SCREENING OF SOYBEAN GENOTYPES FOR WATERLOGGING STRESS TOLERANCE AND UNDERSTANDING THE PHYSIOLOGICAL MECHANISMS

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CERTIFICATE

This is to certify that the thesis entitled ***"SCREENING OF SOYBEAN GENOTYPES FOR WATERLOGGING STRESS TOLERANCE AND UNDERSTANDING THE PHYSIOLOGICAL MECHANISMS"*** *submitted to the Department of Agronomy, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degrees of* ***MASTER OF SCIENCE (MS) in AGRONOMY****, embodies the results of a piece of bonafide research work carried out by* ***KHADEJA SULTANA SATHI,*** *Registration No.* **14-05945** *under my supervision and guidance. No part of the thesis has been submitted for any other degrees or diploma.*

I further certify that such help or source of information, as has been availed during the course of this investigation has been duly acknowledged and style of this thesis has been approved and recommended for submission.

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SCREENING OF SOYBEAN GENOTYPES FOR WATERLOGGING STRESS TOLERANCE AND UNDERSTANDING THE PHYSIOLOGICAL MECHANISMS

ABSTRACT

One of the most exaggerated factors which are liable for diminishing crop yield is abiotic stress that comes up as a potent intimidation to worldwide food security in proceeding decades. Soil waterlogging in cultivated areas is a common abiotic stress which has severe influences on soybean composition and production worldwide. Focusing on that issue, an experiment was carried out at Sher-e-Bangla Agricultural University, during the month from August to November 2019 to screen out the waterlogging tolerance and yield performances of selected soybean genotypes. The experiment was laid out in a completely randomized design with three replications. The experiment consisted of 2 water level conditions (control and waterlogging) and 12 genotypes (Sohag, BARI Soybean-5, BINAsoybean-1, BINAsoybean-2, BINAsoybean-3, BINAsoybean-5, BINAsoybean-6, SGB-1, SGB-3, SGB-4, SGB-5, GC-840). On 15th days after sowing (DAS), plants were revealed to waterlogging for 12 days period. The waterlogging stress reduced plant height, relative water content (RWC), above-ground fresh weight plant^{-1}, above-ground dry weight plant^{-1}, SPAD value, leaf area plant^{-1}, number of leaves plant^{-1}, number of branches plant^{-1}, number of pods plant^{-1}, number of seeds pod^{-1}, 100-seed weight, seed yield plant^{-1}, stover yield, biological yield, whereas increased mortality rate and electrolyte leakage. The waterlogged plants showed delayed flowering and maturity than their respective control plants. It can be concluded that waterlogging remarkably declined the growth and yield of all the soybean genotypes in comparison with the control plants. Among the 12 genotypes Sohag, BARI Soybean-5, GC-840, BINAsoybean-1, BINAsoybean-2 performed better than the other genotypes under waterlogging. These genotypes showed a greater number of adventitious roots in their stem under waterlogging stress, which probably helps the plants to thrive under waterlogging condition.

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
ADH	Alcohol dehydrogenase
APX	Ascorbate peroxidase
AsA	Ascorbic acid/ Ascorbate
ATP	Adenosine triphosphate
BARI	Bangladesh Agricultural Research Institute
CAT	Catalase
Chl	Chlorophyll
cv.	Cultivar
DAS	Days after sowing
DHAR	Dehydroascorbate reductase
DW	Dry weight
et al.	*et alibi* (and others)
FAO	Food and Agriculture Organization
FL	Flooding
FW	Fresh weight
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSSG	Oxidized glutathione
GST	Glutathione *S*-tranferase
H_2O_2	Hydrogen peroxide
LSD	Least significant difference
MDA	Malondialdehyde

LIST OF ABBREVIATIONS (Cont'd)

MDHAR	Monodehydroascorbate reductase
mM	Milimolar
μM	Micromolar
MSI	Membrane stability index
NAD^+	Nicotinamide adenine dinucleotide
$O_2^{\bullet -}$	Superoxide radical
$OH^{\bullet}$	Hydroxyl radical
PAR	Photosynthetically active radiation
PDC	Pyruvate decarboxylase
POD	Peroxidase
POX	Peroxidases
Pro	Proline
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RWC	Relative water content
SOD	Superoxide dismutase
WL	Waterlogging

Chapter I

INTRODUCTION

One of the most exaggerated factors which is liable for diminishing crop yield is abiotic stress (Acquaah, 2007) that comes up as a potent intimidation to worldwide food security in proceeding decades (Hasanuzzaman *et al.,* 2019). Plants are randomly revealed to unfavorable environmental circumstances, which are named as abiotic stresses, for instance, waterlogging, drought, salinity, heavy metal stress, high temperature, nutrient stress, radiation, and environmental pollution (Hasanuzzaman *et al.,* 2018; 2017a,b; 2013a,b; 2012) and as a result posing a serious ultimatum to crop production.

Soil waterlogging in cultivated areas is a common abiotic stress which has severe influences on the composition and production of soybean (Ara *et al.,* 2015; Wu *et al.,* 2017) and most crops species (Alizadeh-Vaskasi *et al.,* 2018; Duhan *et al.,* 2018) worldwide. Waterlogging or flooding is the condition of the soil where water table outreaches the root zone of soil or when soil becomes saturated up to 100% and become unsuitable for crop production. Flooding is of two kinds: i) waterlogging (only the root systems are under anaerobic condition) and ii) submergence; submergence maybe two kinds: a) partial submergence (all roots are immersed in water and just a portion of shoots are covered by water) and b) complete submergence (whole plants are under the water level). Gas exchanges between root systems and porous spaces in waterlogged soils are restricted due to diffusion resistance to oxygen. In water, which is about 10,000 times greater than in air (Bailey-Serres *et al.,* 2012). Excess rainfall, tides, floods, storms, and lack of adequate drainage facilities are the causes of waterlogging stress in plants (Kim *et al.,* 2015). Waterlogging stress reduced the availability of O_2 in plants (Capon *et al.,* 2009). Primarily, waterlogging stress causes hypoxic (O_2 deficient) stress, which affects aerobic respiration. With a course of time, the stress switched to an anoxic (O_2 absent) stress condition, which causes inhibition of respiration (Wegner, 2010), limitation of energy, deposition of noxious compounds (for instance, lactate) and loss of carbon (through the loss of ethanol from the roots) (Tamang *et al.,* 2014). Oxygen is needed for the division of cells, development of cell, respiration, absorption and transportation of nutrients in plants. Due to flooding stress, plants experienced chlorosis, necrosis, defoliation, reduction of growth, reduction in N fixation, yield loss, death of plant at both vegetative and reproductive stages (Hasanuzzaman *et al.,* 2016). Flooding stress-induced ethanol accumulation imposing adverse impacts on various processes (Hasanuzzaman *et al.,* 2017a). Voesenek and Bailey-Serres (2015) stated that waterlogging stress posed O_2 deficiency in the soil, which triggered the accumulation of ethylene, phytotoxic mineral nutrients (Mn^{2+}, Fe^{2+}) and also production of reactive oxygen (ROS) species and reactive nitrogen (RNS) species in plants. Flooding stress-induced production of ROS, which was responsible for oxidative stress as well as cellular damages like lipid peroxidation, damaged nucleic acid, down-regulation of enzyme and activation of programmed cell death (Anjum *et al.,* 2015).

