaly declined under 3, 6 and 9 days of waterlogging. The maximum reduction of leaves number, was recorded in the longest duration (9 days) of waterlogging treatment (Figure 4A and 5A). Leaves number were recorded in waterlogged for 3, 6 and 9 days plants at 30 DAS, 16%, 33% and 41% lower and at 40 DAS 28%, 29% and 25% lower, respectively compared to the control (Figure 4C and 5C). However, the foliar application of both SA and KN effectively increased leaves number by reducing the adverse effects of waterlogging. Protectants caused a significant variation observed for leaves number at 30 DAS and 40 DAS (Figure 4B and 5B). Salicylic acid (12% and 7%) and KN showed (10% and 10%) higher leaves number contrast to their control condition at 30 DAS and 40 DAS, respectively. After 30 DAS, it was 10%, 23% and 15% higher leaves number for SA and 11%, 18%, 18% higher leaves number for KN at 3, 6 and 9 days of waterlogging, respectively in contrast to corresponding control (Figure 4C). Similarly, at 45 DAS, leaves number significantly increase by KN that was 26%, 11%, 18% higher at 3, 6 and 9 days of waterlogging, respectively in contrast to corresponding control (Figure 5C).

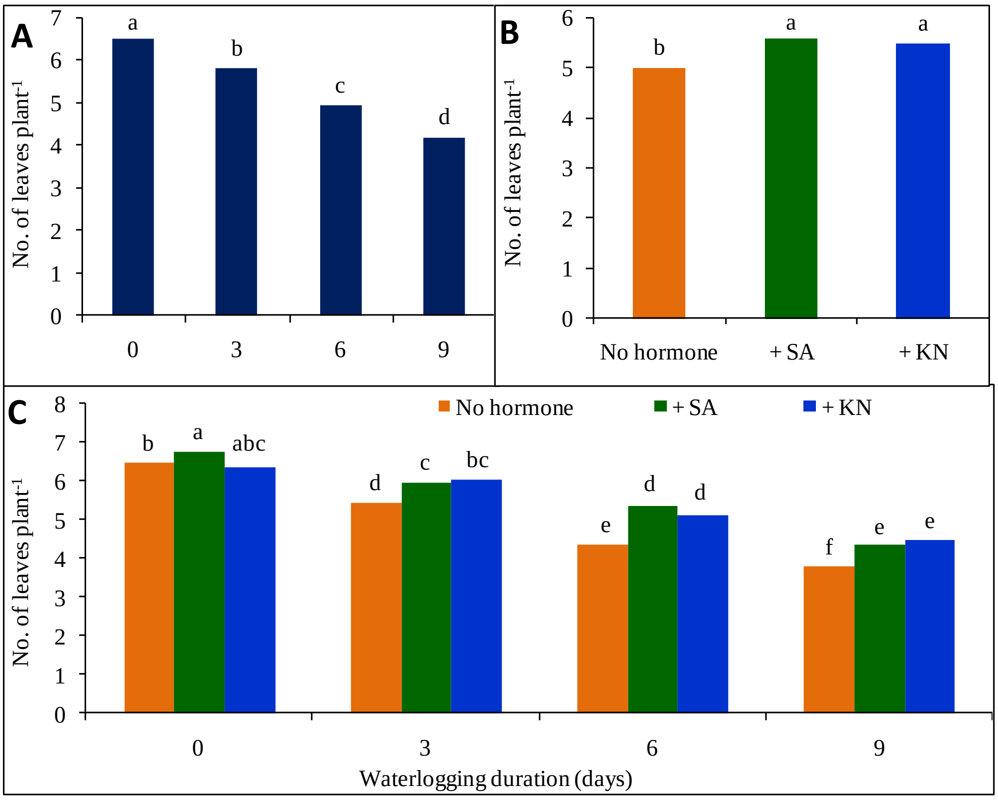

Figure 4. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on leaves number of *G. max* at 30 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

Leaves number was decreased due to waterlogging in a duration-dependent manner at any stages of plants (Figure 4 and 5). Waterlogging brought a reduction in crop growth, net assimilation, leaf expansion and the ultimate result was a reduction of leaf number and leaf area in soybean crops (Ezin *et al*., 2010). Prasanna and Rao (2014) recorded that number of leaves decreased due to waterlogging stress in green gram plants. On the other hand, SA regulated different plant activities such as delay of leaf senescence and enhanced plant tolerance under various types of stresses by improving different physiological activities (Besseau *et al*., 2012). Salicylic acid increased leaves number by reducing protenin synthesis and ABA production (Moradkhani *et al*., 2012; Zeb *et al*., 2017). Kinetin delaying the leaf senescence process by increasing the protein degradation process rather than reducing synthesis of protein (Liu *et al*., 2008). Hence, the foliar application of KN increased leaves number under stresses and the ultimate ultimate result was increased photosynthesis.

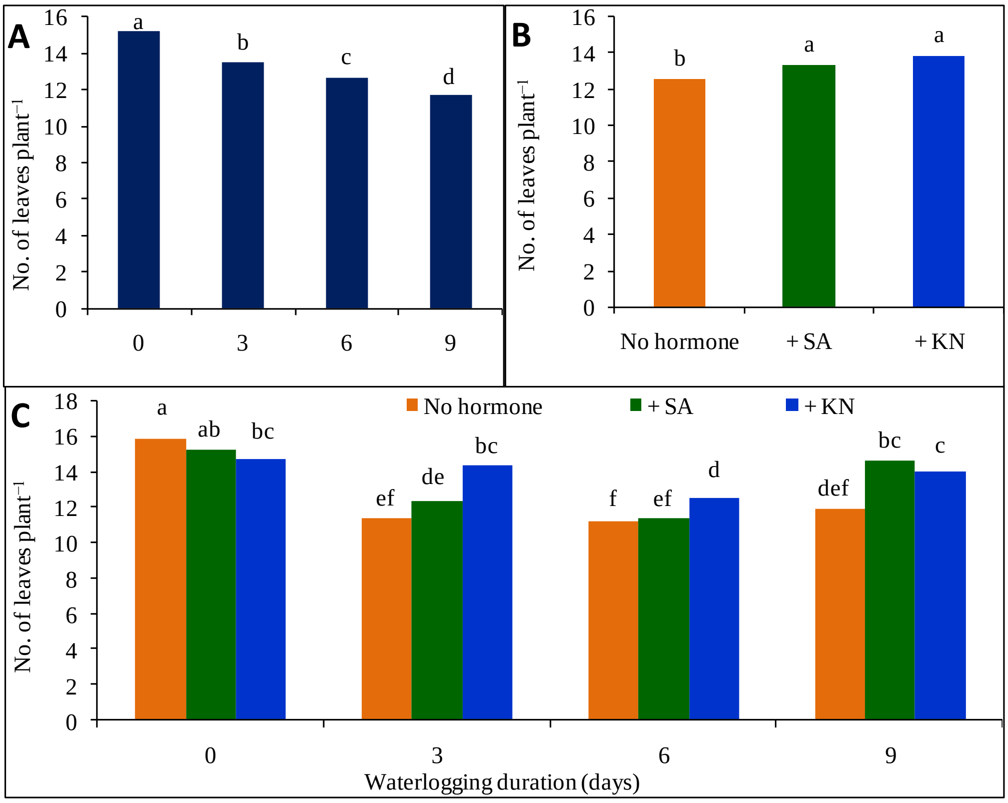

Figure 5. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on leaves number of *G. max* at 40 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.3 Leaf area

Waterlogged *G. max* plants showed remarkably lower leaf area compared to control plants and the increment happened in a duration-dependent manner. The lowest leaf area was recorded in longer duration waterlogged plants (9 days) at any measurement (Figure 6A and 7A). After 40 DAS, the plants which were 3, 6 and 9 days waterlogged 13%, 17% and 22%, respectively lower and after 50 DAS 16%, 20% and 24%, respectively lower leaf area were recorded in contrast to the control (Figure 6C and 7C). In the contrary, SA and KN significantly increased leaf area. Effect of protectants caused a considerable variation observed on leaf area (Figure 6B and 7B). In (Figure 6C) the risen was 7%, 8% and 8% higher for SA, and 10%, 11% and 13%

higher for KN at 3, 6 and 9 days of waterlogging, respectively compared to the same duration of waterlogging treatment alone at 40 DAS (Figure 6C). Similarly, at 50 DAS, the reduction was 8%, 5% and 10% for SA and 9%, 11% and 11% for KN at 3, 6 and 9 days of waterlogging, respectively in contrast to the same duration of waterlogging only (Figure 7C).

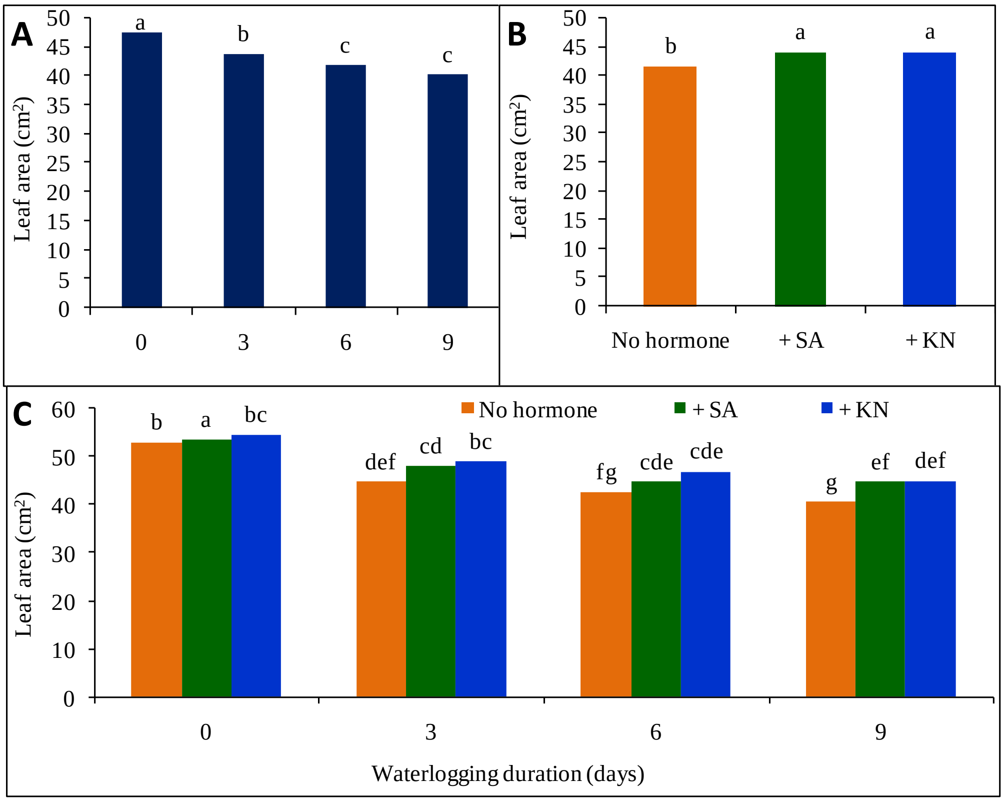

Figure 6. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on leaf area of *G. max* at 40 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

Waterlogging brought reduction in crop growth, net assimilation, leaf expansion and the ultimate result was a reduction of leaf number and leaf area in soybean crops (Ezin *et al.*, 2010). Many scientists showed that leaf area and net photosynthetic rate remarkable reduction in plants due to waterlogging. The reduction of leaf area under waterlogging stress has been exhibited in barley (Zhang *et al.*, 2016), mungbean (Kumar *et al.*, 2013), green gram (Prasanna and Rao, 2014), summer maize (Ren *et al.*, 2016) and sesame (Saha *et al.*, 2016). Our study also supported this hypothesis. In addition, waterlogging decreased the leaf area, intensified the process of senescence and as a result,lower photosynthesis rate. Waterlogging decreased Chl content which leads to a decrease in the photosynthesis rate of plants leaves (Pociecha *et al*., 2008), suggesting that waterlogging affected soybean leaf photosynthesis and inhabited the capacity of photosynthetic assimilation (Nakano *et al*., 2019; Saputro*et al*., 2018). In the contrary, the supplementation of SA and KN increased leaf area at every stage (Fig 6, 7). Several researchers also showed that SA was involved in increasing leaf area under stress in different crops (Zamaninejad*et al*., 2013; Zeb *et al*., 2017). On the other hand, KN also improves Chl which increasedthe photosynthesis rate and finally increased leaf area. Several researchers also showed that KN positive results to increase leaf area (Farber *et al*., 2016).

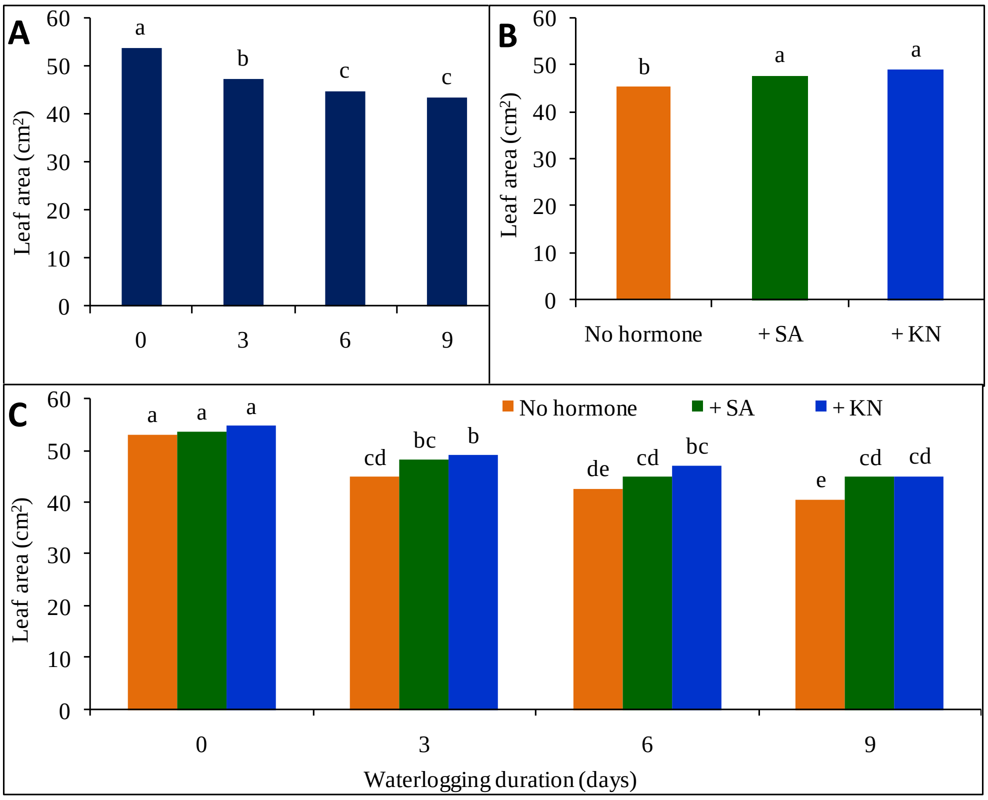

Figure 7. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on leaf area of *G. max* at 50 DAS. Here, SA and KN indicate salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.4 Number of branches plant^{-1}

In response to the different duration of waterlogging (6 and 9 days) branch number in soybean plants significantly decrease in contrast to control plants (Figure 8A, 8C, 9A and 9C). The lowest number of branches showed 9 days waterlogging at any measurement. At 30 DAS, 26% and 35% lower branch number and at 40 DAS, 25% and 44% lower branch number were recorded in plants waterlogged for 6 and 9 days of waterlogging, respectively in contrast to the control (Figure 8C, 9C).). In the contrary, exogenous application of both SA and KN played a reverse role and increased the branch number under non-stress and stress conditions (8B, 9B). SA increased the branch number 31%, 27% and 9% and by KN 12%, 32% and 22% increased for 3, 6 and 9 days of waterlogging, respectively compared to the same duration of waterlogging treatment alone at 30 DAS (Figure 8C). In other hand, KN showed significant result at 40 DAS that was 32%, 33% and 78% higher at 3, 6 and 9 days of waterlogging, respectively in contrast to corresponding control (Figure 9C).

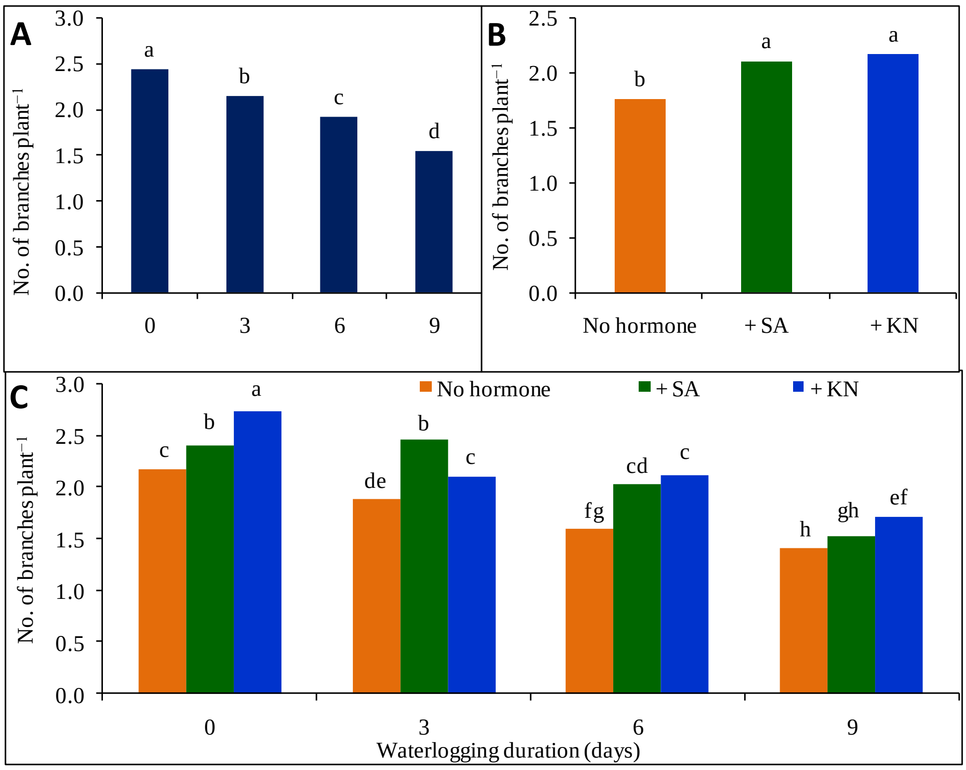

Figure 8. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on branch number of *G. max* at 30 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

Branch numbers plant^{-1} decreased upon exposure to waterlogging stress (Figure 7). Cho and Yamakawa (2006a) showed the number of leaves, branch number, nodulation significantly reduced due to waterlogging in soybean. Miura *et al*. (2012) also reported that waterlogging treatment at 21 days in soybean, reduced the number of branches significantly. At the vegetative stage, prolonged waterlogging greatly reduced branch number in mungbean (Koyama *et al*., 2019; Ahmed *et al*., 2003) and decreased 50% branch number in chickpea (Paltaa *et al*., 2010). It also showed that the N fixation also declined as a result reduction of total biomass. By inhibiting carbon and nitrogen metabolism, waterlogging will limit the ability of plant to assimilate. The reduction of CO_2 assimilation photosynthesis rate drastically decreased, ultimately plant stunted. The stunted plant leads to lower number of branch. Branch number declined in a duration-dependent manner at any stage of plants. Decreased in branch number in response to different stresses in soybean was also reported by several studies (Akter and Nazir, 2013; Hamayun *et al.* 2015). Due to lack of energy plant height reduced and ultimately, reduction of branch number (Nanjo *et al*., 2014). On the other hand, supplementation of SA and KN increased the branch number plant^{-1} at every growth stages (Figure 8B and 9B). Salicylic acid enhanced protein content in cells as a plant growth increase and branch number also increased. Mishra *et al*. (2013) showed the role of SA in waterlogging on soybean total protein content significantly rised by reducing protein degradation, which results in increased branch number. Salicylic acid enhanced bud development, transportation of sugars, metabolism of carbohydrate. Exogenous application of KN promoted bud activation, shoot branching increased by activating axillary buds (Waldie and Leyser, 2018). In this study, KN significantly increased branch number plant^{-1} under waterlogging in a duration-dependent manner at any stages of plants (Figure 8B, 9B, 8C and 9C).