Many countries in Asia experience flooding stress during the rainy season. In Bangladesh, the waterlogging condition is common in *kharif* season due to flash flooding and/or rainwater. Soybean crops are usually not tolerant to waterlogging stress (Tougou *et al.,* 2012). In different parts of the world, soybean growth and production have decreased significantly (Van Nguyen *et al.,* 2017). In different flooding cycles, soybean plants reacted significantly different to flooding stress (Wu *et al.,* 2017). In soybean, waterlogging stress switched aerobic respiration towards anaerobic. Anaerobic respiration triggers alcoholic (C_2H_5OH) fermentation by generating waterlogged-inducible proteins that help in generation of NAD^+ and conveyed sharp increment activity of alcohol dehydrogenase (ADH) in soybean plants (Komatsu *et al.,* 2011a). According to Oosterhuis *et al.* (1990), waterlogging can diminish the yield of soybeans by 17 to 43 percent at the vegetative growth stage and by 50 to 56 percent at the reproductive stage.

In Bangladesh, soybean is a promising crop. It is a versatile, nutritionally and economically vital legume for its seed composition (Shea *et al.,* 2020). Since it contains a considerable source of plant proteins and oils, it has valuable uses as food, feed and oilseed crop. Soybean seed contains about 18-22% oil and 38-56% vegetable protein with favorable amino acid (USDA, 2018). Worldwide total grain production was approximately 338.08 million tons with the coverage of 121.87 million hectares of land and an average yield of about 2.77 metric tons hectare^{-1} (USDA, 2019). The three enormous soybean-growing countries in the world are the United States, Brazil, and Argentina. Globally soybean seed production came to 348.7 million tons with area harvested 124.9 million hectares of land (FAOSTAT, 2018). According to BBS 2017-2018, the total land area harvested 59,490 hectares and 98699 tonnes of soybean seeds produced in Bangladesh. Waterlogging may be harmful to soybean root development, formation and function of the nodule (Cho *et al.,* 2006a). However, The plants response to flooding stress varies among the crop varieties and the duration of stress.

There might be some approaches to mitigate waterlogging stress condition. For optimal production under stress condition, it is essential to identify soybean cultivars that are tolerant to flooding. In developing soybean varieties that can withstand pre-germination conditions of low oxygen during waterlogging, the available genotypic variation could be exploited. The criteria for the choice and development of waterlogging resistant soybean are appropriate screening methods, morpho-physiological characteristics correlated with tolerance and the identification of promising genotypes. The nodules of soybean can respond to a broad range of oxygen concentrations in the rhizosphere (Weisz and Sinclair, 1987). In some soybean cultivars, there are several physical features that might be due to flood resistance. The plants exposed to waterlogging tries to diffuse oxygen in aerial parts of plants for adapting to the stress condition. For the adaptation under stress condition, plants modified morphological structures that include leaves hyponasty, shoot elongation, the formation of aerenchyma in root cells, formation of lenticels in the stem, commencing adventitious roots in the waterlogged stem. These reports revealed that waterlogging stress triggered ethylene production, which drives the plant to epinasty, chlorosis and senescence of leaf (Voesenek and Bailey-Serres, 2015). Under soil flooding conditions, soybean plants inoculated with aerobic bacteria (*Bradyrhizobium elkanii* and *japonicum bradyrhizobium*) may play an important role in increasing N_2 fixation in plants and boosting plant growth (Beutler et al., 2014). Kadempir et al. (2014) also noticed the positive outcome of inoculating *B. japonicum* in soybean plants which enhances the plant's nutritional status as well as improved N_2 uptake and flooding amendment. Another motile, aerobic, free-living bacterium, which can flourish in waterlogged situations is *Azospirillum*, which can stimulate plant growth and development at various stages (Sahoo *et al.,* 2014). Some species of arbuscular mycorrhizal (AM) fungi have the capacity to acclimatize through which they can survive in waterlogging stress conditions (Landwehr *et al.,* 2002). Soybean crops have a symbiotic relationship with AM fungi and rhizobia (*B. japonicum*), so the shared procurement of nutrients regulates seed production and crop growth under conditions of waterlogging (Hattori *et al.,* 2013).

It was hypothesized that if we follow the above-mentioned approaches, plant biologists might be able to mitigate the waterlogged-induced stress to some extent. Thus, improving genetic resources of soybean and developing waterlogged tolerant genotypes is of great importance. In Bangladesh, studies on this aspect have not yet been done extensively. Therefore, this experiment has been designed to screen out waterlogging tolerance and yield performances of selected soybean genotypes. Considering the above mentioned phenomenon the following objectives has been taken:

i. To investigate the waterlogging-induced growth and physiological damages in soybean genotypes
ii. To understand the differences in metabolism under waterlogging stress in different soybean genotypes
iii. To screen the tolerant genotypes of soybean under waterlogging

Chapter II

REVIEW OF LITERATURE

2.1 Soybean: Botany and cultivation status

Soybean (*Glycine max* L.) is a vital legume crop under the Fabaceae family. Depending on the variety, the plant gets taller up to 1.5 m. Stems are covered with dark-colored hairs and bear trifoliate leaves with long petiole. In the axils, flowers are born individually or in small bunches and depending upon variety, the color of flowers are white to purple. The fruit is around 10 cm long and, when fully developed and dried, turns yellow to black (Gupta 2012). The fruits containing 1 to 4 seeds are called pods. Soybeans have various colors of the seed coat, including dark, brown, blue, yellow, green and mottled. This will not sprout if the seed coat is ruptured. The blemish, which is apparent on the seed coat, is called the hilum and there is a micropyle (little opening in the seed coat) on one side of the hilum through which water can be absorbed for the growing of plants (Khojely *et al.,* 2018).

Worldwide total grain production was approximately 338.08 million tons, with the coverage of 121.87 million hectares of land and an average yield of about 2.77 metric tons hectare^{-1} (USDA, 2019). The three enormous soybean-growing countries in the world are the United States, Brazil, and Argentina. Globally soybean grain production came to 348.7 million tons with area harvested 124.9 million hectares of land (FAOSTAT, 2018).

According to BBS (2018), the total land area harvested 59,490 hectares and 98,699 tonnes of soybean seeds produced in Bangladesh. Soybean cultivated area in Bangladesh was 59,445 hectares and seed production was 98,699 tonnes (FAOSTAT, 2018).

2.2 Importance of soybean

Soybean is a versatile, nutritionally and economically vital legume for its fruitful seed composition (Shea *et al.,* 2020). Since their domestication, soybeans have been an important food in Eastern Asia. There are many uses of soybeans, for instance, oil and food for human intake and feed for animal consumption.

As it contains a considerable source of plant proteins and oils, it has valuable uses as food, feed and oilseed crop. Soybean seed contains about 18-22% oil and 38-56% vegetable protein with favorable amino acid (USDA, 2018). Soybean seed comprises of protein, oil, soluble and insoluble carbohydrates, ash, moisture in addition to different functional products, for instance, isoflavones, anthocyanins, saponin and dietary fiber (Bellaloui *et al.,* 2013; Waqas *et al.,* 2014). Both water and oil-soluble vitamins are found in the soybean. During oil extraction, water-soluble vitamins are not lost. Approximately of 3.25 mg vitamin B1 (thiamin), 3.11 mg vitamin B2 (riboflavin), 16.9 mg vitamin B5 (pantothenic acid), and 29.7 mg vitamin B6 (niacin) are found in a kilogram of soy flour (Kumar *et al.,* 2008a,b).