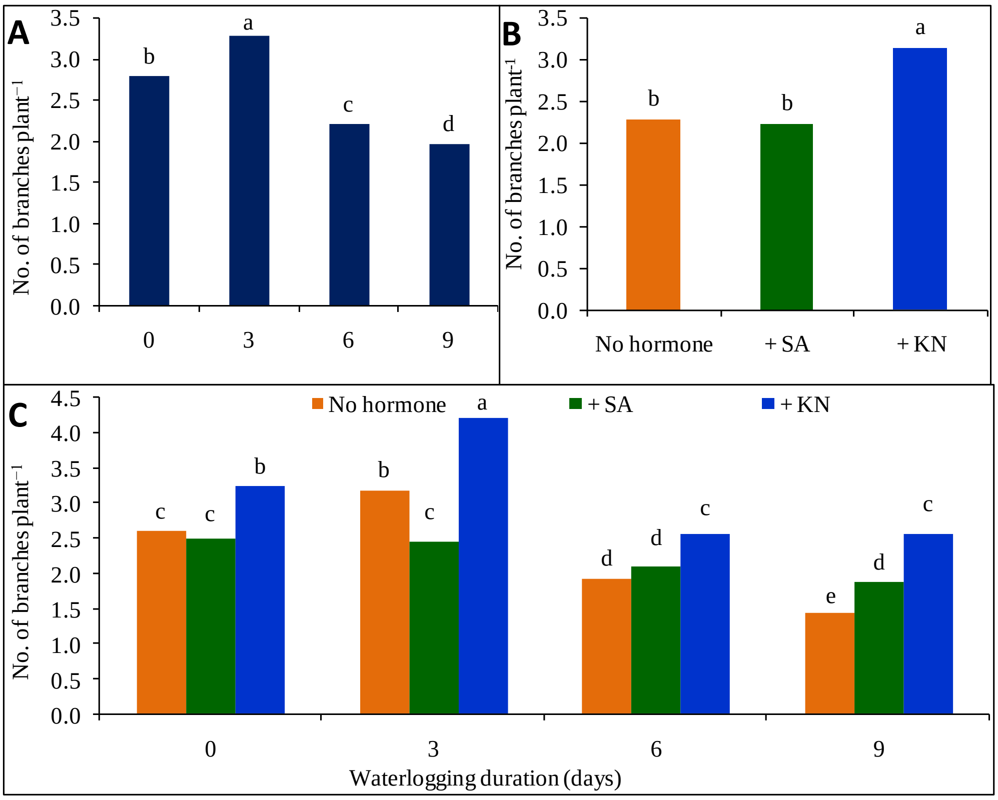

Figure 9. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on branch number of *G. max* at 40 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.6 Shoot fresh weight plant^{-1}

Shoot FW plant^{-1} crucially reduced by waterlogging stress and the highest reduction of FW was recorded in the prolonged duration (9 days) of waterloggingat any measurement. Waterlogging caused 15%, 33%, 56% shoot fresh weight reduction (Figure 10A) and 11%, 32%, 54% fresh weight reduction (Figure 11A). At 30 DAS, 18%, 39%, 58% lower FW and at 40 DAS, 13%, 34% and 56% lower FW were showed in plants waterlogged for 3, 6 and 9 days of waterlogging, respectively compared to the control plants (Figure 10C, 11C). In the contrary, the foliar application SA and KN recovery the negative effect of Waterlogging. The highest fresh weight recorded in SA sprayed plants at any measurement (10B, 11B). Salicylic acid significantly increased FW, which was 13%, 19% and 17% higher at 30 DAS (Figure 10C) and 15%, 11% and 21% higher at 40 DAS (Figure 11B) for 3, 6 and 9 days of waterlogging, respectively compared to the same duration of waterlogging only. On the other hand, KN also increased FW at every growth stages.

It was 11% and 6% higher compared to no hormone applied plants at 30 DAS and 40 DAS, respectively (Figure 10B, 11B).

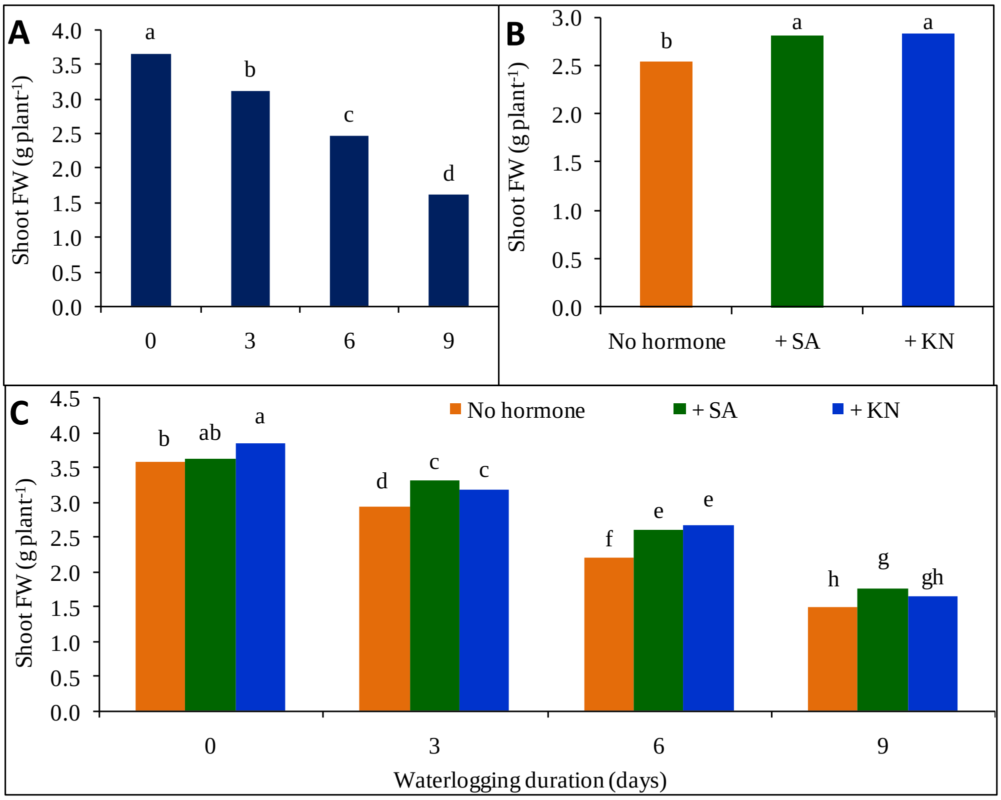

Figure 10. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on shoot FW of *G. max* at 30 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

In this experiment, aboveground FW plant^{-1} was sharply decreased in a duration-dependent manner at any ages of the plants (Figure 10A and 11A). Waterlogging-induced reduction in aboveground FW was recorded in soybean in several studies (Miao *et al*., 2012; Beutler *et al*., 2014; Kim *et al*., 2019). Previous studies showed that waterlogging reduces the content of Chl, which resulted a decrease in photosynthetic activity and rate that inhibited plant growth and biomass accumulation (Ren *et al*., 2014). Under waterlogging the phytology and catabolism of plants are disrupted. Waterlogging stress restricted stomatal conductance, the transition of gases and metabolism of CO_2. Reduction of CO_2 entering the leaf, which reduced transpiration leading to wilting of the leaves and decreased Chl content as a result lower dry matter accumulation (Ashraf, 2012). Under waterlogging, plants limited uptake of N, P and K. Waterlogging condition can inhibit the capacity of plant to carbon assimilate, which result greatly decline of FW (Rhine *et al*., 2010). Decreased shoot FW in response to different stresses were also reported by several studies (Mutava *et al*., 2015; Ahmed *et al*., 2002). In the contrary, SA inhibited the MDA production and increased shoot fresh weight. The foliar application of SA increased fresh weight of plants under stress in soybean supported by (Liu *et al*., 2008). Several researchers also recorded that SA involved to increasing fresh weight under stress in different crops (Bai *et al*., 2009; Moradkhani *et al*., 2012; Zanganeh *et al*., 2019). Kinetin also improves Chl, which increase photosynthesis rate and finally increased fresh weight. Several researchers also showed that positive results of KN to increase fresh weight under abiotic streses supported by (Ahanger *et al*., 2020; Wang *et al*., 2015). In our study, also supported this hypothesis and increased FW by foliar application of SA and KN. In this study, the positive result showed by SA and KN shoot FW under waterlogging stress (Figure 10 and 11).

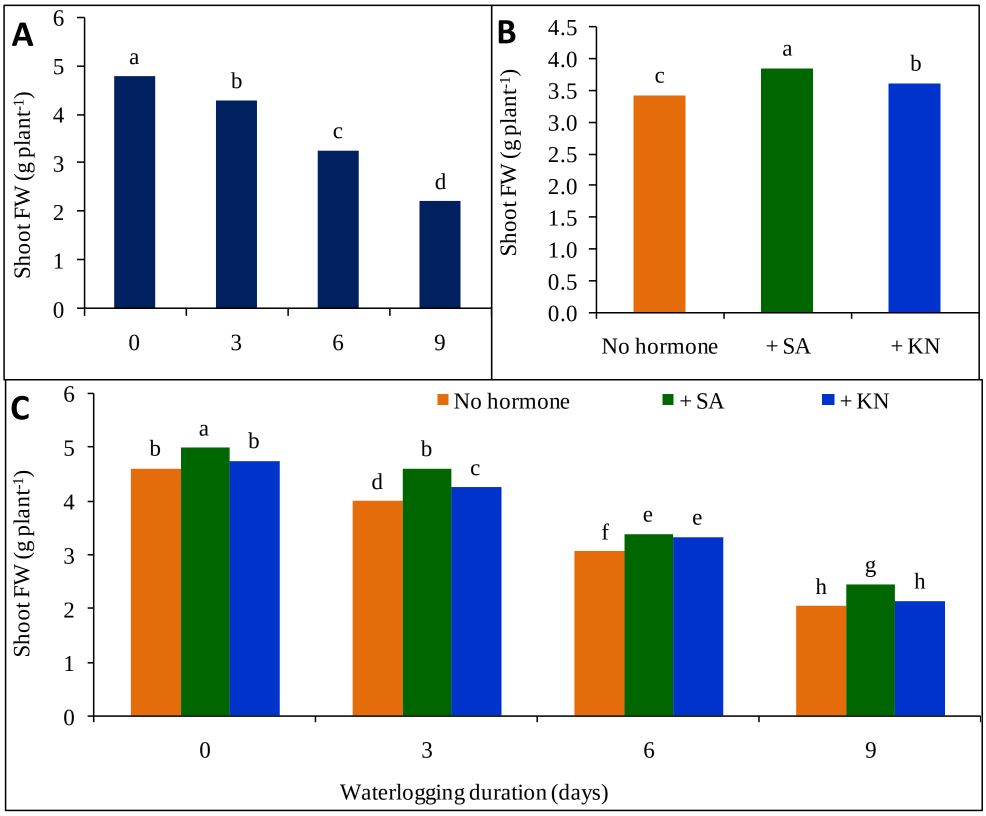

Figure 11. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on shoot FW of *G. max* at 40 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.7 Shoot dry matter weight plant^{-1}

Plant DW showed similar results as plant FW. As shown in figure 12 and 13 DW remarkably reduced in waterlogging for 9 days at 30 and 40 DAS. Compared to control, DW sharply decline at the longer duration (9 days) of waterlogging treatment, which was 51% at 30 DAS and 57% lower at 40 DAS (Figure 12C, 13C). Due to Exogenous application of SA and KN effectively increase DW under 3 and 6 days waterlogging rather than 9 days waterlogging. At the early stage (30 DAS), SA significantly increase DW that was 22% and 16% higher for 3 and 6 days of waterlogging, respectively, compared to the same duration of waterlogging only (Figure 12C). Similarly, KN increase DW at both stages 11% (Figure 12B, 13B). Kinetin significantly increase DW that was 10%, 9%, 16% at 30 DAS and 15%, 30%, 7% at 40 DAS for 3, 6, 9 days waterlogging, respectively (Figure 12C, 13C).

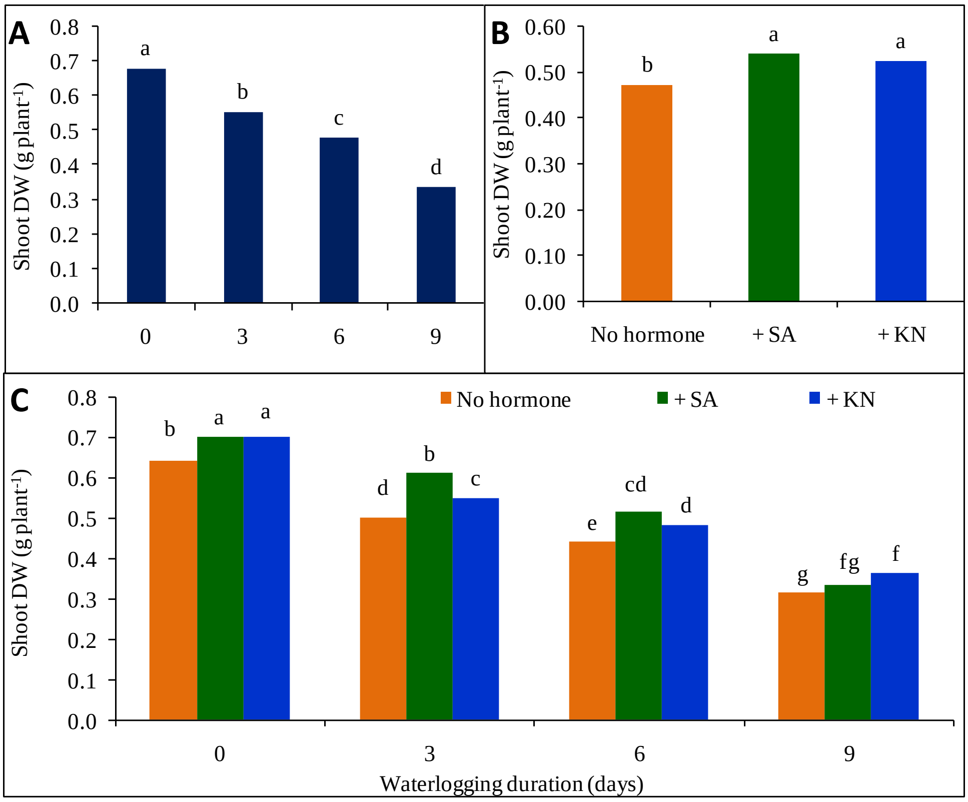

Figure 12. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on shoot DW of *G. max* at 30 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

Under waterlogging, aboveground dry matter accumulation also decline in a duration-dependent manner at any ages of the plants (Figure 12A, 13A). Similar to FW, waterlogging-induced reduction in aboveground DW was observed in soybean in several studies (Miao *et al*., 2012; Beutler *et al*., 2014; Kim *et al.*, 2019). Plants accumulate biomass and increased dry weight by photosynthesis (Ren *et al*., 2016). Previous studies indicated that waterlogging stresss inhibited dry matter accumulation by reducing carbohydrate synthesis (Zhang *et al*., 2016). Several scientists recorded that waterlogging stress reduced dry weight (Tian *et al*., 2019a; Prasanna and Rao, 2014; Kumar et al., 2013). Whereas, foliar application of SA improves photosynthetic attributed and increased RWC and also supply water which increase photosynthetic rate. Previous studies showed that SA significantly increased dry matter supported by (Yang *et al*., 2013; Brodersen *et al*., 2005). Similarly, KN also reverted the negative effects of waterlogging by improving antioxidant enzymes activities. Kinetin increase nutrient uptake, enhanced phosynthetic pigments pigments, as a result, increased photosynthesis and finally increased dry matter accumulation. Ahanger *et al*. (2020) and Wang et al. (2015) showed that KN showed positive result under stress. In the present study, it has been found that SA and KN remarkably improved shoot DW under waterlogging stress (Figure 12B, 12C, 13B and 13C).

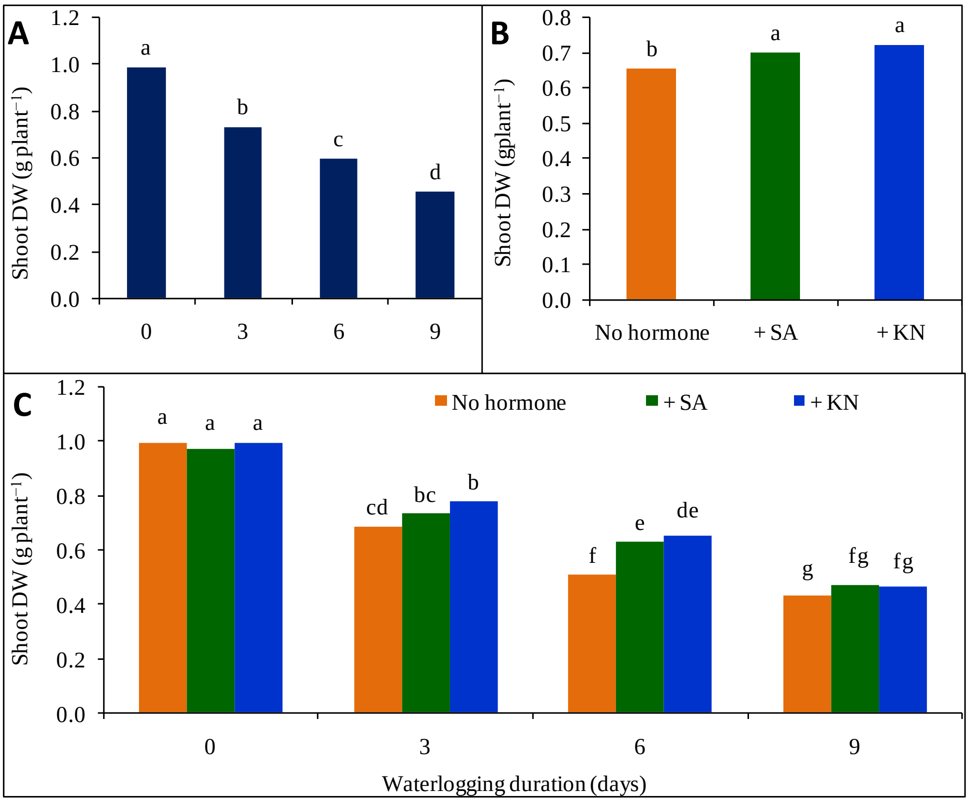

Figure 13. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on shoot DW of *G. max* at 40 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.5 Root length

The highest reduction of root length was recorded in the longest duration (6 and 9 days) of waterlogging treatment (Figure 14A and 14C). The reduction was 29% at 40 DAS for 9 days of waterlogging compared to the control (Figure 14C). However, the supplementation of SA and KN effectively increased the length of root. The increasing amount was 13% higher for SA and 10% higher for KN compared to no hormone sprayed plants (Figure 14B). Sharply increased root length by SA 25% higher at 40 DAS for 3 days waterlogging compared to the same duration of waterlogging treatment alone. Both SA and KN significantly increase the root length which was 15% and 18% higher for SA, 17% and 19% higher for KN at 6 and 9 days of waterlogging, respectively, in comparision to the same duration of waterlogging treatment alone at 40 DAS (Figure 14C).

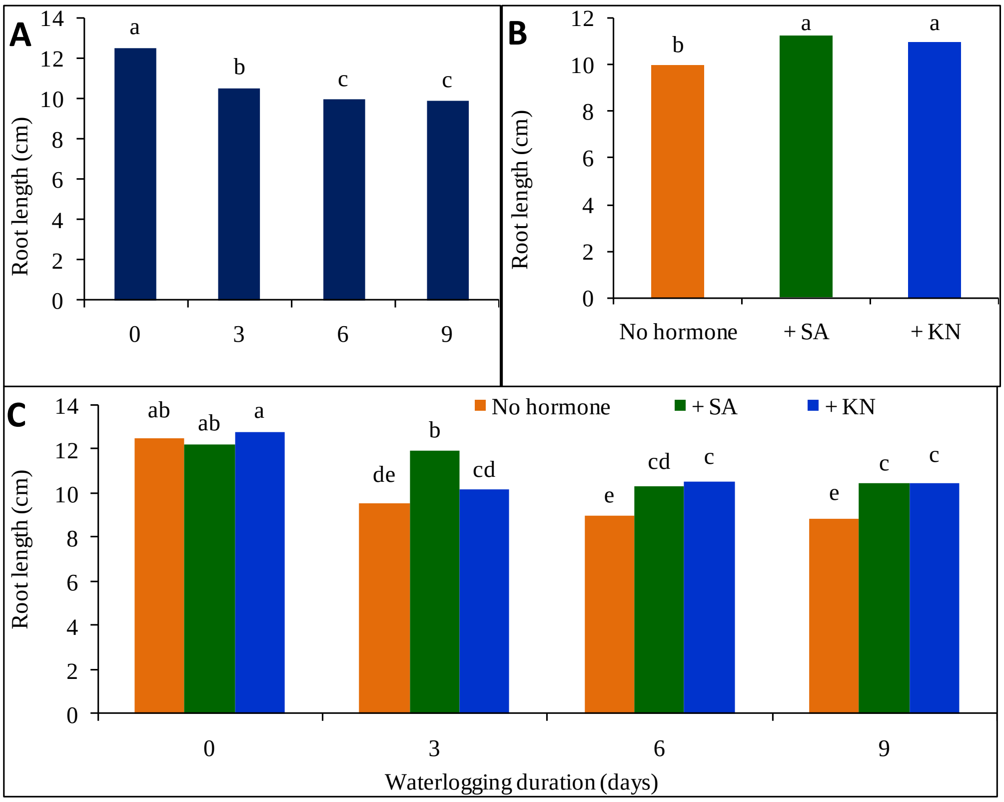

Figure 14. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on root length of *G. max* at 40 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

In this study, root length was decreased in a dose-dependent manner at any age of the plants besides exogenous spray of SA and KN increased root length under waterlogging stress (Figure 14B and 14C). Waterlogging-induced decrease in root length in soybean was observed by (Miao *et al.*, 2012). It is also stated that waterlogging caused a decline in oxygen level as a result inhibited every metabolism, root growth and ion transport. Ultimately, root activities decreased and also nutrient uptake inhibited in consequence nutrient deficiency symptom. The consequence of prolonged waterlogging is the formation of ethylene that inhibited root growth and ultimately cause plant death (Visser and Pierik, 2007). In soybean adventitious roots under waterlogging is very common, which showed high rupture of cortex cells, creating aerenchyma used as pores for O_2 transfer to roots, being a plant survival mechanism under stress conditions (Beutler *et al.*, 2014; Kim *et al.*, 2018). However, the foliar application of SA and KN alleviated the negative effects of waterlogging stress. Exogenous SA increased biosynthetic enzyme activities which helps plant to tolerance under waterlogging. In previous studies, the application of 0.5 mM SA alleviated growth inhibition and reduced injury in *Malus robusta* by inducing the antioxidant system (Bai *et al*., 2009). Similarly, KN regulated physiological mechanisms like nutrient mobilization, cell division and increased GA3 and zeatin (Fahad *et al*., 2015). Several researchers found that KN increased root length under abiotic stress in *Vigna sinensis* (50 ppm) kinetin increased root length and reduced stress injury (Ahanger *et al*., 2020), KN addition ameliorated the deleterious effects of Mn pollution (Gangwar *et al*., 2010) in maize and reverted the negative effects on root growth by increased uptake nutrient (Wang *et al*., 2015).