It is possible to grow soybeans for hay and silage as a pasture crop (Heuze *et al.,* 2015). For the use of good quality hay, some late-maturing varieties are cultivated and harvested at the flowering to maturity stage. As soybean plants are quite coarse and fibrous, which can cause digestive inconvenience if consumed alone as a forage crop. Moreover, an early harvest is recommended in this case and soybean can be grown as monocrop or intercropped with sorghum or maize as a silage crop (Blount *et al.,* 2013). As soybean silage is bitter in taste, it is hardly ensiled alone (Gobetti *et al.,* 2011). Sorghum and soybean (60:40) ensiled with molasses make a high quality silage (Lima *et al.,* 2011). Tobia *et al.* (2008) stated that the combination of inoculants (i.e, *Lactobacillus brevis*) and molasses had a synergistic impact on the quality of soybean silage

The soybean plants can fix nitrogen in their roots. It can be used as a restorative crop, green manuring crop and cover crop and easily can be included in cropping pattern as main crop or inter-crop. The crop can be grown in conjunction with cotton, maize and sorghum as a rotational crop. Soybeans are cultivated as green manure in the wet tropics of Australia to protect fallow paddocks from erosion (DAFF, 2010).

Giller and Dashiell (2007) observed that the fast-growing soybean plants help in reducing the parasitic weed *Striga hermonthica* in Africa. Soybeans can be cultivated in a wide variety of climates and soils in several cropping seasons. Soybean roots develop a symbiotic association with specific rhizobium (*Bradyrhizobium japonicum),* which fixes atmospheric N and contributes to soil fertility. Therefore, the association has economic and environmental significance as it decreases the use of N chemical fertilizer (Miransari *et al.,* 2013; Miransari, 2011).

2.3 Plant abiotic stress: General aspects

The detrimental effect of non-living factors in a given situation on the living organism is collectively referred to as abiotic stress. Plants are randomly revealed to unfavorable environmental circumstances, which are named as abiotic stresses, for instance, waterlogging, drought, salinity, heavy metal stress, high temperature, nutrient stress, radiation, and environmental pollution (Hasanuzzaman *et al.,* 2018; 2017a,b; 2013a,b; 2012) and as a result posing a serious ultimatum to crop production. It remains the pronounced obstruction to crop production worldwide. Acquaah (2007) reported that considerably more than 50% of yield reduced due to abiotic stresses. A variety of morphological, physiological, biochemical and molecular changes are triggered by abiotic stress that adversely perturbs crop growth and production. It may lead to an unendurable metabolic pressure on cells that decreases growth and leads to plant death in extreme cases since the stress becomes excessive and continues for an expanded period (Hasanuzzaman *et al.,* 2012).

Plant stress varies depending upon stressor type and existing period. Plants may be subjected to any extent of stress caused by any factor. Different factors may take different times to become stressful. Like, in a few minutes, air temperature can become stressful where soil waterlogging may take days to weeks and soil mineral deficiencies may take months to turn into stressful (Taiz and Zeiger, 2006).

Due to global warming, crops are continuously exposed to an increased number of abiotic and biotic stress conditions that ultimately affect the growth, development and yield of crops. Suzuki *et al.* (2014) demonstrated that concomitant incidents of drought and heat showed more exterminating effects on crop production than these stresses happening separately at different crop growth stages.

Moreover, it is of great importance to know the morphological, physiological, and biochemical processes that undergo stress injury and to know their adaptation and acclimatization mechanisms against unfavorable environmental ccondition.

2.4 Waterlogging stress

Waterlogging or flooding is the condition of soil where water table outreaches the root zone of soil or when soil becomes fully saturated and become unsuitable for crop production. Flooding is of two kinds: i) waterlogging (only the root systems are under anaerobic condition) and ii) submergence; submergence maybe two kinds: a) partial submergence (all roots are immersed in water and just a portion of shoots are covered by water) and b) complete submergence (whole plants are under the water level). Excessive precipitation, floods, tides, storms, and inadequate drainage facilities are the causes of waterlogging stress in plants (Kim *et al.,* 2015). Waterlogging stress reduced the availability of O_2 in plants (Capon *et al.,* 2009).

Primarily, waterlogging stress causes hypoxia (O_2 deficient), which affects aerobic respiration. With a course of time, the stress switched to an anoxic (O_2 absent) stress condition, which causes inhibition of respiration (Wegner, 2010). Oxygen is needed for the division of the cell, growth of cell, respiration, uptake and transportation of nutrients in plants. Due to flooding stress plants experienced chlorosis, necrosis, defoliation, reduction of growth, reduction in N fixation, yield loss, death at both vegetative and reproductive stage of plants (Hasanuzzaman *et al.,* 2016). Flooding stress induced ethanol accumulation imposing adverse impacts on different processes (Hasanuzzaman *et al.,* 2017a).

Ashraf (2012) observed a reduction of growth and development of plants as there lack oxygen and deficient of micronutrients under waterlogging stress. Flooding stress posed detrimental effects on seed germination, growth, yield, root anatomy etc in plants (Dufey *et al.,* 2009). Voesenek and Bailey-Serres (2015) stated that waterlogging stress posed O_2 deficiency in the soil, which triggered the accumulation of ethylene, phytotoxic mineral nutrients (Mn^{2+}, Fe^{2+}) and also generation of ROS and RNS in plants.

Flooding stress-induced production of ROS, which was responsible for oxidative stress as well as cellular damages like lipid peroxidation, damaged nucleic acid, inactivation of enzyme and activation of programmed cell death (Anjum *et al.,* 2015).

Plants exposed to waterlogging tries to diffuse oxygen in aerial parts of plants for adapting to the stress condition. For adaptation under stress condition plants modified morphological structures that includes leaves hyponasty, shoot elongation, the formation of aerenchyma in root cells, formation of lenticels in the stem, commencing adventitious roots in the waterlogged stem. These workers also reported that waterlogging stress triggered ethylene production, which drives the plant to epinasty, chlorosis and senescence of leaf (Voesenek and Bailey-Serres, 2015).

2.5 Effect of waterlogging on crop attributes

2.5.1 Effect on growth

González *et al.* (2009) demonstrated that waterlogging stress reduced leaf area and specific leaf area (SLA) by 36.2% and 26.2% than the control quinoa (*Chenopodium quinoa* Wild.) plants.

While working with 15 genotypes of maize under waterlogging stress, Lone and Warsi (2009) found that nearly all the tested genotypes showed depletion in the plant height and ear height. In another experiment, Ren *et al.* (2014) observed that the overall growth and development of maize significantly affected due to waterlogging.

Plant height, leaf length and leaf number was observed in a decreasing manner in two cultivars and two wild related species of tomato palnt. But there were increased adventitious root formation under waterlogging. Among the four genotypes, CLN2498E and CA4 showed high waterlogging tolerance, followed by LA1421. The genotype LA1579 showed sensitivity against waterlogging (Ezin *et al.,* 2010).

Paltaa *et al.* (2010) reported that leaf area (56–70%), the number of branches (50%) and root growth were decreased in both chickpea cultivars at flooding stress in comparison with the control plants. The kabuli cultivar (Almaz) showed less depletion in growth parameters than the desi cultivar (Rupali). Shoot dry weight were reduced by 56% and 70% in Almaz and Rupali cultivars, respectively, under waterlogging condition.

In green gram, plant height, leaf area, the number of leaves and dry matter were considerably reduced by flooding stress. They observed that 4 days of waterlogging was more severe than waterlogging for 2 days compared to control. Waterlogging reduced the plant height by 33%, the number of leaves by 31%, leaf area by 31%, number of branches by 34%, and total dry matter by 30% (Prasanna and Rao, 2014).

In some research, the exaggerated effect of waterlogging has been recorded on some water-loving plants. While working on two rice genotypes (Puzhuthiikar and IR72593), Anandan *et al.* (2015) reported that the genotype Puzhuthiikar exhibited noteworthy increment of leaf blade length, sheath length and area but the reduction in leaf blade area than the genotype IR72593 under prolonged flooding stress.

Shin *et al.* (2017) worked with six Korean maize lines (KS85, KS124, KS140, KS141, KS163, KS164) on which they imposed waterlogging for about 30 days at V3 stage. After 30 days of waterlogging treatment, they observed that plant height, the number of fully-expanded leaves were reduced significantly and among the six lines, KS140 performed better.