4.1.8 Root fresh weight plant^{-1}

Upon exposure to waterlogging, root FW sharply decreased with the increment of waterlogging duration. The highest reduction lelevl of root FW was recorded in waterlogged plants for a longer duration (9 days) at any measurement. Waterlogging for 3, 6 and 9 days reduced root FW 7%, 19% and 30%, respectively lower at 30 DAS and 10%, 22% and 33%, respectively lower at 40 DAS were recorded in contrast to the control (Figure 15A and 16A). In figure 15C and 16C the reduction was was 33% and 33% lower, respectively for 9 of days waterlogging in comparision to control. In the contrary, the application of SA and KN increased the root FW. After 30 DAS, 10% for SA and 5% for KN higher, compared to no hormone sprayed plants. Similarly, at 40 DAS the increasing amount was 7% for SA and 4% for KN compared to no hormone sprayed plants (Figure 15B and 16B). Salicylic acid showed more effective results rather than KN that was 4%, 4% and 10% higher for 3, 6 and 9 days waterlogging, respectively, compared to KN treated plants at the same duration of waterlogging only at 30 DAS (Figure 15C).

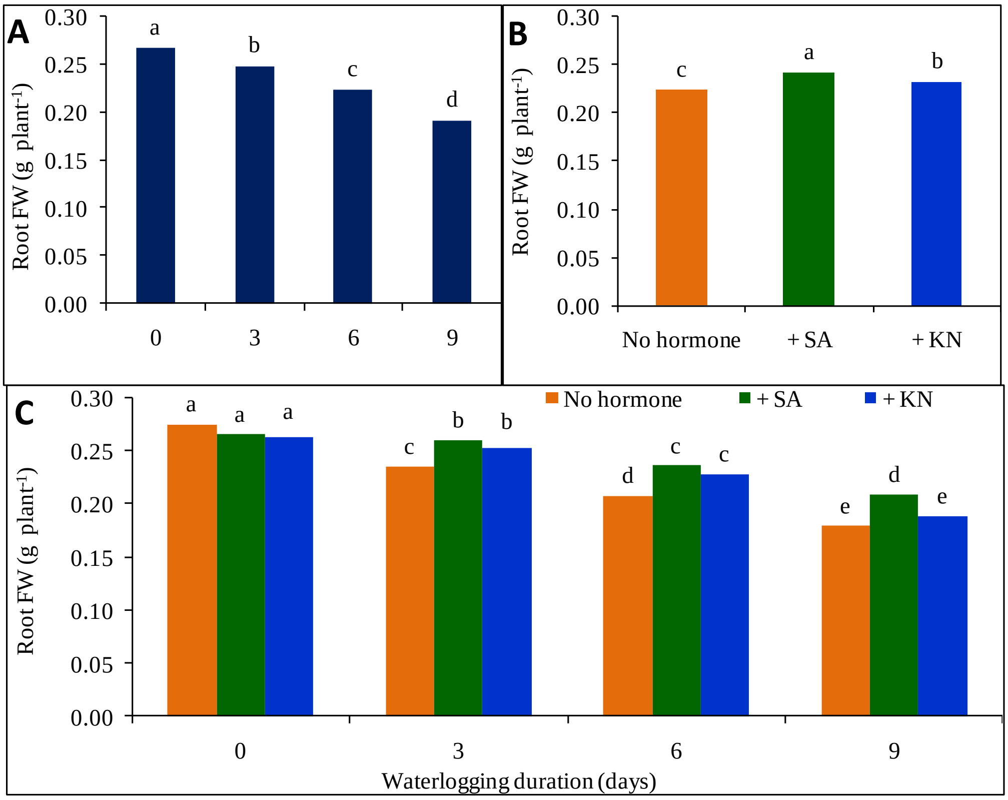

Figure 15. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on root FW of *G. max* at 30 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

In this study, root fresh weight was decreased like shoot fresh weight due to waterlogging stress in a duration-dependent manner at any age of plants (Figure 15A, 15C, 16A and 16C). Waterlogging reduced root FW in soybean (Kim *et al*., 2018; Miura *et al*., 2012; Beutler *et al*., 2014). It was common that waterlogging stress imposed both oxidative and osmotic stress in plants and as a result root weight decline. Waterlogging inhibited root growth and also reduced carbon content and nodule number. Previous studies showed that cotton plants reduced the content of nitrogen, iron, potassium and phosphate (Milroy *et al*., 2009) and potassium concentration decreases (Antheunisse and Verhoeven, 2008). Akhtar and Nazir (2013) also reported waterlogging negatively affected macro and micronutrient uptake, which caused root growth decreased. Oxygen deficiency in the root zone has drastic impacts on plant growth and development. Under waterlogging the phytology and catabolism of plants are disrupted. Reduction of CO_2 entering the leaf, which reduced transpiration leading to wilting of the leaves and decreased Chl content as a result, lower dry matter accumulation (Ashraf, 2012). However, the exogenous spray of SA and KN alleviated the negative effects of waterlogging stress (Figure 15B and 16B). In addition, SA increased biosynthetic enzyme activities, which helps plant to tolerance under waterlogging (Bai *et al*., 2009; Yang *et al*., 2013) and inhibiting K^+ ion loss and also reducing the accumulation of Na^+ under salt stress Jayakannan *et al*., 2013). Kim *et al*. (2018) also showed root surface increase and increased fresh weight by foliar application of SA. Similarly, KN involved increased IAA, GA3 and markedly reduced ABA. Kinetin showed significant result to alleviate negative effects under abiotic stress in several plants such as, ameliorates the deleterious effects of Mn pollution (Gangwar *et al*., 2010) in maize and reverted As negative effects on root growth by increase uptake nutrient (Wang *et al*., 2015), Cadmium effects reverted in *Vigna angularis* (Ahanger *et al*., 2020). After all, SA and KN to revert the harmful effects of waterlogging on soybean (Figure 15B, 15C, 16B and 16C).

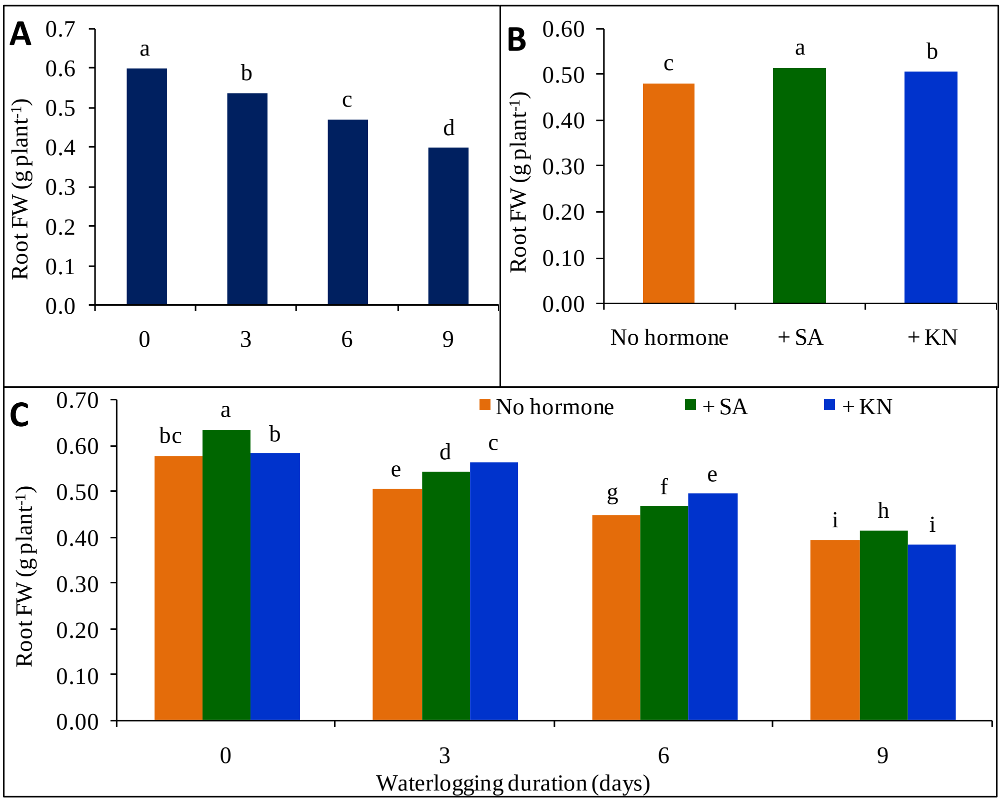

Figure 16. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on root FW of *G. max* at 40 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.9 Root dry weight plant^{-1}

In response to different period of waterlogging (3, 6 and 9 days) root DW was reduced sharply compared to control. Similar to FW, the longest duration (9 days) showed highest reduction in root DW. It was 50% lower at 30 DAS and 40% lower at 40 DAS, compared to control (Figure 17A and 18A). At 30 DAS, 25%, 37%, 50% lower DW and at 40 DAS, 24%, 41%, 45% lower DW were recorded in plants waterlogged for 3, 6 and 9 days, respectively in contrast to control (Figure 17C and 18C). However, the foliar application of SA and KN remarkably increase root DW under waterlogging stress. It was 14% higher for SA and 9% higher for KN at 30 DAS (Figure 17B) and similarly, at 40 DAS 11% higher for SA and 19% higher for KN (Figure 18B). Waterlogging for 3 and 6 days which was 23%, 25% higher for SA and 20%, 22% higher for KN, respectively at 30 DAS in comparision to corresponding control (Figure 17C). Similarly, at 40 DAS root DW increased 24%, 19% for SA and 23%, 29% for KN at 3 and 6 days of waterlogging, respectively compared to the same duration of waterlogging only (Figure 18C).

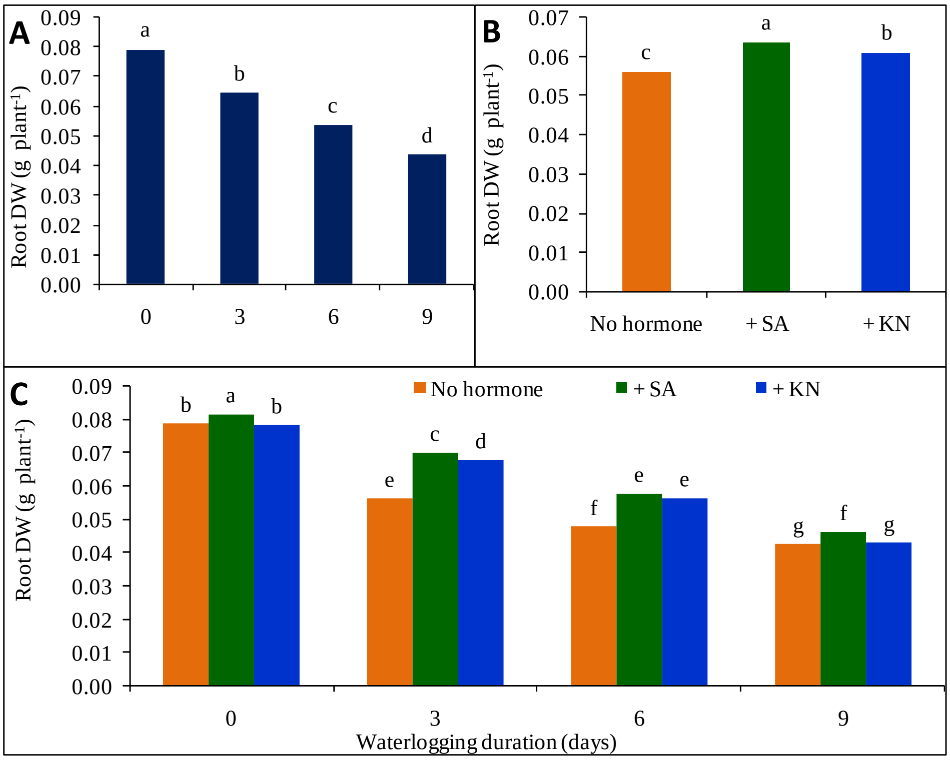

Figure 17. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on root DW of *G. max* at 30 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

The sharp decline was observed in root DW under waterlogging in a duration-dependent manner at any stages of plants (Figure 17A, 17C, 18A and 18C). Waterlogging reduced root dry weight in soybean plants in several studies showed (Zhang *et al*., 2019; Kim *et al*., 2018; Miura *et al*., 2012; Beutler *et al*., 2014). Under waterlogging root damaged as a result, insufficient allocation of water and nutrients, which leads to root DW reduction (Jackson and Ricard, 2003). Waterlogging stress showed similar results in case of root dry weight in previous studies of various crops like sesame (Anee *et al*., 2019), mungbean (Ahmed *et al*., 2015), green gram (Prasanna and Rao, 2014), cowpea (Younis *et al*., 2002). Akhtar and Nazir (2013) also reported that waterlogging negatively affected macro and micronutrient uptake, which ultimate result was decreased plant growth. Waterlogging also reduced the microbial activity which helps to available different niutrient. Root dry matter also declined due to lower uptake of water and oxygen deficiency. But foliar application of KN showed significant result by increasing root growth and weight in *Vigna sinensis* (Younis *et al*., 2002) under waterlogging. Kinetin also alleviated Cadmium effects reverted in *Vigna angularis* (Ahanger *et al*., 2020), Mn pollution (Gangwar *et al*., 2010) in maize. Kinetin was involved in various physiological and developmental mechanisms of plant system, e.g., nutrient mobilization, cell division and also inhibited ethylene production. However, SA also increased biosynthetic enzyme activities which helps plant to tolerance under waterlogging (Bai *et al*., 2009; Yang *et al*., 2013) and also showed root surface increase as a result, root dry weight increased (Kim *et al*., 2018). In the present study, it has been found that SA and KN remarkably improved root DW under waterlogging stress (Figure 17B, 17C, 18B and 18C).

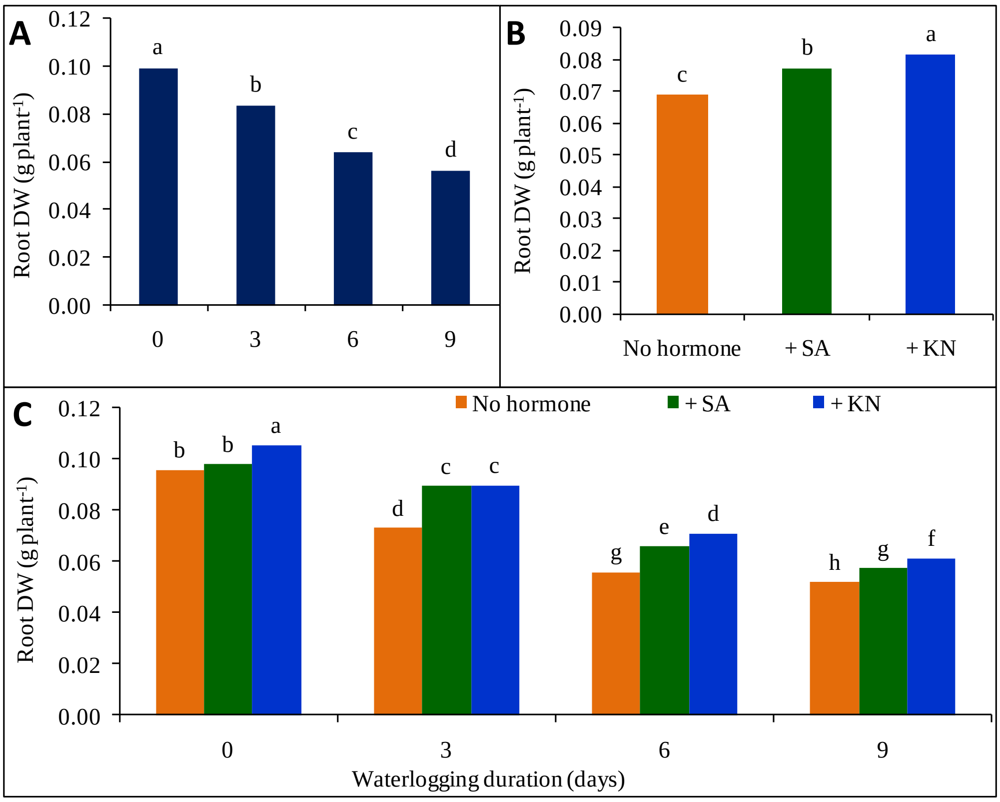

Figure 18. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on root DW of *G. max* at 40 DAS. Here, SA and KN indicate salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.1.10 Root phenotypes

In this study, no adventitious root formed in control plants. Under waterlogging condition adventitious root formed and root growth gradually reduced with increasing waterlogging duration. In the contrary, the application of SA and KN improved the root structure. Salicylic acid showed more prominent result rather than KN.

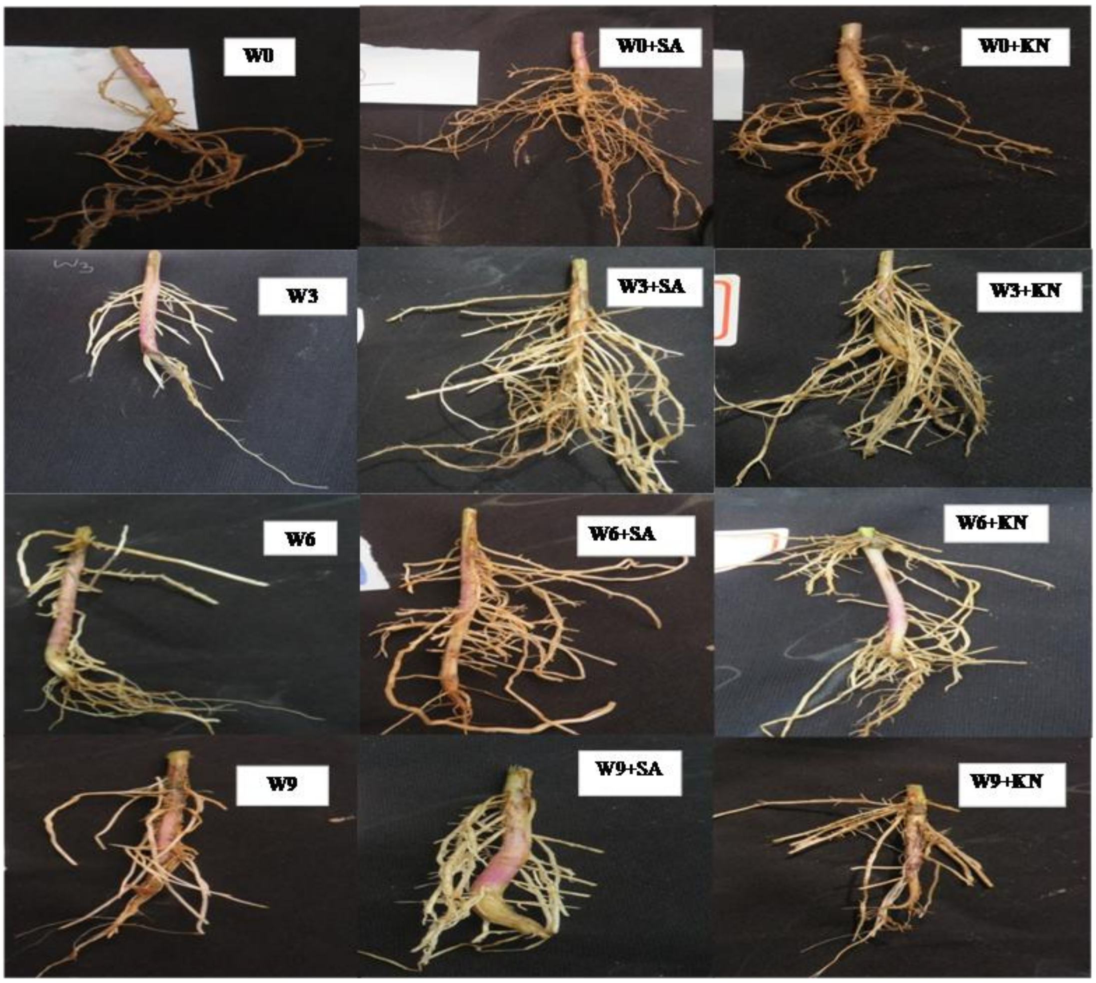

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Figure 19. Root phenotype at 40 DAS of *G. max* as affected by the different duration of waterlogging supplemented with SA and KN. Here, W0, W3, W6 and W9 indicates waterlogging for 0, 3, 6 and 9 days, respectively and SA and KN indicate salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively.

One of the main adaptive responses under waterlogging stress is adventitious root formation (Yin *et al*., 2009; Ahmed *et al*., 2002). In soybean adventitious roots under waterlogging showed high rupture of cortex cells, creating aerenchyma used as pores for O_2 transfer to roots, being a plant survival mechanism under these conditions (Beutler *et al*., 2014). Adventitious roots have not been found in soybean control plants (Kim *et al.,* 2018). This experiment also supported the hypothesis. Morphological acclimatization to waterlogging in soybean appears to adventitious root formation. However, foliar application of SA increased adventitious root development and aerenchyma cells in soybean (Kim *et al*., 2018; Yang *et al*., 2013; Brodersen *et al*., 2005). Kinetin also increased root length and helps to formation of adventitious root by interpretation of carbohydrate in root (El-Shahaby *et al*., 2002).

In this experiment, also showed adventitious root and improved root structure under waterlogging due to foliar application of SA and KN

4.2 Physiological parameters

4.2.1 Electrolyte leakage

Electrolyte leakage activity increased markedly under waterlogging stress comparing to control. When the duration of waterlogging increased the content of EL became gradually high. After 30 DAS, it was 29%, 57% and 144% higher for 3, 6 and 9 days waterlogging, respectively in contrast to control (Figure 20A). Similarly, after 40 DAS 45%, 154% and 280% higher for 3, 6 and 9 days waterlogging, respectively in contrast to control (Figure 21A). The highest content of EL was recorded in the longest duration (9 days) of waterlogging which was 131% at 30 DAS and 303% at 40 DAS (Figure 20C and 21C). In soybean plant, lower accumulation of EL content was observed due to the supplementation of SA and KN under waterlogging. More effective result showed by KN application 20% reduced in comparision to control at any measurement (20A and 21A). Salicylic acid decreased the EL content 28%, 13%, 4% at 30 DAS and 21%, 25% and 18% at 40 DAS for 3, 6 and 9 days of waterlogging, respectively, compared to the same duration of waterlogging treatment alone. Similarly, at 30 DAS EL content reduced 22%, 22%, 11% and at 40 DAS, the reduction was 19%, 4% and 5% for 3, 6 and 9 days of waterlogging, respectively compared to corresponding control (Figure 20C and 21C).

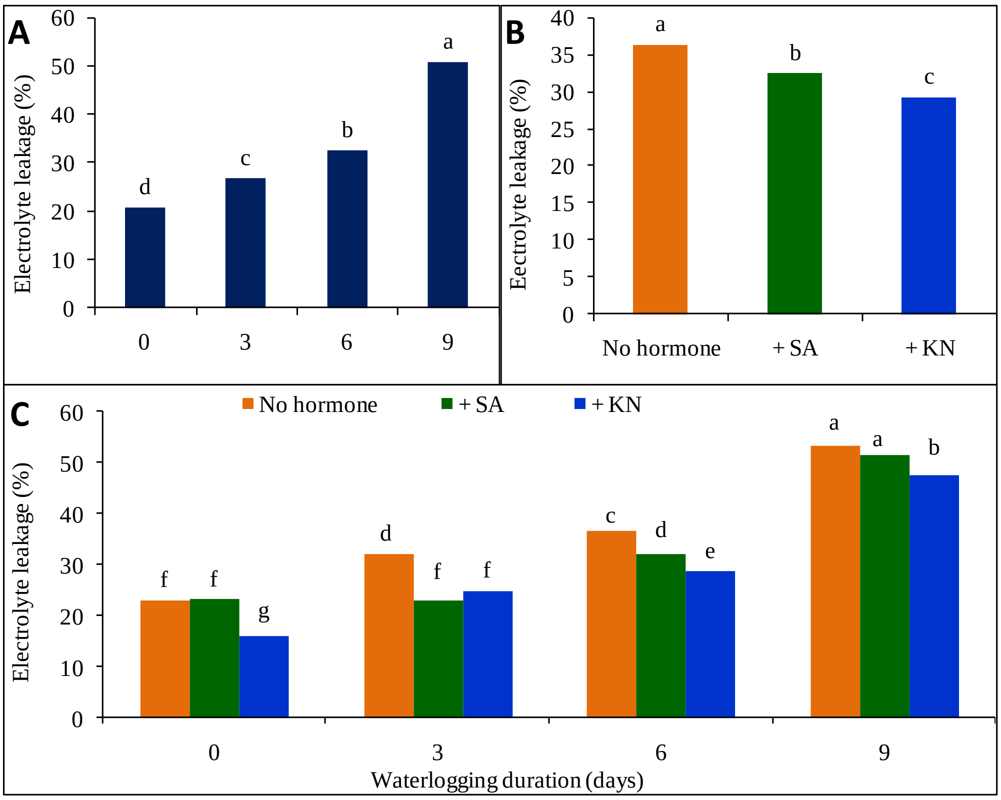

Figure 20. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on Electrolyte leakage of *G. max* at 30 DAS**. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

In this study, EL was increased due to waterlogging in a duration-dependent manner at any ages of plants (Fig 13). Electrolyte leakage was enhanced with increasing stress level as compared to the control. Yordanova and Popova (2007) showed that under waterlogging condition EL significantly increased in maize. Similarly, Khan e*t al.* (2014) and Ghoulam *et al.* (2002) observed the same increasing trend of electrolyte leakage in salt sensitive cucumber and sugar beet cultivar, respectively. On the contrary, the supplementation of SA and KN reverted the negative effects of waterlogging stress in the case of EL. Zhang *et al.* (2019) recorded that the application of phytohormone reduce EL content under waterlogging by reducing H_2O_2. Salicylic acid may alleviate the negative effects of waterlogging stress on plants by enhanching antioxidant mechanism. Previous studies have confirmed these outcomes (Bai *et al.*, 2009). Similarly, KN also declined the EL content during stress supported by (Kaya et al., 2018; Ahanger *et al.*, 2018).

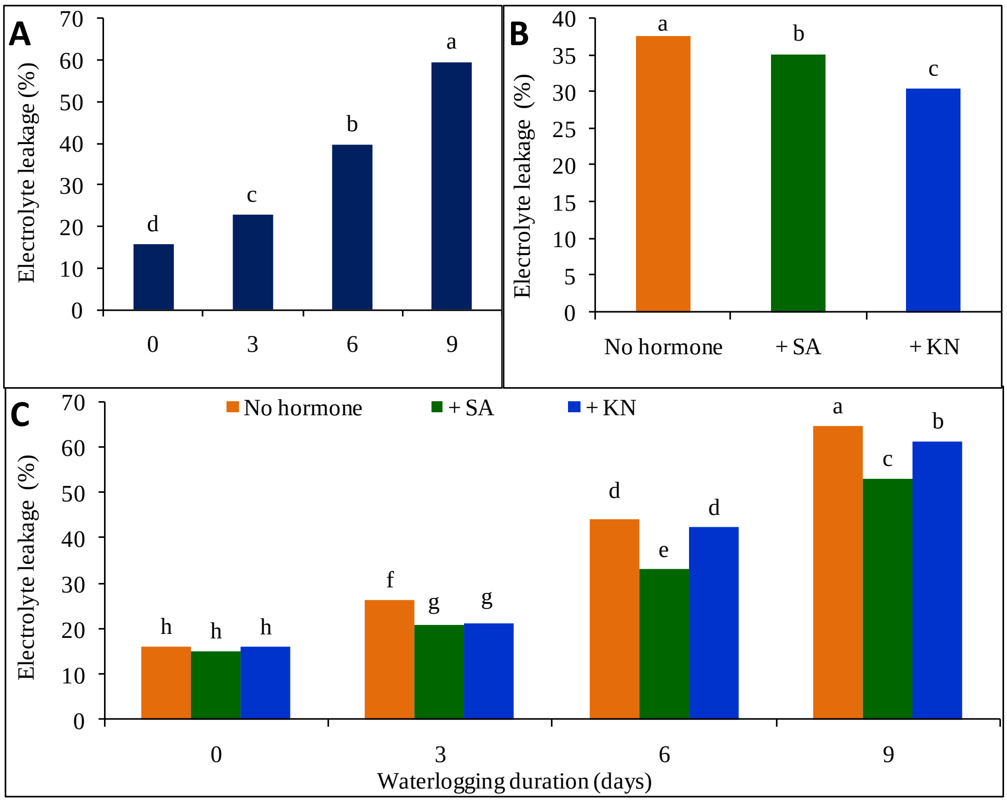

Figure 21. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on EL content of *G. max* at 55 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.2 Relative water content (RWC)

Leaf RWC showed variable responses to waterlogging. The lowest RWC was recorded in the longest duration of waterlogging (9 days) at 30 and 55 DAS which was 21% and 33%, respectively, compared to control (Figure 22A and 23A). In the interaction figure showed RWC gradually declined. It was 7%, 17% and 28% lower for 3, 6 and 9 days waterlogging, respectively in contrast to control at 30 DAS (Figure 22C). Similarly, after 55 DAS the declined amount was 19%, 29% and 35% lower for 3, 6 and 9 days waterlogging, respectively, compared to control at 55 DAS (Figure 23C). However, foliar application of both SA and KN effectively increased RWC contents by reducing the waterlogging negative effects. It was 10% higher for SA and 8% higher for KN at 30 DAS (Figure 22B) and similarly, after 55 DAS 9% higher for SA and 7% higher for KN in comparision to no hormone sprayed plants (Figure 23B). At 30 DAS, 24% higher for SA and 15% higher for KN, compared to 9 days waterlogged without hormone (Figure 22C). Relative water content also increased at 55 DAS, 17%, 20% higher for SA and 13%, 18% higher for KN at 3 and 6 days of waterlogging, respectively, compared to corresponding control (Figure 23C).

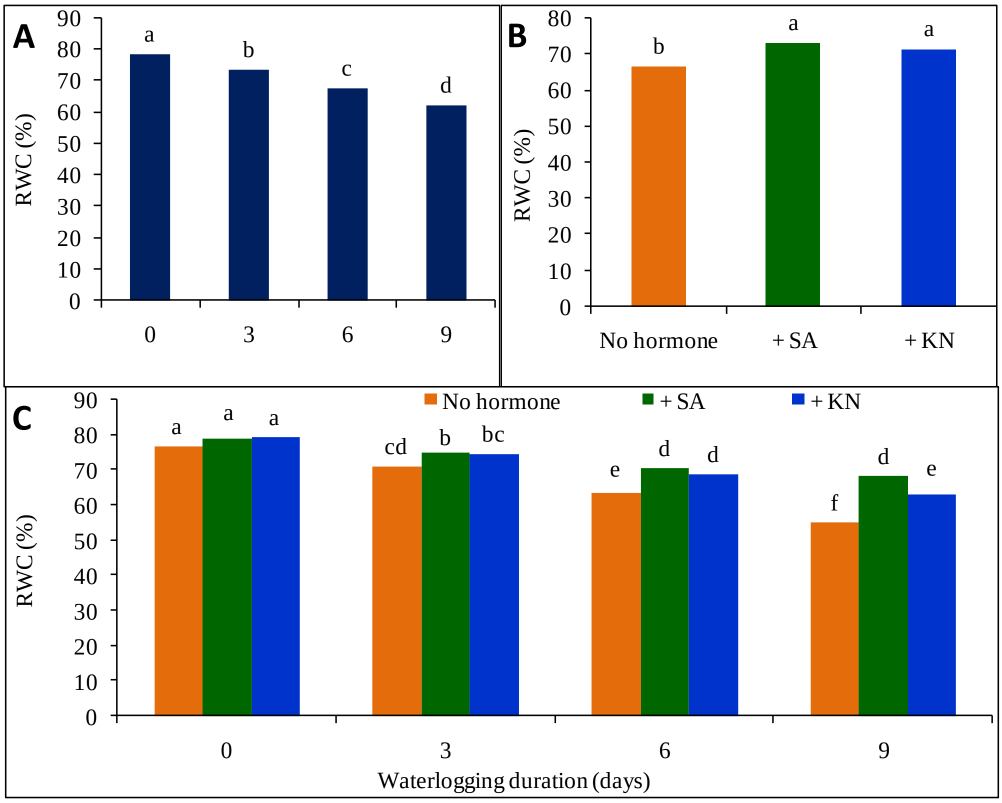

Figure 22. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on RWC content of *G. max* at 30 DAS**. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

In our study, Waterlogging led to a substantial reduction of RWC content in duration depend manner at any age of plants (Figure 22A, 23A). Reduction in leaf RWC suggests an insufficient supply of water for cell expansion. Despite the excess quantity of water available under waterlogged conditions, RWC were reduced of soybean plants. This may be occurred due to domination of waterlogging, which hampered root permeability (Asharf, 2012) and as a result, leaf wilting symptoms were observed on plants. Similar results found in different plants by (Anee *et al.*, 2019; Kumar *et al.*, 2013). However, foliar application of SA and KN increased the RWC of leaves (Alam *et al.*, 2013 for SA; Ahanger *et al.*, 2018 for KN; Kaya *et al.*, 2018 for KN). Salicylic acid may help to reduce adverse effects of stress by increasing water status (Kadioglu *et al.*, 2011), photosynthetic pigment content and antioxidant defense. Smilarly, KN ameliorated waterlogging-induced adverse effects on RWC by stomatal regulation and increased soluble sugar (Pospíšilová *et al.*, 2000). Kinetin enhanced membrane integrity in the conservation of tissue water quality (Ahanger *et al.*, 2018).

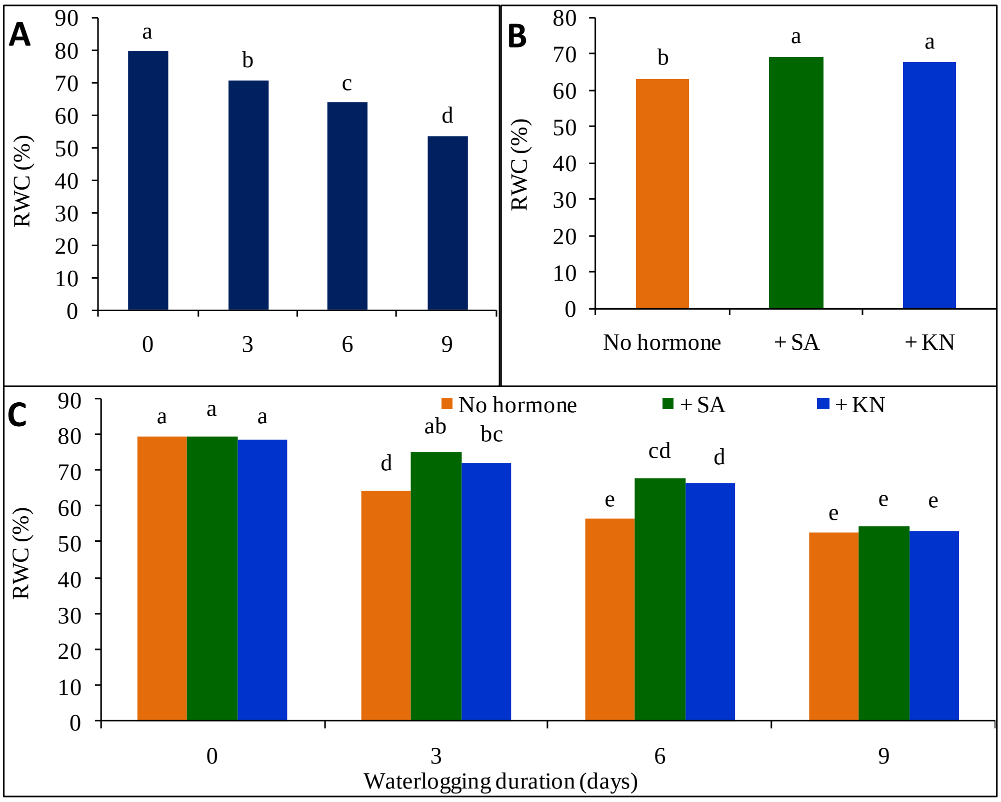

Figure 23. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on RWC content of *G. max* at 55 DAS**. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.2.3 SPAD value

SPAD reading is the indicator of chl content of leaf. Upon exposure to waterlogging stress, SPAD value changed at every growth stage. At 20 DAS, no difference of SPAD value among (Figure 24). But after treatment was showed difference and it was 4%, 5% and 3% for 3, 6 and 9 days waterloggingg compared to control (25A). In the contrary, foliar spray of SA and KN increased the SPAD value at every stage of growth, compared to control plants of all treatments (Figure 25B and 25C).

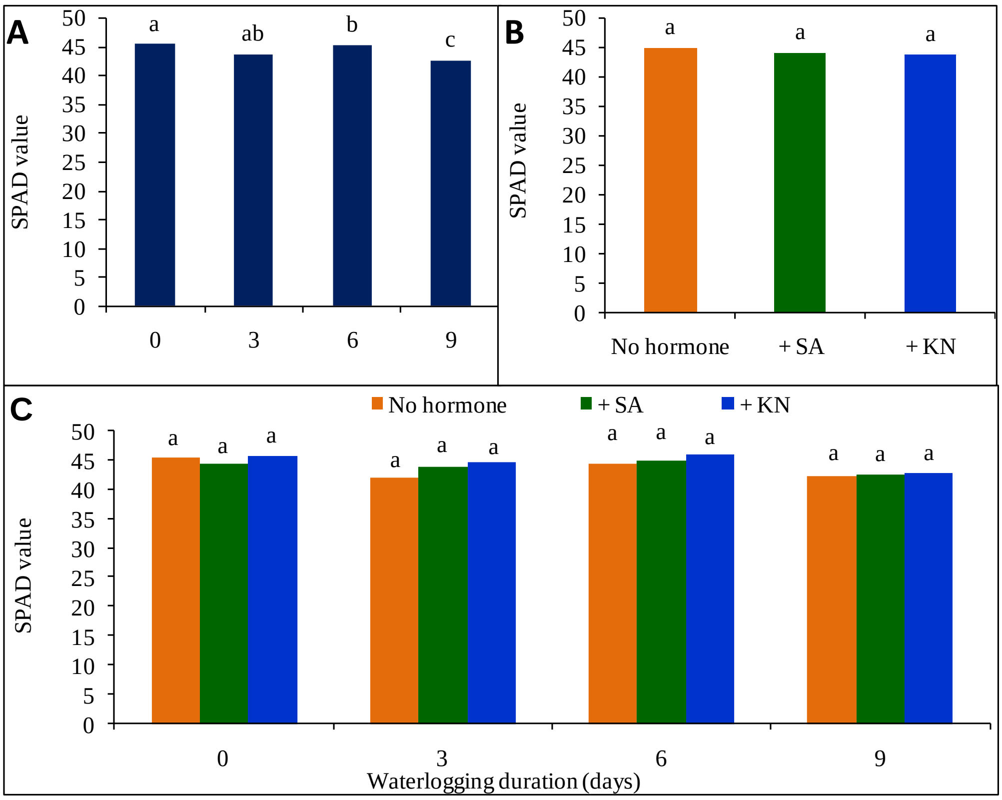

Figure 24. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on SPAD value of *G. max* at 20 DAS**. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

Photosynthesis is one of the most important physiological mechanism. (Ramachandra *et al.*, 2004). In this experiment, SPAD value was decreased due to waterlogging stress (Fig 14). However, at an earlier stage (15 DAS) did not show any differences in SPAD value.Waterlogging in soybeans showed a decrease in photosynthetic activity (Mutava *et al*., 2015). Tian *et al*. (2019) showed that SPAD value reduced 10-38% in KY16 variety and 5-30% in DMY1 variety of maize due to waterlogging. Previous studies recorded that waterlogging reduced the Chl content as a result of the reduction of photosynthetic activity and rate, which decline plant development and biomass accumulation. It has been found that waterlogging remarkably decreased N uptake in soybean leaves and branches. The results of prolonged waterlogging CO_2 assimilation declined, which caused the reduction of photosynthesis (Yordanova and Popova, 2007). In the contrary, supplementation of phytohormones (SA and KN) increased photosynthesis by reducing the negative effect of waterlogging. Salicylic acid protects chlorophyll loss and increase net photosynthesis against different stresses in soybean (Noriega *et al*., 2012; Khan *et* *al*., 2012). The exogenous application of KN significantly improved chlorophylls and carotenoid which is vital elements of photosynthesis. Hence, photosynthesis increased under stress. Similar findings were reported in other studies on different crops under different stresses (Ahanger *et al*., 2020; Wang *et al*., 2015).