Duhan *et al.* (2018) carried out an experiment with four genotypes (ICPH-2431, PARAS, UPAS-120, H09-33) of pigeonpea and found that total plant biomass was declined by 22.3–28.1% under 8 days of waterlogging. Among the tested genotypes, ICPH 2431 exhibited minimum and UPAS 120 exhibited a maximum decline of plant biomass at flooding stress.

Waterlogging stress showed deleterious effects on plant growth parameters of seven different barley genotypes. Among the genotypes TX9425, Yerong and TF58 found tolerant against waterlogging in some extent and Franklin, Naso Nijo and TF57 were found to be waterlogging-sensitive. Waterlogging-tolerant genotypes exhibited less decline in plant height, SPAD value, tillers, shoot and root biomasses than the waterlogging-sensitive genotypes (Luan *et al.,* 2018).

Li *et al.* (2018) performed a study with 18 different maize cultivars and observed plant height, dry weight, root length, root hairs, root surface area and root volume were decreased under flooding stress.

Chávez-Arias *et al.* (2019) aimed to evaluate the impacts of waterlogging stress (flooding for 4, 6 and 8 days with and without Foph) in cape gooseberry plants infected with *Fusarium oxysporum* f. sp. Physali (Foph). With waterlogging for 6 and 8 days inoculated plants showed a higher disease progress curve (55.25 and 64.25) in comparison with the inoculated plants but without waterlogging (45.25). They noticed that waterlogging significantly affected stem diameter, leaf area, DW. Cape gooseberry plants exhibit a less acclimation to flooding stress for more than 6 days in soil with Foph.

Liu *et al.* (2020) aimed to distinguish the variation between lowland (YueFu (YF)) and upland (IRAT109 (IR)) rice genotypes in terms of flooding tolerance. Waterlogging for 7 days were applied to 28 days old rice seedlings. They found that root length (11.8% and 16.0%), root dry weight (9.9% and 10.6%) and shoot dry weight (13.3% and 25.3%) were reduced under flooding stress in YF and in IR genotypes, respectively.

2.5.2 Effect on plant physiology and metabolism

Waterlogging stress has a significant influence on plant physiology and metabolism. Lone and Warsi (2009) reported that waterlogging remarkably reduced transpiration rate, stomatal conductance, SPAD value in maize genotypes. The net photosynthetic rate of the leaf of the wheat plant showed a decreased manner when the plants were exposed to hypoxia (Zheng *et al.,* 2009).

Ashraf (2012) reported that primarily under waterlogging condition plants showed a lessened gas exchange, stomatal conductance, CO_2 absorption and hydraulic conductivity of roots. Flooding impedes photosynthetic rate in plants. Akhtar and Nazir, (2013) also stated that in C_3 plants, waterlogging stress impelled stomatal closure.

Kumar *et al*. (2013) performed research with four genotypes of mungbeans, including two tolerant genotypes (T-44 and MH-96-1) and two susceptible to waterlogging (MH-1K-24 and Pusa Baisakhi). Plants were exposed to 3, 6 and 9 days of waterlogging at the vegetative stage. Relative water content (RWC), membrane stability index (MSI), photosynthetic rate, chlorophyll and carotenoid content were decreased at different durations of waterlogging stress. Nevertheless, the effects were more noticeable in sensitive genotypes than the tolerant genotypes.

While working on six wheat genotypes Amri *et al.* (2014) reported that after 28 days of waterlogging chl content decreased minima of 41.3% and 44.5% for cv. Salammbô and cv. Utique against maxima of 58.5%, 58.9% and 60.7% for cv. Vaga, FxA and cv. Ariana, respectively. Leaf water potential, net photosynthesis, *chl* concentration showed a decreased manner due to recurrent flooding in *Cichorium intybus* plant (Vandoorne *et al.,* 2014).

Two sugarcane varieties, one is an early maturing (V1) and another is a mid-late maturing (V2) on which 96 h waterlogging stress is imposed. The results showed increased RWC (85% to 90% in the V1 variety and 87% to 90% in the V2 variety), proline content (V1 than V2). They also resulted in reduction of chl *a*, chl *b* and carotenoids contents under waterlogging treatment (Bajpai and Chandra, 2015).

Anandan *et al.* (2015) conducted an experiment with 2 rice genotypes (Puzhuthiikar and IR72593) under prolonged waterlogging stress. The genotypes in a long time waterlogging demonstrated elevated photosynthetic rate, stomatal conductance to CO_2, reduced transpiration rate and intercellular CO_2 concentration than the control plants. Even so, Ci in Puzhuthiikar under prolonged hypoxia (316.5 µmol CO_2 mol air^{-1}) was not notably differed from control (316.6 µmol CO_2 mol air^{-1}).

Shin *et al.* (2017) worked with 6 Korean maize lines (KS85, KS124, KS140, KS141, KS163, KS1644) on which they imposed waterlogging for about 30 days at V3 stage. After 30 days of waterlogging treatment, they observed that SPAD values were reduced significantly and among the six lines, KS140 performed better.

Duhan *et al.* (2018) performed an experiment with four genotypes (ICPH-2431, PARAS, UPAS-120, H09-33) of pigeonpea plants and found that proline and membrane injury (MI) extended under all waterlogging treatment. A maximum elevation of proline content was recorded in genotype ICPH 2431 and the minimum was in UPAS 120. An elevation of 101–128% proline and 110–121% MI in waterlogging (W) (8 days) 1 DAR (day after removal) of treatments in 20 days old plants were observed. Though at 8 DAR, a partial recovery was observed in MI and proline content, which was 65–110% and 37–60% increase under W in 20 days aged plants. At 12 days W 8 DAR, no plant was survived. 40 days old plants showed less elevation of proline content (81–109%) for waterlogging 8 days 1 DAR. Membrane injury was more detrimental for 40 days old plants under the same treatment. Genotype UPAS 120 (165%) showed maximum and ICPH 2431 (125%) minimum increase. Waterlogging (8 days) treatment resulted in a decrease of 23–38% and 31–39% Chl content and RWC respectively at 1 DAR of treatments which furthermore declined to 16–30% and 28–38% at 8 DAR in 20 days old plants. In 20 days old plants, 12 days waterlogging treatment resulting in 42-49% and 40-47% reduction at 1 DAR, while no survival was observed at 8 DAR. More chl content and RWC decreased in 40 day old plants, resulting in a 51-56% and 44-51% decrease of 1 DAR from waterlogging (8 days) treatment, respectively. No survival in 40-days old pigeonpea plants was observed at any duration of waterlogging treatment

Alizadeh-Vaskasi *et al.* (2018) noticed that, waterlogging treatments declined chl *a* and *b* and carotenoids and enhanced proline contents in three wheat genotypes (N-93-19, N-93-9 and N-92-9) in tillering and stem elongation stages. In the tillering stage, carotenoids contents declined in N-93-19 genotype (16, 38 and 67%), N-93-9 genotype (15, 27 and 54 %), and N-92-9 genotypes (11, 16 and 29%) for 7, 14 and 21 days of WL, respectively in comparison with the corresponding controls. In the stem elongation stage, a reduction of carotenoid content was observed at 7 d of waterlogging, but there was no noteworthy differences between the three wheat genotype. carotenoids contents declined in N-93-19 (49 %), N-93-9 (36%) and N-92-9 (32%), respectively in comparison with the corresponding controls under 14 d flooding treatment.

During waterlogging stress conditions of nine wild solanaceous plants, there observed a rapid stomatal closure and reduced photosynthesis and stomatal conductance. Among them, *Solanum torvum* species appeared photosynthetically better under waterlogging and had greater stomatal conductance as well (Kumar *et al.,* 2018).

While working with 6 maize genotype Akter *et al.* (2018) found the total chl content was decreased by 12%, 9% and 8% in CML54 × CML487; 8%, 5% and 3% in P18; 30%, 12.5% and 43% in CML 54; 18%, 20% and 14% in CML 486 × CML 487; 19%, 14% and 27.2% in CML 486 followed by 12%, 10% and 13% in CML 487, respectively on the 2, 4 and 6 days of waterlogging stress condition.

Waterlogging treatment significantly reduced MSI, chl content, and fluorescence in four blackgram genotypes (Uttara, T-44, IC530491, IC519330) for about 10 days of waterlogging at vegetative stage. In comparison with Uttara, MSI and chl content was greater in IC530491, IC519330 and T44 (Ruchi *et al.,* 2019).

Anee *et al.* (2019) conducted an experiment with sesame plants which were exposed to waterlogging for about 2, 4, 6 and 8 days at the vegetative stage of plants. They showed a reduction of RWC (75%), proline content (20%), chl *a*, chl *(a + b)* and carotenoid content under waterlogging compared to their respective controls for up to 8 days.

2.5.3 Effect on plant anatomy

Ashraf (2012) stated that plants commence adventitious root, hypertrophied lenticels and aerenchymatous cells for adapting with the adverse waterlogging situation. Shiono *et al.* (2011) also reported that both in wetland and dryland species, aerenchymatous tissue formation was augmented.

Garcinia brasiliensis (Mart.) seedlings were supplemented with waterlogging for 90 days. Waterlogging exhibited thicker exodermis, higher xylem number, thicker phloem and fewer xylem fibers than the control. The width of exodermis was elevated by 24% compared to control (de Souzaa *et al.,* 2013).

Zhang *et al.* (2015) observed adventitious root porosity was significantly greater in waterlogging-tolerant barley genotypes than sensitive genotypes and development of aerenchyma was much faster in tolerant genotypes than the sensitive one. In another experiment Broughton *et al.* (2015) also found that under flooding treatment, the porosity of adventitious roots were notably higher than the control of two barley varieties, which were selected in screening experiments. Flooding induced NO formation, which helps in the initiation of aerenchyma in wheat roots (Wany *et al.,* 2017).

Luan *et al.* (2018) observed morphological and anatomical adaptations in 7 barley genotypes under waterlogging stress. The tolerant genotypes (TX9425, Yerong, TF58) displayed a much higher number of adventitious roots under waterlogging stress conditions than the sensitive genotypes (Franklin, Naso Nijo, TF57). There were observed more intercellular spaces and better integral membrane structures of chloroplast in the leaves of the waterlogging-tolerant genotypes as there extended ethylene content declined ABA content and less $O_2^{\cdot -}$ accumulation.

Liu *et al.* (2020) worked on two rice genotypes (YueFu (YF) and IRAT109 (IR)) where they found that aerenchyma formed under 7 days of flooding in both genotypes. They observed that the generation was 1.5 fold higher in YF than in IR genotype.

At flooding conditions, roots of plants exhibit different apparatus. Due to waterlogging stress conditions, radial O_2 was reduced from the roots and there found tangential diffusion barriers (Sauter, 2013).

2.5.4 Effect on nutrient availability

Smethurst *et al.* (2005) observed that nutrient constituents (K, P, Cu, Ca, Mg, Zn, and B) of leaves and roots notably decreased in *Medicago sativa* due to flooding stress. The uptake of both the macronutrients and micronutrients of plants got disturbed due to waterlogging stress (Akhtar and Nazir, 2013).

Ashraf (2012) reported that waterlogging might disintegrate the availability of various crucial macro- and micronutrients in the soil which deleteriously affect many physiological and biological mechanisms in plants. As there lack essential plant nutrients like N, K, Ca, Mg, etc.

2.5.5 Effect on yield

Waterlogging stress significantly reduced yield at both vegetative and reproductive stages except for some water craving plants.

Lone and Warsi (2009) conducted experiments with 15 genotypes of *Zea mays.* Among them, 5 were parents and 10 were their single crosses in both winter and summer season. They observed that excess soil moisture exhibited a drastic reduction in grain yield of all the genotypes. Yield reduction was higher in the winter trial than in the summer trial. In winter trials, reduction of yield ranged between 19% in YHPP45 (tolerant) to 53% in Pop 3121 × YHPP45. Whilst the reduction of yield observed highest in Tarun83 (susceptible genotype) that is 66 % and lowest in YHPP45 (tolerant) that is 2% in summer trial.

Forty days old tomato plants (two cultivars and two wild related species) were exposed to continuous waterlogging for 2, 4, 6 and 8 days duration by Ezin *et al.* (2010). Waterlogging for 8 days showed a drastic reduction in yield of all the genotypes. Yield reduction was observed 22.82%, 69.235%, 89.55%, and 100% in CLN2498E, CA4, LA1421, LA1579 genotypes, respectively upon exposure to 8 days of waterlogging. Among the four genotypes, LA1579 was waterlogging sensitive, CLN2498E, and CA4 showed high tolerance and LA1421 showed tolerance in some extent. Waterlogging for 12 days reduced seed yield of kabuli cultivar (Almaz) and desi cultivar (Rupali) of chickpea by 54 and 44%, respectively (Paltaa *et al.,* 2010).

Yaduvanshi *et al.* (2010) worked with eight wheat genotypes exposing 15 days of waterlogging. They detected grain yield reduction of the genotypes due to waterlogging stress. Yield reduced in the genotypes by 12% (KRL 3-4), 9.8% (NW 1076), 9.0% (KRL 146), 190% (Brookton), 162% (PBW 343), 3.2% (KRL 200), 1.7% (KRL), 100% (HD 2009), respectively. In another experiment, Rasaei *et al.* (2012) detected a yield reduction of wheat for 10, 20 and 30 days of waterlogging. They reported that the highest yield reduction (45%) was observed for 30 days of waterlogging.

Few mungbean genotypes, among them two flooding tolerant (T 44 & MH–96–1) and two flooding sensitive (Pusa Baisakhi & MH–1K–24) were exposed to different duration of waterlogging (3, 6, 9 days). The treatment was imposed at 30 days old mungbean seedling. The average yield reduction of the genotypes were observed 20%, 34%, and 52% at 3, 6, and 9 days of waterlogging, respectively. The yield losses during 3 days of waterlogging were almost recovered by tolerant genotypes. Sensitive genotypes showed upto 20% yield reduction for 3 days of waterlogging. Sensitive genotypes showed 70% (Pusa Baisakhi) and 85% (MH–1K–24) yield reduction when exposed to 9 days of c (Kumar *et al.,* 2013).

Amri *et al.* (2014) carried out experiments with six bread wheat and imposed 28 days of waterlogging. They reported 56% yield reduction on an average, maximum reduction by 74% (in cv. Ariana and cv. Vaga) and lowest reduction by 39% (in, Salammboˆ and Utique). Prasanna and Rao (2014) detected that 2 and 4 days of waterlogging declined yield by 25% and 71% in *Vigna radiata.*

Ren *et al.* (2014) worked with 2 summer Maize genotypes (cv. Denghai 605 (DH605) and Zhengdan 958 (ZD958)) in field condition exposing 3 and 6 days of waterlogging at three-leaves stage (V3), six-leaves stage (V6), tenth day after tasseling stage (10VT). Waterloggigng reduced yield by 23%, 32%, 20%, 24%, 8%, and 18% in Denghai 605 (DH605) genotype and 21%, 35%, 15%, 33%, 7%, and 12%

in Zhengdan 958 (ZD958) genotype at V3-3, V3-6, V6-3, V6-6, 10VT-3, and 10VT-6 stages when compared with their control plants.