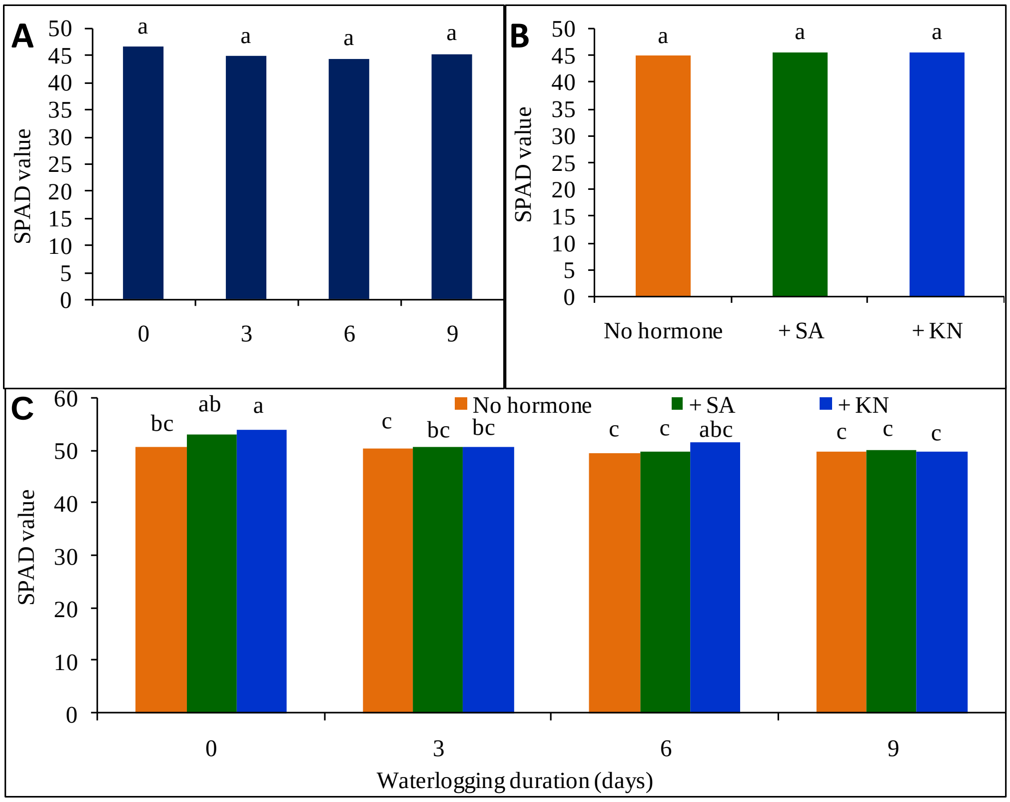

Figure 25. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on SPAD value of *G. max* at 40 DAS**. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.3 Oxidative stress indicators

4.3.1 Lipid peroxidation

Waterlogged *G. max* plants showed remarkably higher MDA contents compared to control plants and the increment happened in a duration-dependent manner. The highest MDA was recorded in longer duration waterlogged plants (9 days) at any measurements. It was 80% higher at 30 DAS and 51% higher at 55 DAS in comparision to the control (Figure 26A and 27A). After 30 DAS, the plants which were 3, 6 and 9 days waterlogged 41%, 75% and 106%, respectively higher MDA and after 55 DAS 26%, 59% and 60%, respectively higher MDA were recorded in contrast to the control (Figure 26C and 27C). In the contrary, SA and KN significantly reduced the accumulation of MDA content. It was 16% lower for SA and 16% lower for KN at 30 DAS (Figure 26B) and 9% lower for SA and 12% lower for KN at 55 DAS (Figure 27B) compared to the no hormone sprayed plants. On other hand, the reduction was 22%, 25% and 27% lower for SA, and 25%, 12% and 23% lower for KN at 3, 6 and 9 days of waterlogging, respectively, compared to the same duration of waterlogging treatment alone at 30 DAS (Figure 26C). Similarly, at 55 DAS, the reduction was 14%, 23% and 6% for SA and 14%, 15% and 6% for KN at 3, 6 and 9 days of waterlogging, respectively, compared to the same duration of waterlogging only (Figure 27C).

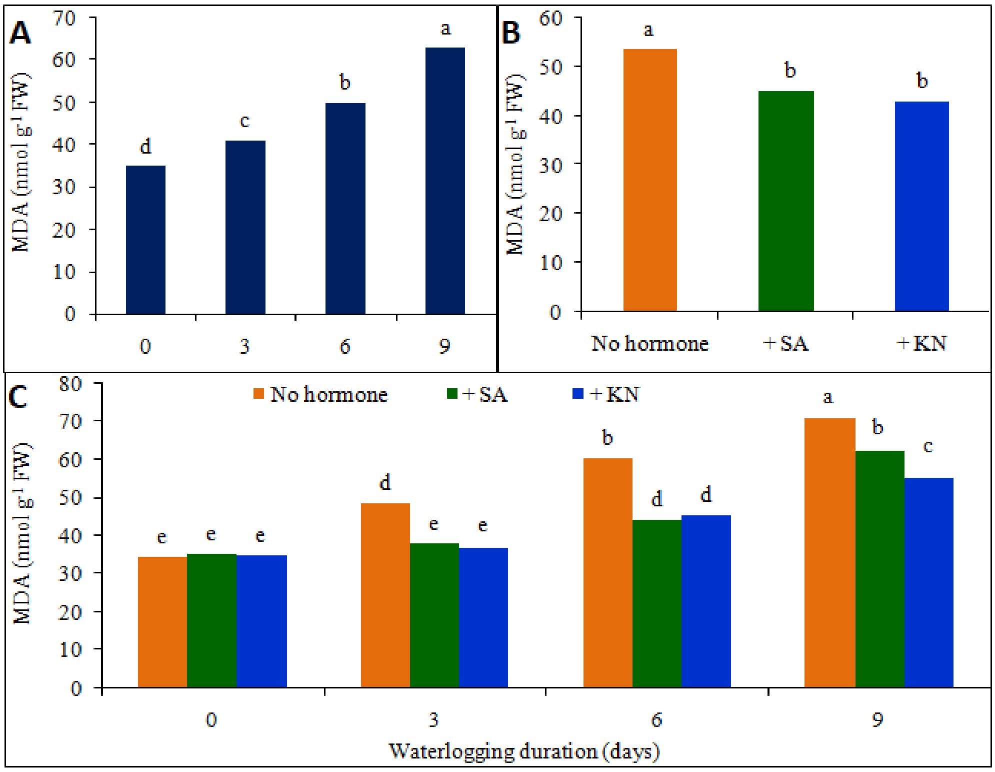

Figure 26. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on MDA content of *G. max* at 30 DAS**. Here, SA and KN indicate salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

A well-known index for evaluating the extent of oxidative stress is lipid peroxidation (Hasanuzzaman *et al.*, 2012). The integrity and function of cell membranes is damaged by MDA and finally cell death (Panda and Choudhury, 2005). In this study, MDA was increased sharply due to waterlogging at any age of plants (Figure 27 and 27). Waterlogging-induced increase in MDA was observed in soybean in several plant studies (Da-Silva and Do Amarante, 2020). Several scientists also have found MDA increase in waterlogging condition in different crops in a duration-dependent manner (Duarte *et al*., 2019; Souri *et al*., 2020; Saha *et al*. 2016; Wei *et al*., 2013; Xu *et al*., 2012; Yin *et al*., 2009). On the other hand, exogenous application of SA and KN alleviated the negative effect of waterlogging stress on soybean in the case of MDA content. Salicylic acid regulates different roles under abiotic stress conditions. Salicylic acid reduced oxidative stress by scavenging ROS. MDA content declined in SA treated plants under abiotic stress were observed in soybean (Liu *et al*., 2017; Kim *et al*., 2018; Noriega *et al*., 2012) and also found significant result in different plant studies (Hayat *et al*., 2012, Alam *et al*., 2013, Kadioglu *et al*., 2011). Similarly, KN also reduced the accumulation of ROS by reducing MDA in tomato under salt stress (Ahanger *et al*., 2018) and in maize under heavy metal stresses (Wang *et al*., 2015).

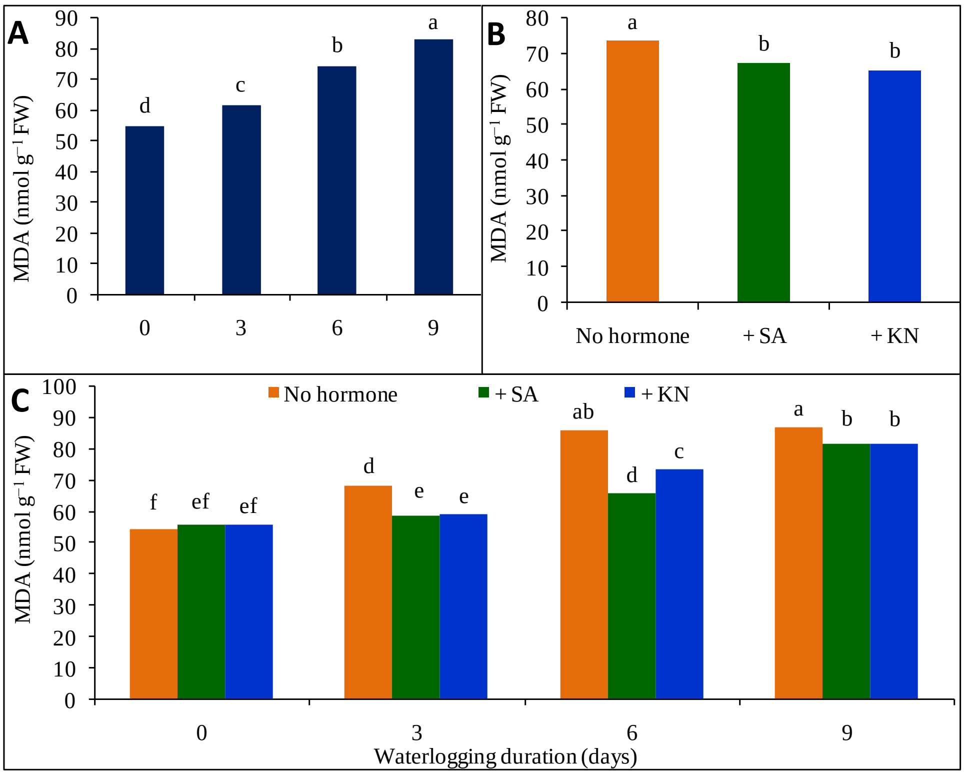

Figure 27. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on MDA content of *G. max* at 55 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.3.2 H_2O_2 content

In any kind of abiotic stress, H_2O_2 production is a common phenomenon. The H_2O_2 level in *G. max* leaves increased significantly under waterlogging especially, at a longer duration of waterlogging. At 9 days the highest H_2O_2 level was determined in waterlogging plants compared to control. The amount was 195% higher at 30 DAS and 88% higher at 55 DAS compared to control (Figure 28A and 29A). At 30 DAS, 109%, 179% and 227% higher H_2O_2 and at 55 DAS 36%, 72% and 103% higher H_2O_2 were observed waterlogged for 3, 6 and 9 days, respectively in contrast to control (Figure 28C and 29C). On other hand, the application of SA and KN diminished the level of H_2O_2 content under waterlogging compared to corresponding control plants. In figure 28B and 29B showed 21% lower for SA and 26% lower for KN at 30 DAS and 12% lower for SA and 13% lower for KN in contrast to without hormone applied plants. However, after 30 DAS 31%, 37%, 20% lower and at 55 DAS 14%, 24%, 9% lower for SA at 3, 6 and 9 days of waterlogging, respectively in contrast to corresponding control (Figure 28C and 29C). Similarly, the reduction of H_2O_2 level was 33%, 26%, 9% at 30 DAS and 20%, 17%, 12% lower at 55 DAS for KN foliar spray at 3, 6 and 9 days of waterlogging, respectively in contrast to corresponding control (Figure 28C and 29C).

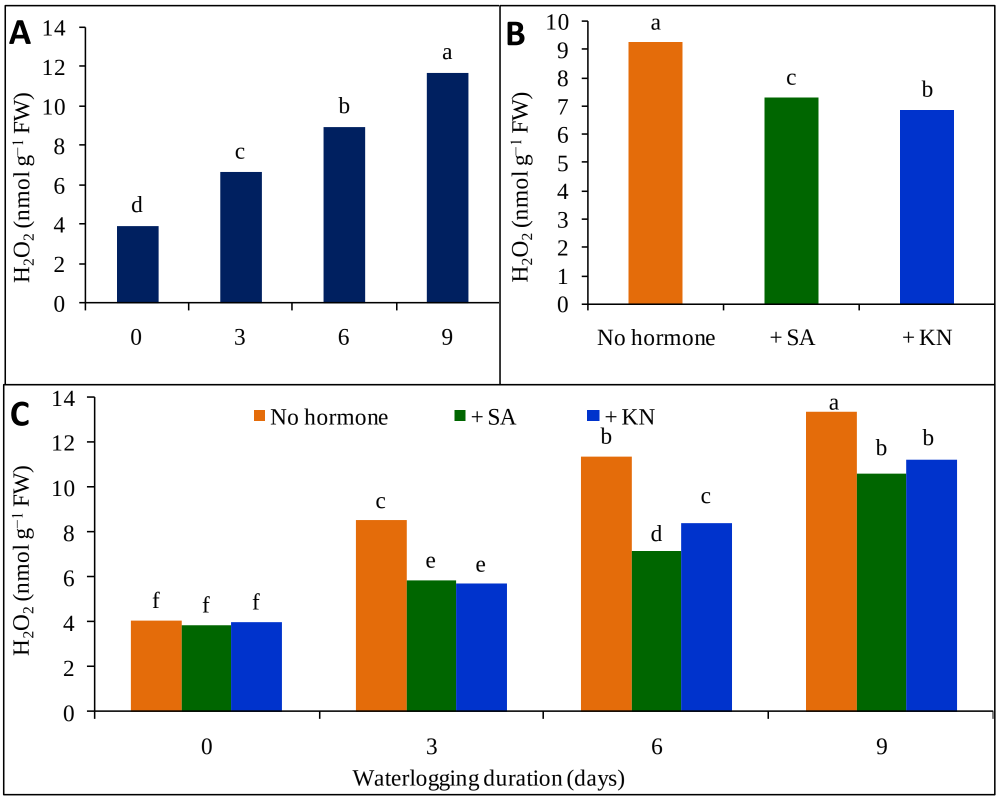

Figure 28. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on H_2O_2 content of *G. max* at 30 DAS**. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

In this study, H_2O_2 was increased like MDA due to waterlogging stress in a duration-dependent manner at any age of plants (Figure 11). H_2O_2 may inactivate enzymes by oxidizing their thiol groups. Waterlogging-incited H_2O_2 was observed in soybean (Da-Silva and Do Amarante, 2020). Waterlogging accumulated excess amount of ROS in different forms and different subcellular compartments. These ROS include both free radicals ($O_2^{\bullet -}$ and $OH^{\bullet}$) and molecules such as H_2O_2 and $^1O_2$ (Jaspers and Kangasjärvi, 2010). Several researchers have reported greater accumulation of H_2O_2 under anaerobic conditions (Hasanuzzaman *et al.*, 2012; Kumutha *et al.*, 2009). However, the foliar application of SA and KN enhanced the activity of antioxidant enzymes which helped in detoxifying H_2O_2. Salicylic acid reduced H_2O_2 by increasing enzymatic activities in soybean leaves under waterlogging stress (Mishra *et al.*, 2013). Salicylic acid also showed the significant result in different crops under different stresses like under waterlogging stress in *Malus robusta* (Bai *et al.*, 2009), drought stress in wheat (Waseem *et al.*, 2006), salinity stress in maize and wheat

(Arfan *et al.*, 2007), drought stress in mustard (Alam *et al.*, 2013). The membrane stability, antioxidant mechanism (both enzymatic and non-enzymatic) and osmotica accumulation significantly rise as the supplementation of KN. Kinetin also reduced the accumulation of ROS by reducing $O_2^{\bullet -}$ and H_2O_2 content. Ahanger *et al.* (2018) was recorded significant result under salinity stress in tomato and Singh and Prasad (2014) also showed the KN exogenous application result in egg plants under Cd stress and heavy metal stress in maize (Wang *et al.*, 2015).

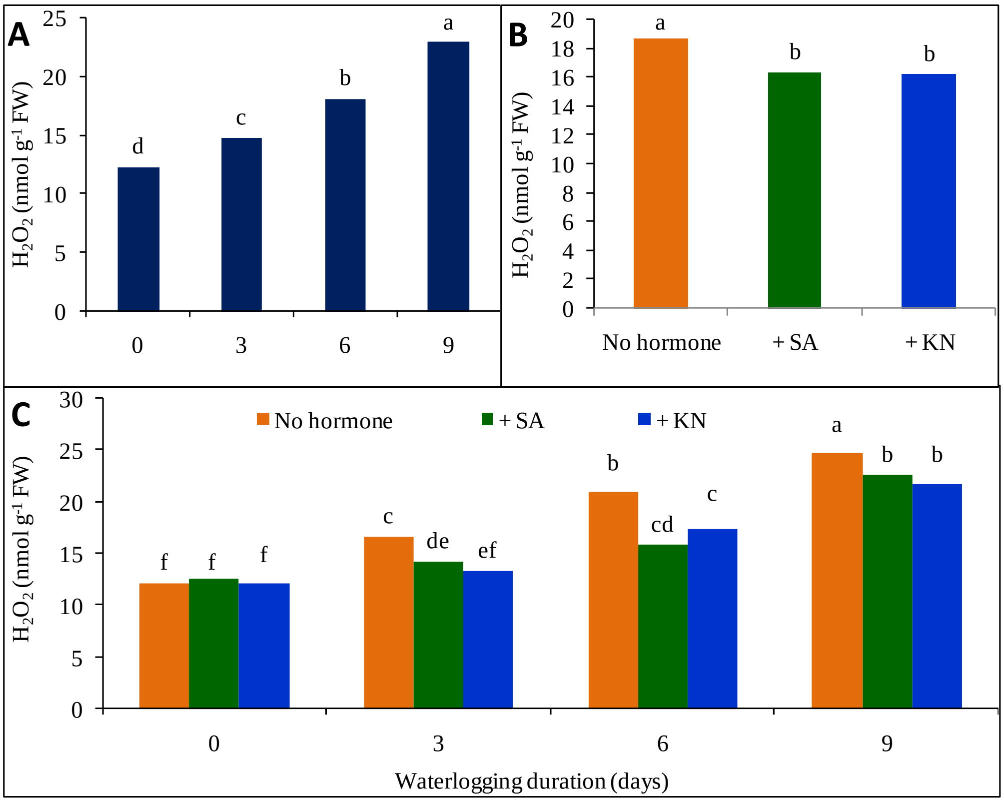

Figure 29. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on H_2O_2 content of *G. max* at 55 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.3.3 Proline content

Figure 30A and 31A showed that Pro content increased significantly upon exposure to waterlogging compared to control plants. Pro content increase in plants gradually with increasing waterlogging duration. After 30 DAS, 30%, 43% and 79% higher for 3, 6 and 9 days waterlogging in comparision to control (Figure 30A) and similarly, after 55 DAS 22%, 34% and 55% higher for 3, 6 and 9 days waterlogging in contrast to control (Figure 31A). The highest level of Pro content was determined at 9 days waterlogged and the lowest was determined at 3 days waterlogged which was 108% and 58%, respectively at 30 DAS (Figure 30C). Similarly, at 55 days also recorded the highest level of Pro level at 9 days waterlogged was 77% and the lowest recorded at 3 days was 38% compared to control (Figure 31C). However, supplementation with SA and KN significantly reduced Pro content compared to control. It was 15% lower for SA and 20% lower for KN (Figure 30B). After 30 DAS, the crucial reduction of Pro by SA at 3 days waterlogging 29.45%. Similarly, the reduction at 6 and 9 days was 27% and 17%, respectively. Kinetin also reduced the Pro content effectively 15%, 26%, 18% for 3, 6 and 9 days of waterlogging, respectively, compared to the same duration of waterlogging treatment alone at 30 DAS (Figure 30C). Similarly, after 55 DAS, the reduction was 16%, 20% and 17% for SA and 19%, 26%, 20% for KN at 3, 6 and 9 days of waterlogging, respectively, compared to the same duration of waterlogging only (Figure 31C).