Yield reduction in *Sesamum indicum was* reported by Sarkar *et al.* (2016) upon exposure to 12, 24 36 h of waterlogging. The author used two cultivars of sesame (BARI til 2 and BARI til 3). Upon exposure to 12, 24 36 h of waterlogging yield declined by 24, 38 and 39.41% in BARI til 2 and 29, 46 and 53% in BARI til 3, respectively in comparison with their control plants. Maximum reduction was observed at 36 h of waterlogging duration.

Saha *et al.* (2016) conducted an experiment with four sesame genotypes (BD-6980, BD-6985, BD-6992 and BD-7012) imposing 3 days of waterlogging. They noticed yield reduction was minimum in BD 7012 (24%) and maximum in BD 6980 (44%) genotypes. Shin *et al.* (2017) observed Decreased yield in *Zea mays* when imposed 30 days of waterlogging.

Grain yield reduction was demonstrated in three wheat lines (N-93-9, N-92-9 and N-93-19) under 7, 14 and 21 days of waterlogging (WL). The highest reduction of grain yield was observed for 21 days of WL. Grain yield declined by 60.15, 56.01 and 37.08 % in N-93-9, N-92-9 and N-93-19 genotypes, respectively upon exposure to WL for 21 days in comparison with their respective control plants (Alizadeh-Vaskasi *et al.,* 2018).

Duhan *et al.* (2018) stated that the genotypes of pigeonpea showed a decline of yield as they were exposed to 8 days of waterlogging stress. In UPAS 120, the reduction is 61.5% and in ICPH 2431 (27.4%) due to waterlogging stress.

2.5.6 Waterlogging-induced oxidative stress and antioxidant defense system in plants

Flooding/waterlogging stress causes oxidative damages, as there generate ROS. Overproduction of ROS causes harm to the plants. Plant exhibit some antioxidant defense system to counteract the negative effects of ROS. The plant has two kinds of the antioxidant defense systems, one is enzymatic and another is nonenzymatic antioxidants. Catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione *S*-transferase (GST), glutathione reductase (GR), peroxidase (POX) are enzymatic and glutathione (GSH), ascorbate (AsA), tocopherols, carotenoids are nonenzymatic antioxidants (Hasanuzzaman *et al.,* 2012; Khan *et al.,* 2016a,b; 2015; 2014). All these antioxidants act coordinately in scavenging ROS, which gives protection to tissues from oxidative stress.

Zhang *et al.* (2007) stated that waterlogging for about 18 days increased lipid peroxidation in the membrane of two barley genotypes: Xiumai 3 (tolerant) and Gerdner (sensitive). Upon exposure to waterlogging, SOD activity increased and was greater in the sensitive genotype than the tolerant one. Both POD and CAT activity was increased in the tolerant genotype than the sensitive one. GR activity increased in both the genotypes.

Two genotypes of wheat: Yangmai 9 (waterlogging tolerant) and Yumai 34 (waterlogging sensitive), were exposed to waterlogging stress for about 15 days. After 15 days of waterlogging treatment MDA content increased by 15 and 22%, SOD activity decreased by 27 and 30%, CAT activity by 51 and 20% in Yangmai 9 and Yumai 34, respectively than the control plants. In sensitive genotype (Yumai 34), POD activity was reduced by 14% than the control. At the same time, tolerant genotype showed higher POD activity (10%) under waterlogging than the control (Tan *et al.,* 2008).

While working on two genotypes (ICPL 84023 and ICP 7035) of *Cajanus cajan,* Sairam *et al.* (2009) observed that H_2O_2 and $OH^{\bullet}$ contents increased under 6 days of flooding stress. SOD, APX, GR, CAT activity increased upon exposure to waterlogging. When applied flooding stress for about 10 days, H_2O_2 content increased 290% than the control *Allium fistulosum* plants. The activity of SOD, POD, CAT, GR also enhanced when exposed to flooding stress (Yiu *et al.,* 2009).

Research work was conducted by Zheng *et al.* (2009) with two wheat genotypes (Huaimai 17 and Yangmai 12) under hypoxia stress. They noticed an enhancement of lipid peroxidation and a reduction of ATP synthesis in the chloroplasts under 5 days of waterlogging. Damanik *et al.* (2010) conducted an experiment with *Oriza sativa* (cv. FR13A) and observed that 8 days of waterlogging enhanced the activity of APX and SOD.

Bin *et al.* (2010) worked with 2 maize genotypes: HZ32 (flooding-tolerant) and K12 (flooding-sensitive), exposing 2, 4, 6, 8 and 10 days of waterlogging. Lipid peroxidation was increased considerably in K12 while in HZ32 showed no difference upto 6 days of waterlogging. The activity of SOD, POD, APX, GR and CAT was higher in the tolerant genotype than the sensitive one.

In maize (two genotypes viz. HZ 32 and K 12), lipid peroxidation elevated notably when exposed for 10 days of waterlogging (Tang *et al.,* 2010). Enhanced MDA content was observed in wheat (cv. Yangmai 9) for 7 days of waterlogging (Li *et al.,* 2011).

Sairam *et al.* (2011) performed an experiment with *Vigna luteola*, a highly tolerant wild species, and two mung bean (*V. radiata*) varieties: T 44 (tolerant) and Pusa Baisakhi (susceptible). *Vigna luteola* and T 44 showed an increased SOD and APX gene expression, while Pusa Baisakhi showed a little expression when exposed to waterlogging for 8 days than their control plants.

Xu *et al.* (2012) reported 29% increase of MDA content when 2 days of waterlogging was applied in sesame (cv. WSG-EZhi2). The activity of SOD increased in the WSG-EZhi2 genotype while the activity of POD and CAT declined in WTG-2541 and WTG-2413 genotypes upon exposure to 48 h of waterlogging stress.

El-Enany *et al*. (2013) also found enhancement of MDA and H_2O_2 content by 32% and 43%, respectively, for about 30 days of waterlogging in cowpea plants. de Souzaa *et al.* (2013) demonstrated a conspicuous increment of H_2O_2 content for prolonged waterlogging (60 days) in *Geophagus brasiliensis.* Waterlogging for 80 days enhanced SOD and APX activity in *G. brasiliensis.*

In sesame (cv. Ezhi-2) plants, waterlogging for 6 days increased MDA content by 1.8 fold than the control plants. waterlogging for 8 days increased SOD and APX activity

(Wei *et al.,* 2013). In another experiment with two genotypes of sesame (BD 6980 and BD 1012), Saha *et al.* (2016) found that MDA content increased by 5.79% and 48.2% in BD 6980 and BD 1012, respectively, for 2 days of waterlogging stress condition.

Two sugarcane varieties, one is an early maturing (V1) and another is a mid-late maturing (V2) on which 48 and 96 h of waterlogging stress are imposed after 60 days of planting. Upon exposure to waterlogging, early maturing (V1) showed a higher SOD gene expression, while mid-late maturing (V2) showed a lower SOD gene expression than the control plants (Bajpai and Chandra, 2015). Duhan *et al.* (2018) demonstrated that 12 days of flooding increased MDA content by 59–91% in pigeon pea plants.

Alizadeh-Vaskasi *et al.* (2018) performed a study with three wheat genotypes (N-93-19, N-93-9 and N-92-9), which were exposed to waterlogging stress (7, 14 and 21 days) in both tillering and stem elongation stages. They found that MDA content increased in all the tested genotypes for 21 days of flooding and the highest increase was observed in N-93-19 genotypes. The activity of SOD enzyme increased by 73.67, 79.55 and 66.99 % in N-93-9, N-92-9 and N-93-19 genotypes, respectively. the activity of POD enzyme decreased in all the three wheat genotypes while N-93-19 genotype showed the highest decrease upon exposure to 14 days of waterlogging treatment. However, N-93-19 and N-93-9 genotypes showed a significant decrease in CAT activity but N-92-9 genotype showed a considerable increase in CAT activity under waterlogging compared to control.

Luan *et al.* (2018) carried out an experiment with seven different barley genotypes under waterlogging stress. Waterlogging for 21 days increased superoxide radical ($O_2^{\cdot -}$) in leaves 9, 8, 29, 28, 27 and 20% in genotypes TX9425, Yerong, YYXT, Franklin, Naso Nijo and TF57, respectively, while no notable variation was found in TF58.

In cape gooseberry plants, MDA content increased for 8 days of waterlogging stressed plants inoculated with *Fusarium oxysporum* f. sp. Physali (Foph) (Chávez-Arias *et al.,* 2019).

Anee *et al.* (2019) found that MDA (39%), H_2O_2 content increased and AsA (38%), GR (23%) content decreased in sesame (cv. BARI Til-4) plants when exposed to 8 days of flooding. The author reported rising of APX (61%), MDHAR (55%), DHAR (59%), GPX (47%), CAT (33%), GSSG activity upon exposure to 8 days of waterlogging in sesame plants. In blackgram, MDA content and SOD activity also increased under 10 days of waterlogging treatment (Ruchi *et al.,* 2019).

While working on 18 maize genotypes, Li *et al.* (2018) reported a reduction of MDA contents in all the genotypes except the GT2, HMT8, YT13 and TBN6 genotypes of maize. Notably, waterlogging declined MDA contents by 24.49%, 33.68% and 24.67% in XMXZ, XMXN and YXN2 genotypes, respectively, under 2 days of waterlogging stress which was due to higher antioxidant enzymes activities that help plants to tolerate under hypoxia condition. Activity of SOD enhanced by (19.16% to 56.89%), POD (19.16% to 106.96%) and CAT (26.08% to 57.29%) under flooding for 2 days.

In an experiment, Kumar *et al.* (2018) found MDA content to be increased under 7 days of waterlogging stress in *Solanum torvum* while working with 9 wild solanaceous species. Also, SOD, CAT, POD activity increased under flooding stress.

Liu *et al.* (2020) worked on two rice genotypes (YueFu (YF) and IRAT109 (IR)), where they found that IAA, ethylene and H_2O_2 content were increased under 7 days of flooding.

2.6 Abiotic stress responses in soybean

Different adverse environmental stresses cause detrimental effects on soybean plants. Even so, the stresses greatly vary on genotypes, nature and duration of the stress.

Salinity stress adversely affects in morphological, physiological and biochemical processes of soybean plants. Shu *et al.* (2017) reported that salt stress hampered soybean seed germination, seedling establishment. They also stated that MDA, CAT, SOD, POX were increased under salinity stress. Soybean plants showed 30-76% reduction of plant height and fresh weight when 50-200 mM NaCl was applied

(Amirjani, 2010). Katerji *et al.* (2003) observed that seed yield of soybean was reduced by 20% and 56% when applied salt stress of 4.0 dS m^{-1} and 6.7 dS m^{-1}, respectively.

Like salinity stress, drought stress also showed an adverse influence on soybean production (El Sabagh *et al.,* 2018). Research work was conducted by Anjum *et al.* (2011) under drought condition where they observed a reduction in pod number $plant^{-1}$ (18.22%), number of seed $plant^{-1}$ (15%), number of seed pod^{-1}, 100 seed weight (13%), biological yield $plant^{-1}$ (16%) and seed yield $plant^{-1}$ (22%), respectively than the control soybean plants. Under drought stress, net photosynthesis was significantly decreased in soybean plants (Anjum *et al.,* 2013). An experiment was conducted with three genotypes (M7, L17 and Hamilton) of soybean in drought stress. The drought was created by applying PEG 4000. He observed that with the enhancement of drought levels (0, −3, −6 and −9 bar) reduced percent germination. Among the all tested genotypes, M7 performed better than the rest two genotypes (Salimi, 2015). BARI Soybean-5, BARI Soybean-6, Shohag and BD2331 were found as tolerant to drought stress, which was screened out from fifty soybean genotypes under drought condition (Chowdhury *et al.,* 2016).

Metal or metalloid toxicity also negatively affects on plant growth, development and productivity. Farooq *et al.* (2013) stated plant height (40% and 74%), root length (32% and 67%) and leaf area (34% and 62%) of cotton plant reduced significantly during exposure to different level of cadmium (Cd) concentration (1 and 5 µM) respectively compared to control. Cd stress (5 µM) increased the amount of MDA, H_2O_2 and EL by 72%, 67% and 77%, respectively in plants which indicate the increased production of oxidative stress in cotton plants than the control. MDA content also increased in rapeseed seedlings by 37 and 60% at 0.5 and 1 mM $CdCl_2$ stress, respectively. DHA, APX, GST, GSH, GSSG and GR activity also increased in stress condition where activity of CAT, MDHAR and DHAR reduced significantly at both stress but GPX activity only increased at mild stress (0.5 mM) (Hasanuzzaman *et al.,* 2017c).

Temperature stress could include two kinds, one is high temperature (HT) stress and another is low temperature stress (chilling stress). High temperature stress can pose impacts to plants with a short period of time. For soybean emergence, a suitable temperature ranges from 15-22° C (Liu *et al.,* 2008). Anto and Jayaram (2010) conducted an experiment with soybean seeds to investigate high temperature stress. They imposed high temperature 50, 60 and 70° C for 10 h and observed that seeds could withstand up to 70° C. But there reduced germination percentage, moisture content, the vigor of soybean seedlings in comparison with the control. The thickness of palisade and spongy parenchyma soybean leaves were increased by increasing temperature (Djanaguiraman *et al.,* 2011). Snider *et al.* (2009) mentioned that high temperature distressed pollen viability and stigma receptivity that reduced fruit set and ultimately decreased yield. A suitable temperature ranges from 25-29° C had found to be optimum for pod setting in soybean, which got affected above 37° C (Lindsay and Thomson, 2012).

Exposure to ultraviolet (UV) radiation may have a negative impact on plants. Baroniya *et al.,* (2011) performed a study to ascertain the effect of solar UV-B radiation in 8 genotypes of soybean. A reduction of plant height, leaf area, number of nodes, number of pods and weight of seed were observed under UV-B radiation stress than the control plants.

Soybean crops are usually not tolerant to waterlogging stress (Tougou *et al.,* 2012). Some literature on the consequences of waterlogging on soybean plants have been presented below.

2.7 Effect of waterlogging on soybean plants

2.7.1 Effect on growth

Linkemer *et al.* (1998) reported that plant height, leaf area and dry weight were decreased when flooding stress induced at the vegetative stage of soybean plants. Youn *et al.* (2008) carried out an experiment with supernodulating mutants (SS2-2 and Sakukei 4) and their wild type (Sinpaldalkong 2 and Enrei) of soybean. The plants were exposed to 15 days of flooding stress at the beginning flowering (R1) stage. After the removal of water, they observed that root dry mass was reduced by 62-67% in supernodulating mutants and 41-45% in wild types, respectively. 30 days after removal of water presented 64-75% and 51-64% in supernodulating mutants and wild types, respectively.