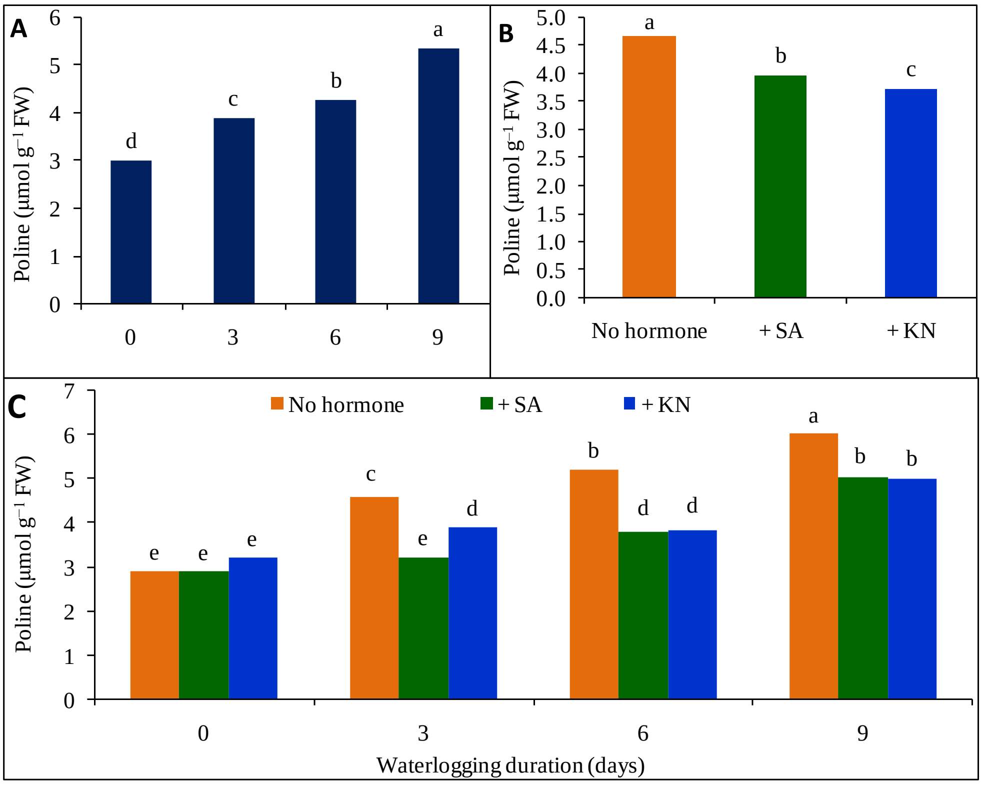

Figure 30. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on proline content of *G. max* at 30 DAS**. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

Proline plays very important role under abiotic stress conditions, including waterlogging stress. An increased of Pro level under waterlogging conditions was considered to be involved in osmoregulation and restoration of water status. It also acts as a ROS scavenger and acts as an antioxidant (Hoque *et al*. 2007) regulator under stress conditions. Previous studies showed that Pro content increased with increasing waterlogging duration in maize (Tian *et al*., 2019) and also increased under drought stress in rapeseed (Bhuiyan *et al*., 2019). In this study, Pro was increased due to the imposed of waterlogging in a duration manner at any age of plants (Figure 30 and 31). The accumulation of Pro is also a sign of stress introduction (Rampino *et al*., 2006). Upon exposure to waterlogging, Pro content increased in some case. The result was supported in a previous study (Barickman *et al*., 2019) in cucumber. In other hand, SA and KN both reduced the accumulation of Pro content under abiotic stresses, including waterlogging. Besides, it increased the enzymatic activities supported by (Alam *et al*., 2013; Moumita *et al*., 2019).

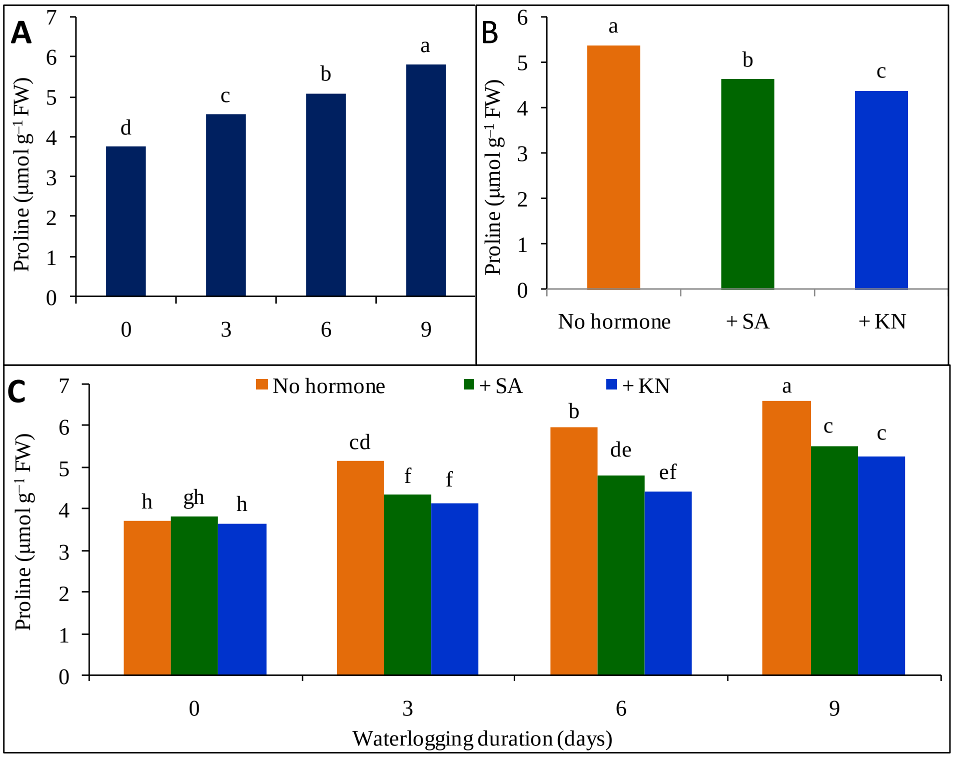

Figure 31. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on proline content of *G. max* at 55 DAS. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.4 Anatomy

In this experiment, we made the thin transverse sections of waterlogged soybean stem and root at the level of waterlogging. Later, it was observed under a digital microscope, which showed the formation of distinct aerenchyma in shoot (Figure 32) and root (Figure 33) due to waterlogging. Under waterlogging aerenchyma formed and the number and diameter of aerenchyma increased with increasing waterlogging duration (Figure 32 and 33).

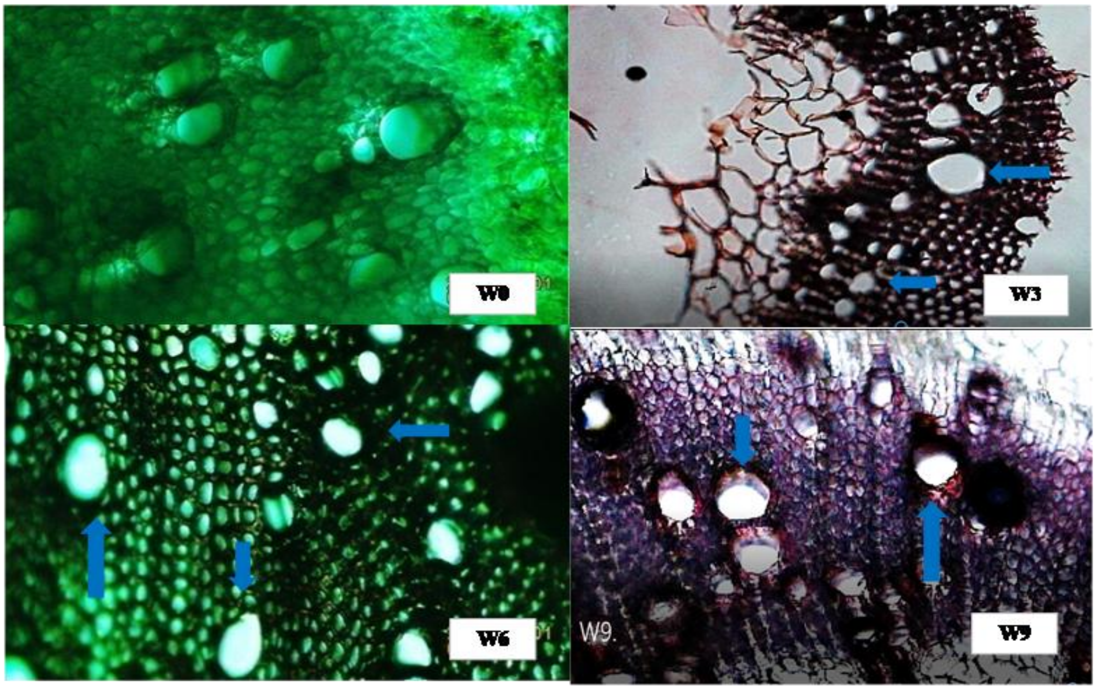

Figure 32. Shoot anatomy of *G. max* as affected by the different duration of waterlogging. Here, W0, W3, W6 and W9 indicates waterlogging for 0, 3, 6 and 9 days, respectively.

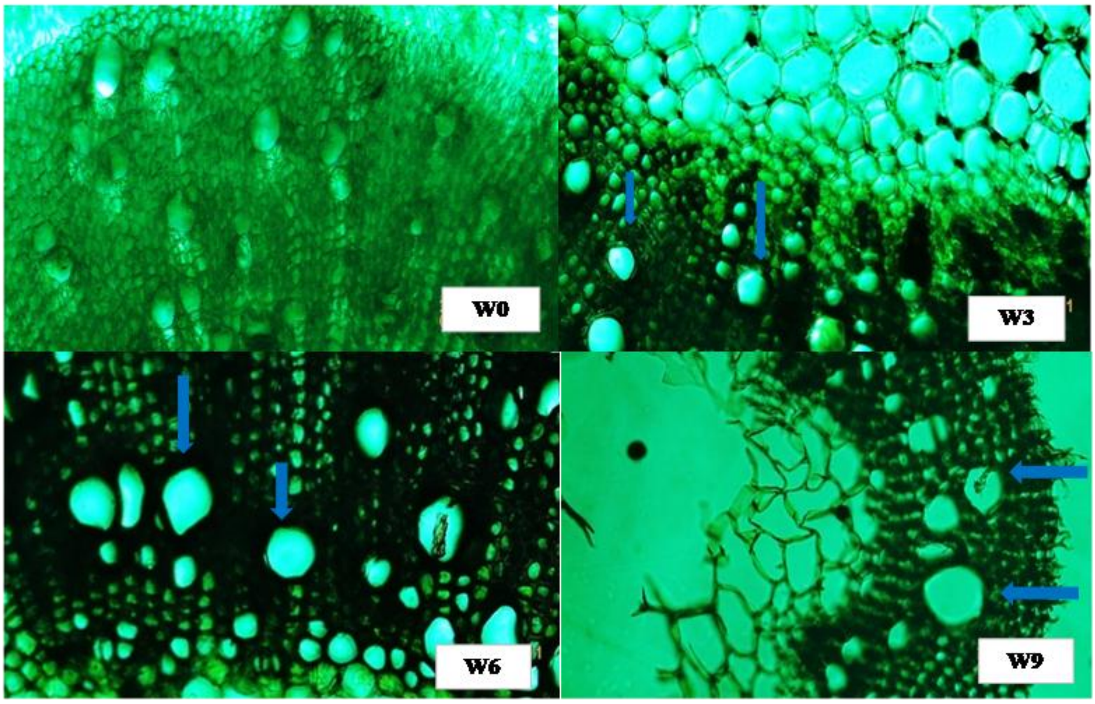

Figure 33. Root anatomy of *G. max* as affected by the different duration of waterlogging. Here, W0, W3, W6 and W9 indicates waterlogging for 0, 3, 6 and 9 days, respectively

The gass-filled spaces in cell or aerenchyma have been demonstrated in the swollen root of plants (Jackson, 2006). The most common anatomical responses of plants in waterlogging conditions are characterized as the formation of aerenchyma in both root and shoot. These responses at the anatomical level enable the plants to facilitate the oxygen capture for submerged tissues, which ultimately alleviated the hypoxic conditions (Wei *et al*., 2013).

Stomatal density significantly increased with increasing waterlogging duration and smaller stomata found in control plants. Maximum stomata number found waterlogging for 9 days (Figure 34). Wang *et al*. (2008) recorded that the stomatal density increased with increasing waterlogging duration.

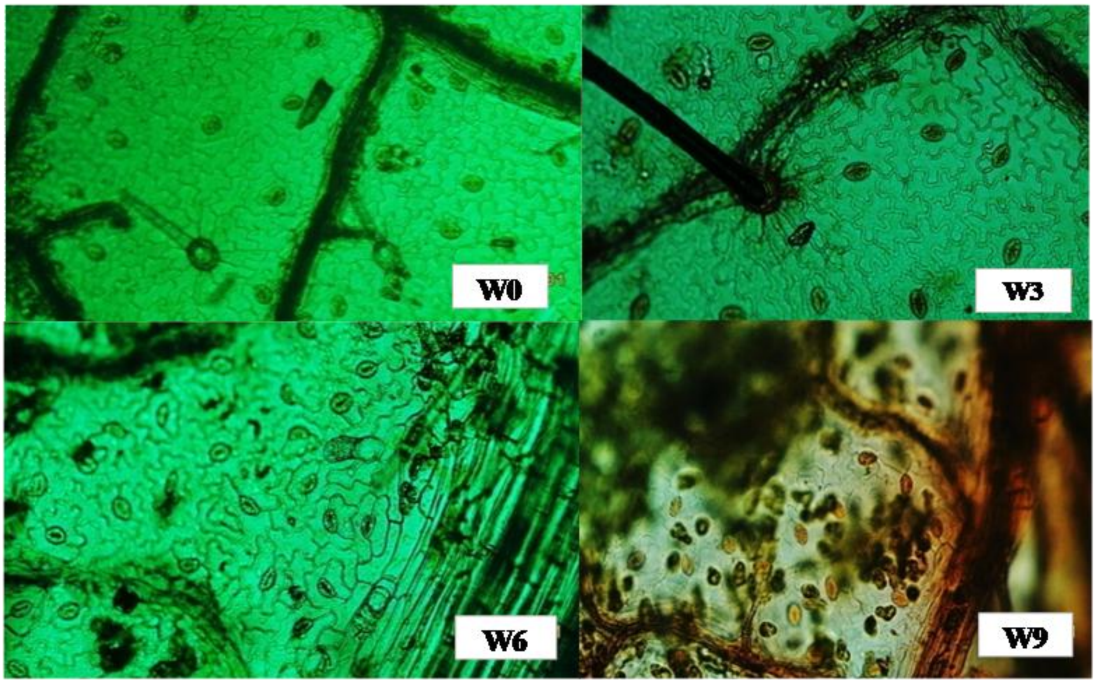

Figure 34. Leaf anatomy of *G. max* as affected by the different duration of waterlogging. Here, W0, W3, W6 and W9 indicates waterlogging for 0, 3, 6 and 9 days, respectively.

4.5 Yield contributing parameters

4.5.1 Pod length

Pod length of *G. max* was dramatically changes upon exposure to waterlogging. Compared to control, pod length decrease waterlogging for 3 and 6 days but increase at 9 days waterlogging (35A and 35C). However, supplementation of SA and KN significantly increase the pod length at 3 and 6 waterlogging rather than 9 days waterlogging treatment compared to the same duration of waterlogging only (Figure 35B and 35C).

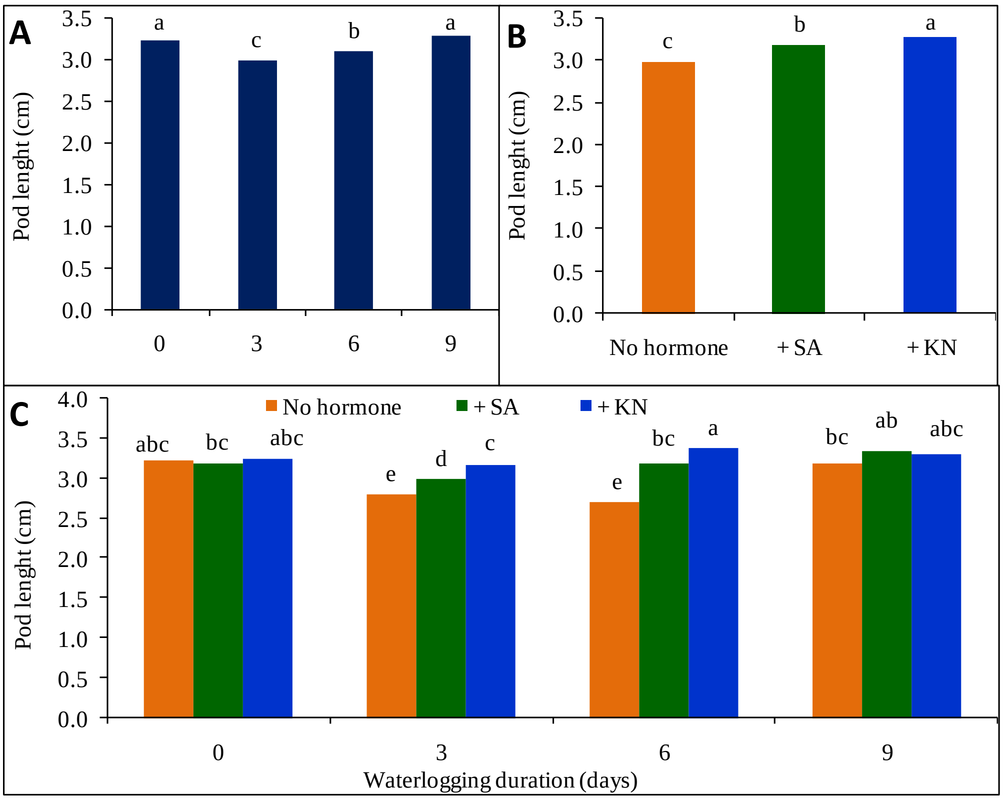

Figure 35. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on the pod length of *G. max***. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.2 Number of pods plant^{-1}

The reduction of number of pods was gradualy declined with increasing waterlogging duration (Figure 36A). The plants waterlogged for 9 days showed the lowest number of pod number per plant (Figure 36C), which was 39% lower than the control plants. But the foliar spray of SA and KN increased the pod number at all duration and significantly increased pod number at 3 and 6 days waterlogging than 9 days waterlogging plants. It was 17%, 22% higher for SA and 14%, 16% higher for KN at 3 and 6 days waterlogging, respectively, compared to the same duration of waterlogging only (Figure 36C).

In this study, pod number per plant and pod setting significantly reduced due to waterlogging. But pod number increased significantly by foliar application of SA and KN (Figure 36B and 36B). Branch numbers correlated to pod number increasing

(Koyama *et al*., 2019). Similar reduction in plant yield have been reported in soybean (Mustafa and Komastsu, 2014; Miao *et al*., 2012; Beutler *et al*., 2014; Rhine *et al*., 2010). Waterlogging also reduced the pod number other crops were observed in green gram (Kumar *et al*., 2013). However, the exogenous application of SA and KN decreased the dropping of flowering and reduced the loss of pod setting. Fariduddin *et al*. (2003) reported that SA increased number of pods in mustard by enhancing the number of flowers setting per plant. Kinetin also increased pod number by improving flower setting on axillary branches and ultimately increased yield (Atkins *et al*., 2011).

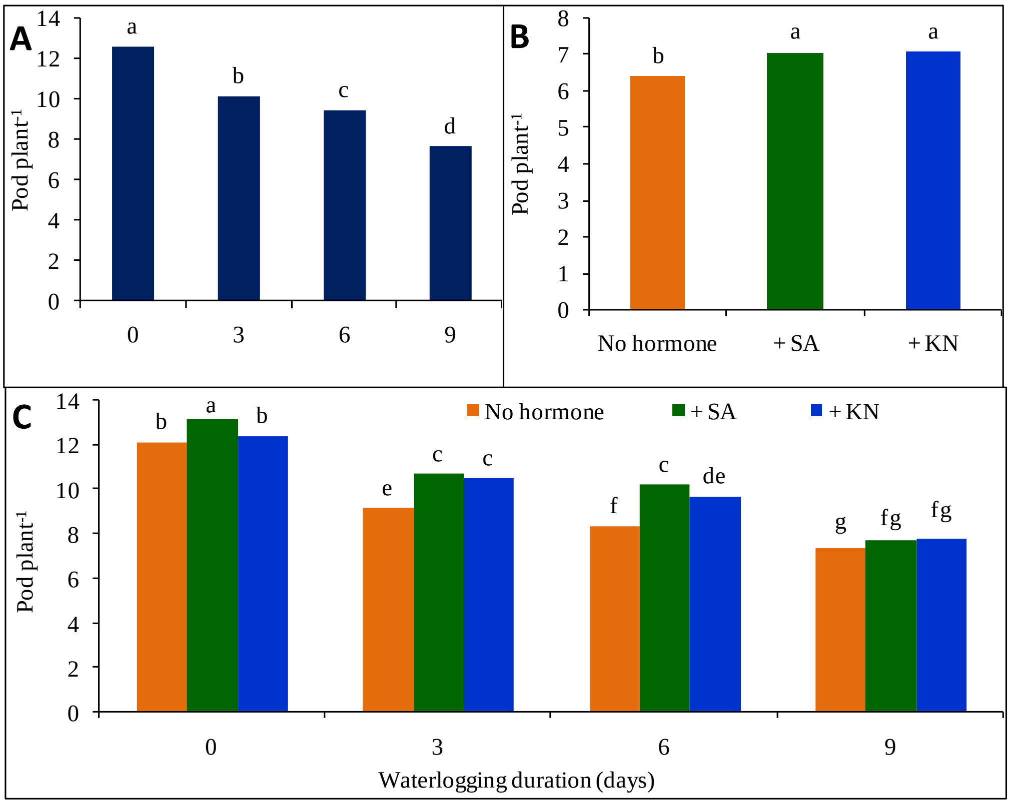

Figure 36. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on the pod number of *G. max***. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.3 Number of seeds pod^{-1}

The imposition of waterlogging stress dramatic result showed in the number of seed pod^{-1}. But the application of foliar spray (SA and KN) significant result showed at all treatment (Figure 37B and 37C).

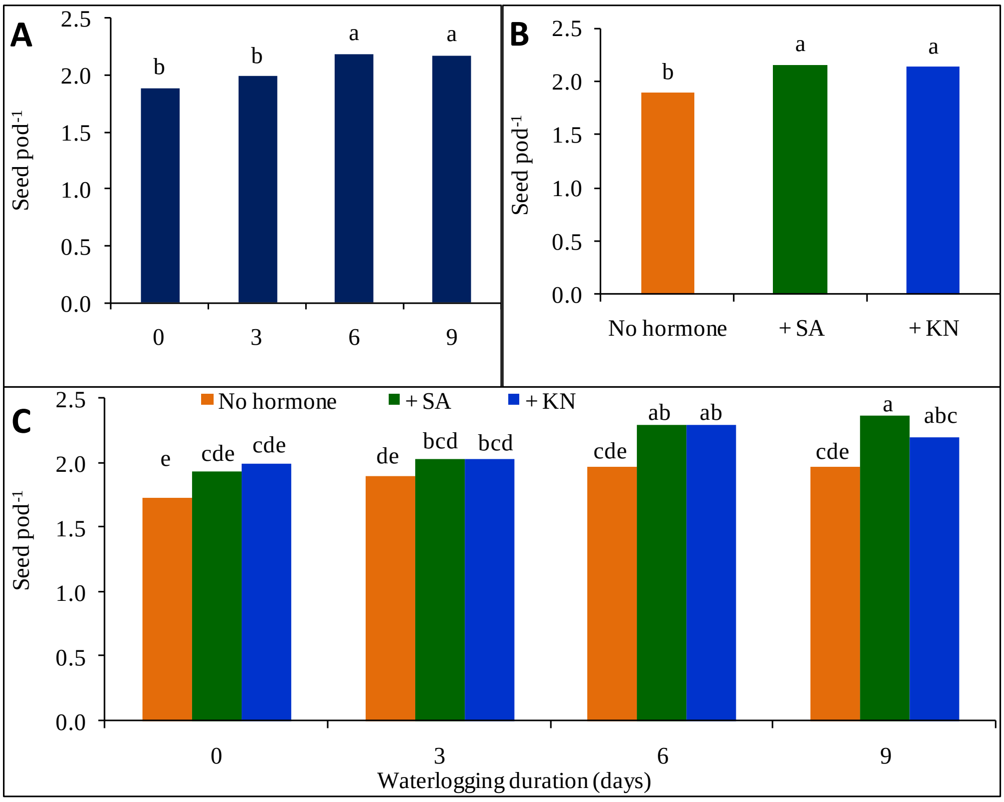

Figure 37. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on the seed number of *G. max***. Here, SA and KN indicate salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.4 Weight of 1000-seed

Upon exposure to waterlogging, 1000-seed weight was sharply reduced compared to control (Figure 38A). The lowest value of 1000-seed weight was recorded in plants waterlogged for 9 days (4.87 g) which were much lower than the control plants (8.02 g) (Figure 38C). However, the SA and KN treated plants increased the seed weight compared to control plants. It was 10% higher for SA and 13% higher for KN compared to control (Figure 38B). Foliar application of SA and KN increased 1000 seed weight under waterlogging and control plants.

This study showed that the decrease in grain yield corresponded to the decrease in the 1000-seed weight (Figure 38). 1000-seed weight gradually decreasing trend in response to an increase in the duration of waterlogging in soybean (Miao *et al.*, 2012; Beutler *et al.*, 2014). Similar results were observed in some other crops like maize (Tian *et al.*, 2019a), wheat and barley (De San Celedonio *et al*., 2014). Waterlogging reduced shoot length, biomass, photosynthesis and pod formation and ultimate result is lower yield. However, foliar application of SA and KN improved 1000-seed yield by reverting the harmful effects of waterlogging. As a result, grain weight decreased. Previous studies showed that KN significantly increased grain yield by improving grain filling, increased 1000-seed weight, endosperm cell division and maximum endosperm cell number, increased the sink capacity, resulting in more assimilate accumulation (Yang *et al*., 2016). In this experiment, KN plays a vital role to increase 1000-seed weight under stress (Figure 39). Zhang *et al*. (2016) showed that KN increase 1000 seed weight in wheat. Moreover, SA also plays an important role to increase 1000-seed weight by improving tolerance capacity, increase flower number, reducing ABA formation and increase grain filling (Khan *et al*., 2003). Previous studies showed that SA increase 1000-seed yield in wheat (Kareem *et al.*, 2017), corn (Zamaninejad *et al*., 2013).

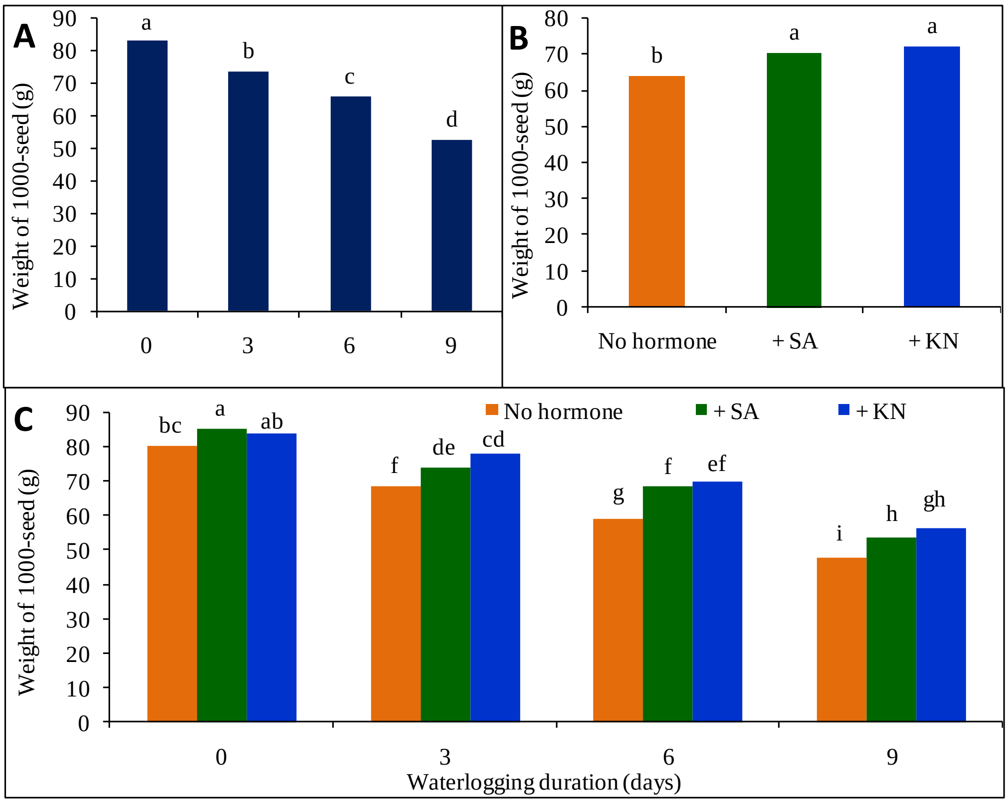

Figure 38. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on 1000 seed weight of *G. max***. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.5 Seed yield plant^{-1}

In case of waterlogging stress caused a significant loss of seed yield compared to control. It was 13%, 28% and 40% yield reduction for 3, 6 and 9 days waterlogging, respectively in comparision to control (Figure 39A). On the other hand, the exogenous application of SA and KN increased seed yield 10% higher for both SA and KN, compared to control plants (Figure 39B). Maximum seed yield plant^{-1} was observed in SA supplemented control plant (8.67 g), which was greatly reduced by waterlogging treatments irrespective of different duration. The lowest value of grain yield plant^{-1} was recorded in plants waterlogged for 9 days (4.81 g). The foliar application of SA and KN effectively increased the seed yield plant^{-1} under 3, 6 and 9 days waterlogging even control plants which was not treated with waterlogging (Figure 39C).

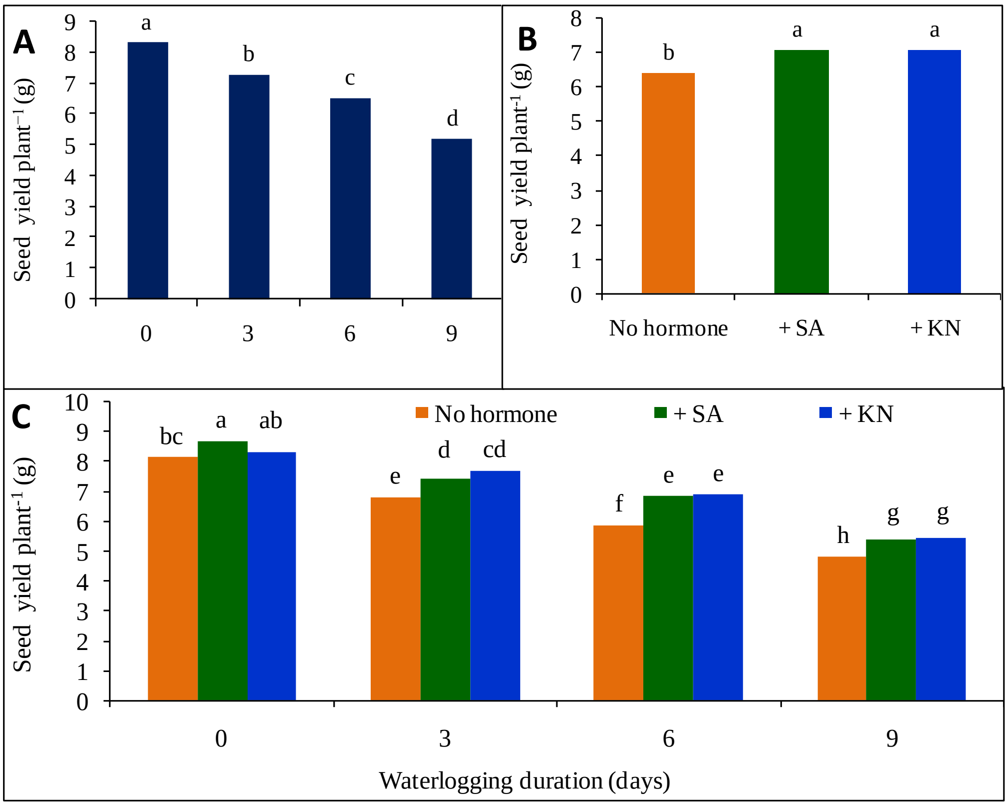

Figure 39. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on the seed yield of *G. max*. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

Many researchers found that waterlogging decreased the Chl content as a result, the reduction of photosynthetic activity and rate and ultimate result was yield loss (Ren *et al.*, 2014). Yield production gradually decreased with increasing waterlogging duration. In this experiment, grain yield decreased due to waterlogging stress (Figure 38). Similar results were observed in soybean under waterlogging (Miao *et al.*, 2012; Beutler *et al.*, 2014). Previous studies also showed the reduction of yield in different crops under waterlogging stress in maize (Tian *et al.*, 2019a), wheat and barley (De San Celedonio *et al.*, 2014), green gram (Kumar, 2013). Maximum photosynthesis rate increased seed weight by storing carbohydrates in the kernels (Serrago *et al.*, 2011). In addition, under stress decreased enzymatic activities highly responsible for the reduction in starch accumulation in grain filling. On the other hand, supplementation of SA and KN alleviated the negative effects of waterlogging on grain yield. Salicylic acid application enhanced rate of assimilation, net photosynthetic rate, internal CO_2 concentration, fruit yield. Salicylic acid increased yield under stress in several crops like wheat (Kareem *et al.*, 2017), corn

(Zamaninejad *et al.*, 2013). Kinetin increased grain yield in soybean. Previous studies showed that KN significantly increased grain yield by improving grain filling, endosperm cell division and also increased the sink capacity, as a result, higher accumulation of dry matter (Yang *et al.*, 2016). Here, both SA and KN increased the grain yield in a duration-dependent manner under stress (Figure 38).

4.5.6 Stover yield plant^{-1}

Stover yield plant^{-1} dramatically changes under waterlogging stress in contrast to control plants. The lowest value of stover yield was recorded in plants waterlogged for 9 days (11.01 g). Dramatically, 3 days waterlogging increased stover yield. The exogenous application of SA and KN showed change upon exposure to waterlogging (Figure 40). Moreover, compared to SA supplementation, KN showed better results under waterlogging and normal condition.

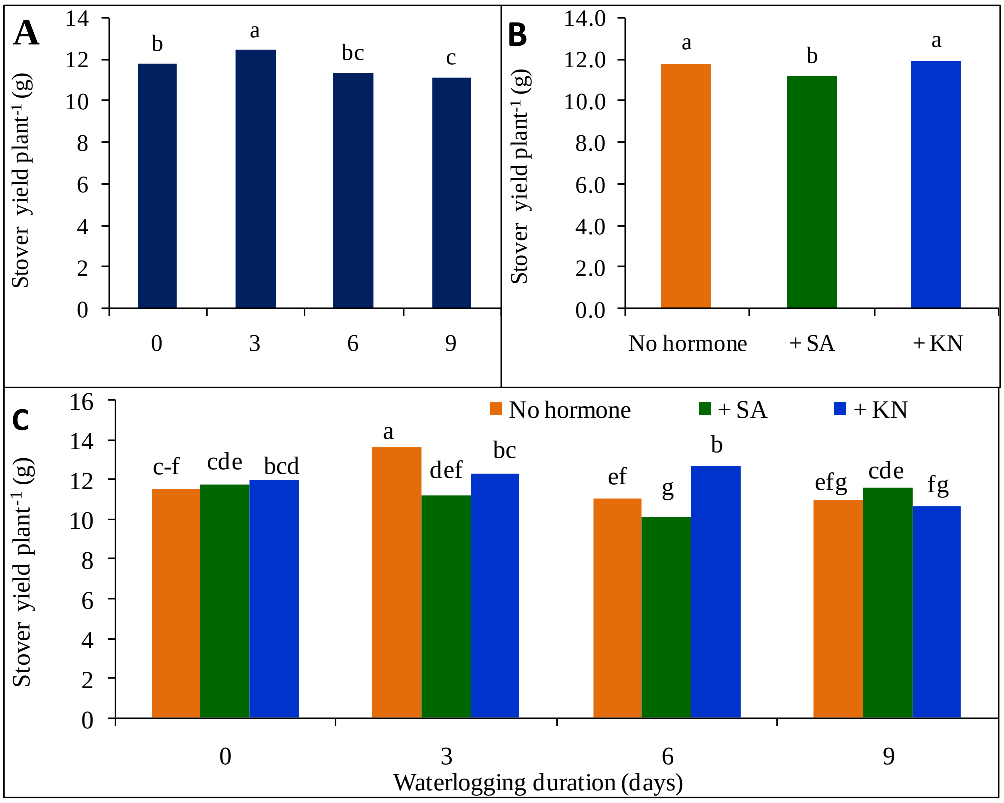

Figure 40. Effect of waterlogging (A), phytohormones (B) and their interaction (C) on the stover yield of *G. max*. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

4.5.7 Biological yield plant^{-1}

The longest duration of waterlogging resulted lowest yield (15.82 g) of soybean. Moreover, foliar spray of SA and KN effectively increased the yield at all duration (Figure 41C).

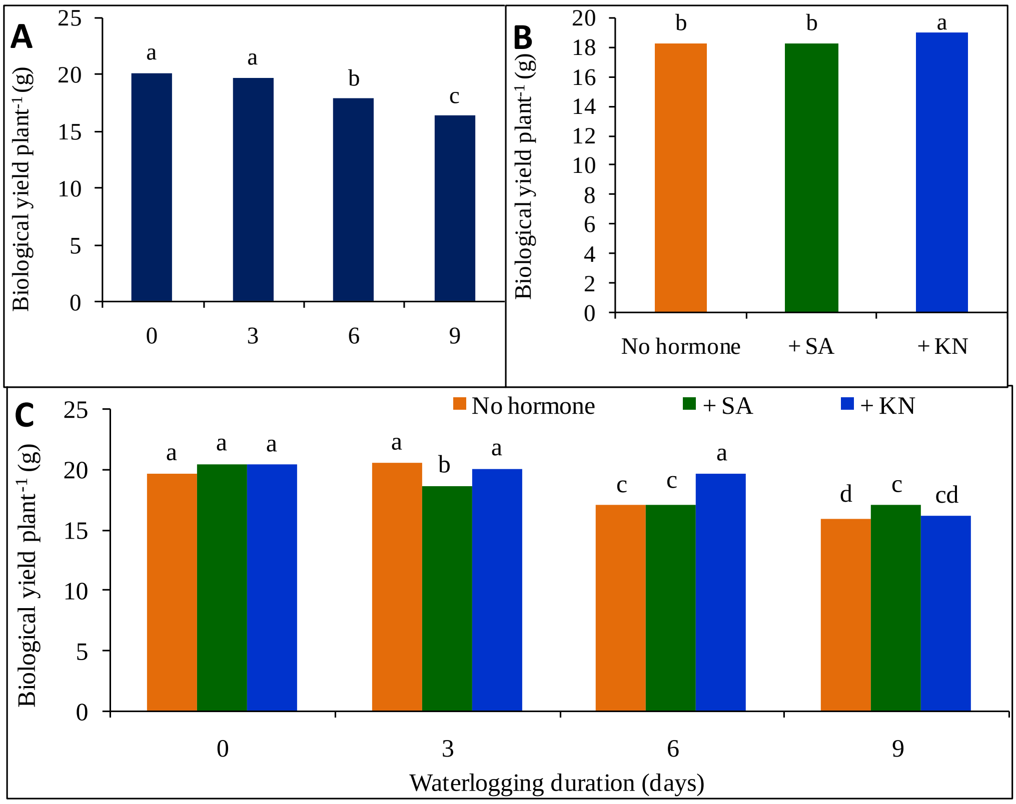

Figure 41. **Effect of waterlogging (A), phytohormones (B) and their interaction (C) on the biological yield of *G. max***. Here, SA and KN indicates salicylic acid (0.5 mM) and kinetin (0.1 mM), respectively. Bars with different letters are significantly different at $p \leq 0.05$ applying LSD test

Waterlogging stress has shown mostly negative effects on yield attributes (pod length, number of pod plant^{-1}, the number of seed pod^{-1}, grain yield plant^{-1}, stover yield plant^{-1} and biological yield) of soybean plants at different stages and durations. All yield attributes reduced significantly under prolonged waterlogging. Such negative effects of waterlogging stress on yield of soybean were also proved in earlier studies (Miao *et al*., 2012; Beutler *et al*., 2014). In contrary, the exogenous spray of SA and KN increase yield attributes at all duration of waterlogging.

Previous study also proved that SA improved yield under different stresses like under drought in wheat (Kareem *et al*., 2017), corn (Zamaninejad *et al.*, 2013). KN also improved yield in wheat (Daskalova *et al*., 2007), tobacco (Ma *et al*., 2008), lupin (Atkins *et al*., 2011), wheat (Yang *et al*., 2016). Kinetin significantly influenced on yield attributes by modifying seed size and grain filling (Jameson and Song, 2016).

4.6 Correlation among oxidative stress markers and growth parameters

From the correlation analysis the oxidative stress markers (MDA, H2O2, Pro content and EL) was significantly negative. In addition, seedling growth parameters and yield attributes were correlated negatively with the oxidative stress markers. Thus, osmotic, ionic, and oxidative stress indicators are negatively associated with growth performance (seedling length and biomass content), while growth is positively linked with growth parameters and yield attributes. The stress markers were positively inter-correlated while these were negative correlated with growth parameters and biomass content (Hasanuzzaman *et al*., 2018; Bhuyan *et al*., 2020; Anee *et al*., 2019).

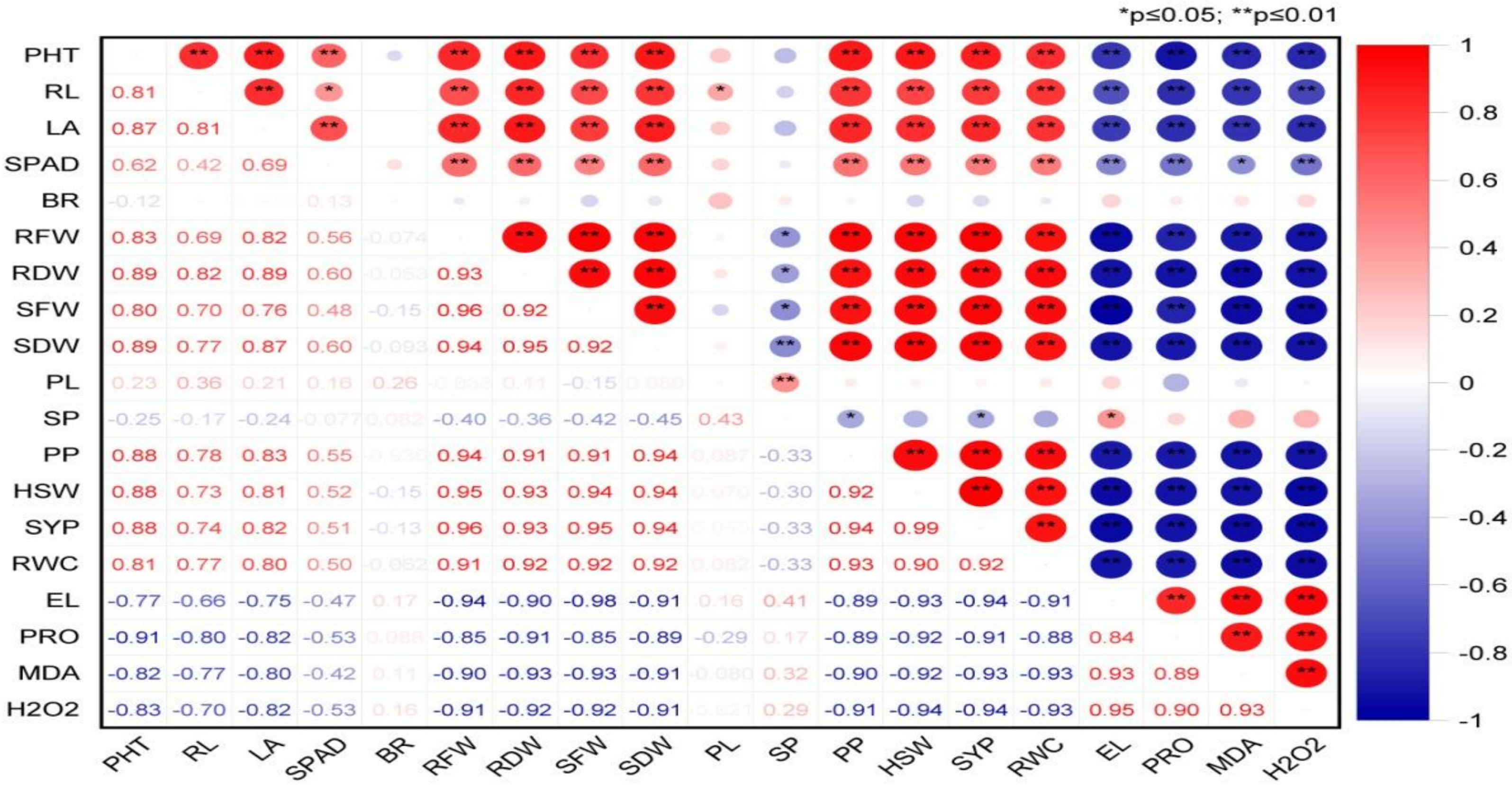

Figure 42. Correlation analysis (n=36) among different studied parameters of *G. max* under waterlogging stress. Red represents a positive correlation and blue represents a negative correlation among the studied parameters. Here, PHT- Plant height at 40 DAS, RL- Root length at 40 DAS, LA- Leaf area at 40 DAS, SPAD value at 40 DAS, BR- Branch number at 40 DAS, RFW- Root fresh weight, RDW- Root dry weight at 40 DAS, SFW- Shoot fresh weight at 40 DAS, SDW- Shoot dry weight at 40 DAS, PL- Pod length, SP- seed pod^{-1}, PP- Pod plant^{-1}, HSW- 100 seed weight, SYP- seed yield, RWC- Relative water content at 55 DAS, EL- Electrolyte leakage at 55 DAS, PRO- Proline content at 55 DAS, MDA content at 55 DAS and H_2O_2 content at 55 DAS.

Chapter V

SUMMARY AND CONCLUSION

The present experiment was conducted at Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from August to November, 2019 where we studied the morpho-physiological, biochemical, anatomical and yield attributes of soybean crop as affected by different durations of waterlogging (3, 6 and 9 days) and also studied the role of phytohormones (SA and KN) in mitigating waterlogging stress-induced damages which was applied exogenously.

The experiments were arranged in a Completely randomized design (CRD) with three replications. Plastic pots were used to facilitate the development of waterlogging stress. The experiment consisted of 12 treatments: no waterlogging (W0), SA spray (SA), KN spray (KN), 3 days waterlogging (W_3), 3 days waterlogging with SA spray (W_3SA), 3 days waterlogging with KN spray (W_3KN), 6 days waterlogging(W_6), 6 days waterlogging with SA spray (W_6SA), 6 days waterlogging with KN spray (W_6KN), 9 days waterlogging (W_9), 9 days waterlogging with SA spray (W_9SA), 9 days waterlogging with KN spray (W_9KN). Foliar application started at 16 DAS and waterlogging treatments were imposed at 19 DAS. There were five seedlings maintained in each pot.

The data on growth parameters were plant height, number of leaves plant^{-1}, number of branching plant^{-1}, leaf area, shoot fresh weight and dry weight plant^{-1}, root length plant^{-1}, root fresh weight and dry weight plant^{-1}. Physiological parameters viz, electrolyte leakage, SPAD value of leaf, relative water content were also recorded. R was measured to understand the effects on plant water relations. Biochemical parameters including lipid peroxidation, H_2O_2 content and Pro content were measured. At harvest, number of pod plant^{-1}, seed pod^{-1}, pod length, grain yield plant^{-1}, 1000 seed weight**,** stover yield plant^{-1} and biological yield plant^{-1} were measured to assess the effect on yield.

Waterlogging showed remarkable decrease in plant height in compared to control and in a duration-dependent manner. The tallest plants were recorded in KN supplemented control plants at all growth stages. Number of leaves and branch number were noticeably decreased at 9 days waterlogging at every growth stages. However, the foliar application of both SA and KN effectively increased leaves number, branch number of soybean plants significantly decreased at 9 days of waterlogging. Kinetin significantly increased the branch number at all growth stages and the maximum branching was recorded at control with KN spraying plants at 30 DAS and 40 DAS. Leaf area significantly readuced at 9 days waterlogging plants 22% at 40 DAS and 24% at 50 DAS, compared to control. In the contrary, the foliar application of SA and KN increased leaf area at all growth stages. Both SA and KN remarkably increase the leaf area which was 8% and 10% higher for SA, 13% and 11% higher for KN at 9 days of waterlogging at 40 DAS and 50 DAS, respectively compared to the same duration of waterlogging treatment. The highest shoot FW and DW was recorded at SA spraying control plants. After 9-day of waterlogging, FW was reduced by 58 and 56% and DW was reduced by 51 and 57% at 30 DAS and 40 DAS, respectively compred to control. In the contrary, the foliar application of SA and KN increased FW and DW under waterlogging. The highest root length was recorded in KN sprayed control plants (9.90 cm at 30 DAS and 12.83 cm at 40 DAS) and the lowest 9 days waterlogging without spray (7.70 at 30 DAS and 8.83 cm at 40 DAS). Both SA and KN remarkably increase the root length which was 34% and 52% higher for SA, 16% and 70% higher for KN at 6 and 9 days of waterlogging, respectively compared to the same duration of waterlogging treatment alone at 30 DAS. Root FW and DW reduced 33% and 50% compared to control, respectively waterlogging for 9 days at 30 DAS. In contrary, the foliar application of SA and KN remarkably increase root fresh weight and dry weight.

Abiotic stress-induced oxidative stress is a common phenomenon and waterlogging as an abiotic stress also increased MDA and H_2O_2 and Pro contents of soybean leaves significantly. MDA, H_2O_2 and Prolin content sharply increased compared to control plants and the highest value showed at a longer duration of waterlogging at 9 days. In the contrary, SA and KN remarkably reduced the value and helped plant to become tolerance against waterlogging stress.

Electrolyte leakage increased markedly under waterlogging stress comparing to control. The highest content of EL was recorded in the longest duration (9 days) of waterlogging which was 131% at 30 DAS and 303% at 40 DAS. In soybean plant, lower accumulation of EL content was observed due to supplementation of SA and KN under waterlogging. Waterlogging results in leaf wilting, which was evident from the significant reduction of RWC in waterlogged plants with the lowest value in plants waterlogged for 9 days (35%). Foliar application of both SA and KN effectively increased RWC contents by reducing the waterlogging negative effects. At 30 DAS, 24% higher for SA and 15% higher for KN compared to 9 days waterlogged without hormone. SPAD value gives a Proportional value of the leaf Chl content. Waterlogged plants leaves decreased SAPD value in a time-dependent manner with increasing duration of waterlogging which verifies the damaging effect of stress on leaf photosynthetic apparatus.

From the anatomical study of the experiment, it was evident that soybean plants formed aerenchyma in their stem and root cortex to capture oxygen. This proved the ability to possess some adaptive mechanisms in soybean plants.

Adventitious root formation is one of the major adaptation responses under waterlogging stress. In soybean adventitious roots showed under waterlogging. But the exogenous application of SA and KN significantly increased adventitious root and also improved root structure.

At harvest, the number of pods plant^{-1}, number of seeds pod^{-1}, pod length, seed yield plant^{-1}, 1000 seed weight**,** stover yield plant^{-1}and biological yield plant^{-1} were decreased in a time-dependent manner with increasing duration of waterlogging with compared to control plants. However, the foliar application of SA and KN improved them. The pod number significantly reduced waterlogging for 9 of days which was 39% lower than the control plants. As a result**,** the lowest grain yield (4.81 g) was recorded at 9 days of waterlogging.

From the above results, it was observed that plants showed negative reactions to waterlogging stress in a duration-dependent manner. More damage effects occurred at the longest duration of waterlogging (9 days) and yield declined 45%. All parameters decreased at any level of waterlogging stress. On the other hand, the foliar application of SA significantly increased leaves number, branch number, shoot FW, shoot DW, root FW, root DW, root length, RWC, SPAD, number of pods number, seed pod^{-1} and grain yield and also decreased adverse effects of osmotic stress by reducing MDA, H_2O_2, Pro content and EL under waterlogging and control plants. Similarly, KN also increased plant height, branch number, leaf area, shoot FW, shoot DW, pod length, seed pod^{-1}, seed yield and 1000 seed weight and also decreased adverse effects of osmotic stress by reducing MDA, H_2O_2 and Electrolyte leakage under waterlogging and control plants. After all, our results showed the ability of SA and KN to revert the harmful effects of waterlogging on soybean and increased yield by improving all parameters.

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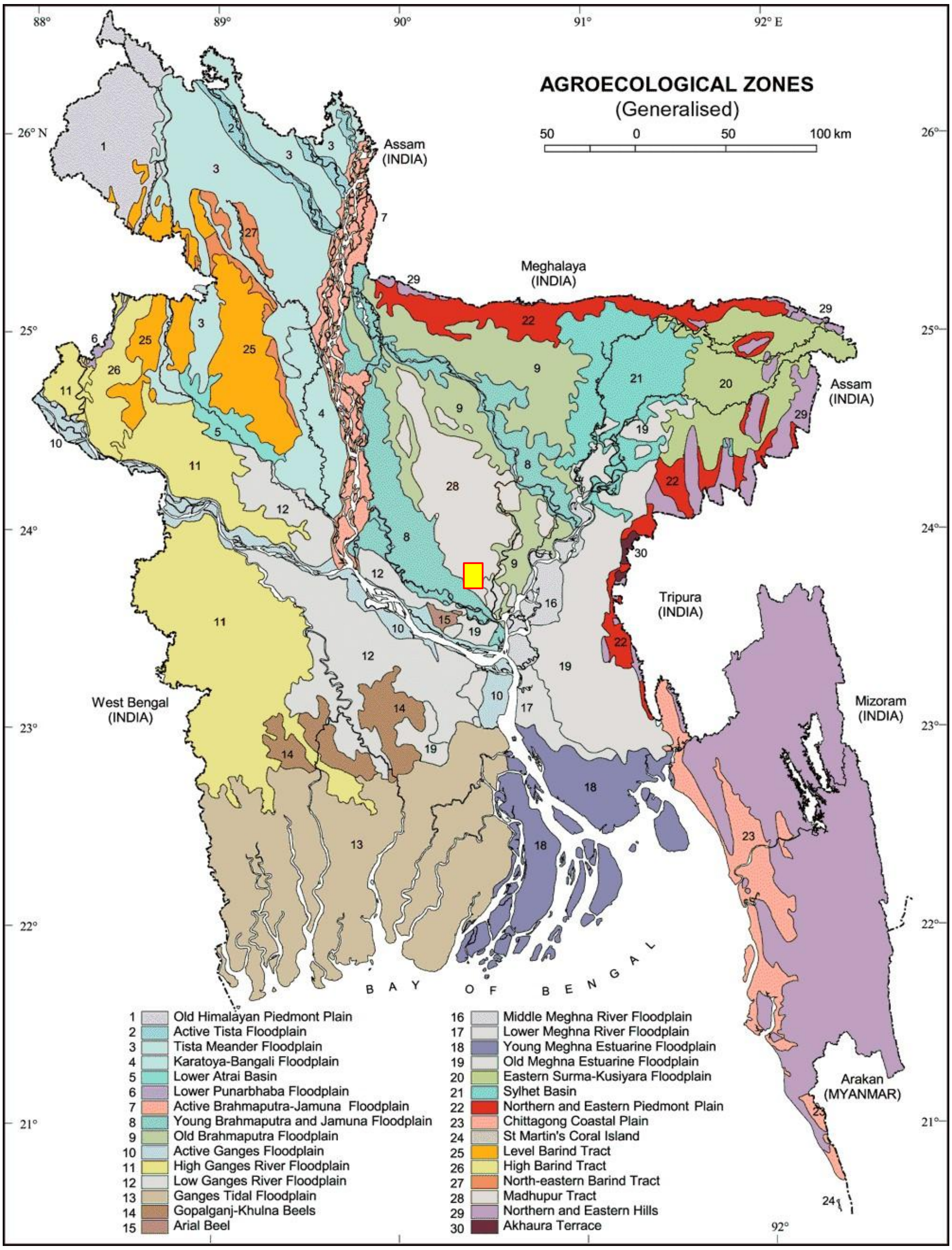
APPENDICES

Appendix I. Map showing the location of experiment.



Appendix II: Mean square values and degree of freedom (DF) of plant height, at 20, 30 and 40 DAS of soybean by foliar application of phytohormones under different duration of waterlogging stress

Source of varience	DF	Mean square values		
		Plant height		
		20 DAS	30 DAS	40 DAS
Waterlogging	3	78.178	264.509	756.954
Phytohormones	2	16.639	86.177	83.071
Waterlogging × Phytohormones	6	0.503	2.312	9.050
Error	24	0.814	2.248	3.221

Appendix III: Mean square values and degree of freedom (DF) of root length at 40 DAS, root FW and root DW at 30 and 40 DAS of soybean by foliar application of phytohormones under different duration of waterlogging stress

Source of varience	DF	Mean square values				
		Root length	Root FW		Root DW	
		40 DAS	30 DAS	40 DAS	30 DAS	40 DAS
Waterlogging	3	13.538	0.010	0.067	0.002	0.003
Phytohormones	2	5.554	0.001	0.004	0.000	0.001
Waterlogging × Phytohormones	6	1.393	0.000	0.001	0.000	0.000
Error	24	0.252	0.001	0.001	0.001	0.001

Appendix IV: Mean square values and degree of freedom (DF) of shoot FW and shoot DW at 30 and 40 DAS of soybean by foliar application of phytohormones under different duration of waterlogging stress

Source of varience	DF	Mean square values			
		Shoot FW		Shoot DW	
		30 DAS	40 DAS	30 DAS	40 DAS
Waterlogging	3	6.991	11.672	0.185	0.462
Phytohormones	2	0.315	0.557	0.014	0.014
Waterlogging × Phytohormones	6	0.034	0.020	0.001	0.004
Error	24	0.021	0.016	0.001	0.001

Appendix V: Mean square values and degree of freedom (DF) of leaves number and branch number at 30 and 40 DAS of soybean by foliar application of phytohormones under different duration of waterlogging stress

Source of varience	DF	Mean square values			
		Branch number		Leaves number	
		30 DAS	40 DAS	30 DAS	40 DAS
Waterlogging	3	1.255	3.187	9.199	20.181
Phytohormones	2	0.567	3.132	1.191	5.131
Waterlogging × Phytohormones	6	0.075	0.332	0.154	3.316
Error	24	0.011	0.030	0.080	0.428

Appendix VI: Mean square values and degree of freedom (DF) of SPAD value at 20 and 40 DAS and RWC at 30 and 55 DAS of soybean by foliar application of phytohormones under different duration of waterlogging stress

Source of varience	DF	Mean square values			
		SPAD value		RWC	
		20 DAS	40 DAS	30 DAS	55 DAS
Waterlogging	3	15.159	7.731	445.870	1082.298
Phytohormones	2	4.486	2.504	138.332	113.698
Waterlogging × Phytohormones	6	1.479	2.715	17.816	32.882
Error	24	6.089	5.662	4.516	11.197

Appendix VII: Mean square values and degree of freedom (DF) of leaf area value at 40 and 50 DAS and EL at 30 and 55 DAS of soybean by foliar application of phytohormones under different duration of waterlogging stress

Source of varience	DF	Mean square values			
		Leaf area		EL	
		40 DAS	50 DAS	30 DAS	55DAS
Waterlogging	3	85.932	186.373	1512.955	3449.270
Phytohormones	2	25.091	42.191	150.628	164.649
Waterlogging × Phytohormones	6	8.683	2.561	15.218	25.397
Error	24	3.062	4.425	3.629	2.191

Appendix VIII: Mean square values and degree of freedom (DF) of MDA, H_2O_2 and Pro content at 30 and 55 DAS of soybean by foliar application of phytohormones under different duration of waterlogging stress

Sourec of varience	DF	Mean square values					
		MDA		H_2O_2		Pro	
		30 DAS	55 DAS	30DAS	55DAS	30DAS	55DAS
Waterlogging	3	1333.74	1433.78	97.64	194.81	8.480	6.771
Phytohormones	2	381.417	230.581	20.360	22.928	2.820	3.152
Waterlogging × Phytohormones	6	60.466	67.296	2.420	4.557	0.517	0.387
Error	24	6.625	7.007	0.227	1.041	0.072	0.059

Appendix IX: Mean square values and degree of freedom (DF) of pod length, seed pod^{-1} and pod plant^{-1} of soybean by foliar application of phytohormones under different duration of waterlogging stress

Sourec of varience	DF	Mean square values		
		Pod length	Seed pod^{-1}	Pod plant^{-1}
Waterlogging	3	0.010	0.194	37.573
Phytohormones	2	0.159	0.260	4.446
Waterlogging × Phytohormones	6	0.273	0.016	0.458
Error	24	0.069	0.028	0.190

Appendix X: Mean square values and degree of freedom (DF) of 100 seed weight, seed yield plant^{-1}, stover yield C, biological yield plant^{-1} of soybean by foliar application of phytohormones under different duration of waterlogging stress

Source of varience	DF	Mean square values			
		1000 seed weight	Seed yield plant^{-1}	Stover yield plant^{-1}	Biological yield plant^{-1}
Waterlogging	3	1503.176	15.975	2.982	27.985
Phytohormones	2	217.711	1.798	2.014	2.405
Waterlogging × Phytohormones	6	9.636	0.126	2.817	3.016
Error	24	8.635	0.066	0.282	0.308