VanToai *et al.* (2010) conducted both screen house (SP05 and SU05) and field tests (FD06) with 21 soybean genotypes to analyze waterlogging tolerance. Plants height were affected in both tests. In SP05 experiment, plant height ranged from 22 to 69 cm with an average of 47 cm in control plants of 21 genotypes when waterlogging decreased the average to 30 cm. In SU05 experiment, the average in control was 55 cm, whereas it dropped to 40 cm under flooding condition, the reduction was estimated 27% as compared to control. In FD06 experiment, plants grew 13% taller under flooding stress, in control, the average height was 45 cm whereas 50 cm in waterlogging treatment. In all the three experiments, genotype Nam Vang and VND2 grew tall under waterlogging stress.

In a field experiment, four soybean genotypes (AGS 313, G 00351, BD Soybean-4, G 00197) were exposed to 3 waterlogging stages, i) Control (no waterlogging) ii) waterlogging at R1 stage (blooming) iii) waterlogging at R4 stage (full pod) for 7 days. Shoot dry matter was accumulated by 69, 67, 65 and 54% in genotypes AGS 313, G 00351, BD Soybean-4 and G 00197, respectively, at R4 stage waterlogging (Ara *et al.,* 2015).

While working on two soybean lines, PI408105A (waterlogging tolerant line (WTL)) and S99-2281 (waterlogging susceptible line (WSL)) in waterlogging stress Kim *et al.* (2015) found that the shoot length (SL), shoot width (SW) of WTL did not appear a notable variation between the control and waterlogging treatments, but SL and SW of WSL were slightly decreased 10 days after treatment (DAT) compared to control plants. Root length (RL) did not vary in control and treatment. Whereas shoot fresh weight and root fresh weight varied. In WTL, no significant difference was found in SFW between control and treatment for 5 DAT but there observed a reduction at 10 DAT.

Andrade *et al.* (2018) analyzed the role of pretreatment of soybean seeds with hydrogen peroxide (70 mM H_2O_2 solution) for stimulating the tolerance of soybean seedlings under flooding stress. They observed that biomass accumulation in roots and shoots, stem diameter were increased in plants as the seeds were pretreated with H_2O_2.

Kim *et al.* (2018) reported that the root surface area (RSA) of control soybean plants was significantly increased at 5 DAT (days after treatment), 10 DAT and 15 DAT, compared to waterlogged (WL) and WL with ethylene (ETP) applications. Compared to WL -only treated soybean plants, RSA was increased in WL with ETP treated soybean plants. They also noticed that ETP application induced adventitious root initiation in soybean plants under waterlogging stress.

2.7.2 Effect on physiology and metabolism

Yordanova and Popova (2007) reported that photosynthesis and chl content dropped remarkably for prolonged waterlogging stress.

Due to waterlogging stress, several proteins regulating glucose degradation, sucrose accumulation, signal transduction, cell wall relaxing and alcohol fermentation were changed in soybean plants using proteomics (Komatsu *et al.,* 2015; 2012). However, Nanjo *et al.* (2013) stated that waterlogging stress decreased the proteins involved in conserving cell structures and increased the proteins involved in production of energy.

While working on two soybean lines, PI408105A (waterlogging tolerant line (WTL)) and S99-2281 (waterlogging susceptible line (WSL)) in waterlogging stress (5 cm from the soil surface) Kim *et al.* (2015) found that proline contents were not significantly different at 5 days after treatment (DAT) but showed a significant reduction at 10 DAT in both WTL and WSL. Proline content showed less reduction in WSL than the WTL during flooding stress at 10 DAT. Photosynthetic activity was reduced in soybean plants under hypoxia stress conditions (Mutava *et al.,* 2015).

While working on 40 soybean genotypes, Wu *et al.* (2017) observed that 3 days of waterlogging notably reduced leaf chl content resulting color variation in leaves.

Andrade *et al.* (2018) analyzed the role of pretreatment of soybean seeds with hydrogen peroxide (70 mM H_2O_2 solution) for stimulating the tolerance of soybean seedlings under flooding stress. 12 days aged soybean seedlings were exposed to waterlogging (0, 16 and 32 days). Since 16 days of waterlogging, gas exchange and chl content were higher in the pretreated plants than the control. At 32 days, they tended to decrease. The authors also found that pretreated plants showed the least electrolyte leakage in the cells of the root.

While working on soybean Kim *et al.* (2018) aimed to apply ethylene (ETP) on waterlogging stressed plants and found that application of ETP lessened flooding stress and significantly enhanced photosynthetic activity as well as bioactive GA_4 content in comparison with untreated plants. 100 µM ETP- treated plants showed enhancement of total amino acid contents.

2.7.3 Effect on plant anatomy

Under the waterlogging stress condition, the formation of secondary aerenchyma was found in soybean seedlings. After 3 weeks of waterlogging, secondary aerenchyma (a white and spongy tissue) was formed in the hypocotyls, tap root, adventitious roots and root nodules. At 14 days of waterlogging, 30% porosity increased in waterlogged hypocotyl developing secondary aerenchyma where 10% porosity was observed in the hypocotyl of irrigated plants that did not develop aerenchyma (Shimamura *et al.,* 2003).

While working on two soybean lines, PI408105A (waterlogging tolerant line (WTL)) and S99-2281 (waterlogging susceptible line (WSL)) in waterlogging stress Kim *et al.* (2015) observed that number and size of aerenchymatous cells were greater in the flooding than the control plants of WTL. The WSL did not show any considerable difference between treatment and control plants. Therefore, the formation of secondary aerenchyma played an important role in adapting hypoxia stress.

2.7.4 Effect on nutrient availability

Davanso *et al.* (2002) reported that waterlogging stress inhibited the uptake of carbon (C), nitrogen (N) and other macronutrients. On the other hand, it enhanced the uptake of iron (Fe), which causes iron toxicity.

In a field trial Cho and Yamakawa (2006b) stated shoots faced more starvation than roots under the waterlogging condition as there prevailed nutrient deficiency.

Content of N, P, K in soybean leaves were obtained lower when imposed waterlogging stress for 8 days (Rhine *et al.,* 2010). Two cultivars of soybean (Hefeng 50 and Kenfeng 16) were exposed to hypoxia stress and it was observed that number of nodule reduced by 84% and 64% in Hefeng 50 and Kenfeng 16, respectively. Reduction of the number of nodules were observed at flowering, pod bearing and grain filling stages thus causing distressed in N fixation in roots (Miao *et al.,* 2012).

While conducting proteomic analysis on waterlogged soybean cotyledons Komatsu *et al.* (2013) obtained a decreased number of calcium oxalate crystals in cotyledons of soybeans. Soybean plants developed a symbiotic relationship with arbuscular mycorrhizal (AM) fungi and rhizobia (*B. japonicum*). Through this symbiosis, plants could acquire nutrient which helped the plants under water-stressed condition (Hattori *et al.,* 2013). Kadempir *et al.* (2014) also stated that inoculation of *B. japonicum* improved nutritional status mitigating waterlogging stress in soybean plants.

Under waterlogging stress, plants show a reduction of nutrient uptake. Content of N and K were significantly increased under waterlogging stress when soybean plants were supplemented with ETP in comparison with waterlogged only. Total macro element content was decreased in waterlogged-only and ETP-treated soybean plants in comparison with the control at 10 DAT (days after treatment) and 15 DAT (Kim *et al.,* 2018).

2.7.5 Effect on yield

Three soybean cultivars were exposed to waterlogging and waterlogging for 9 days decreased seed yield by 38, 44 and 66% in Saebyeolkong, Sobaeg- namulkong and Pungsan-namulkong, respectively. Increasing waterlogging duration also decreased the number of pods m^{-2} in all three cultivars (Cho and Yamakawa, 2006a).

Rhine *et al.* (2010) held an experiment under waterlogging and demonstrated that 20 to 39% yield reduced in several soybean cultivars at R5 stage for 8 days of waterlogging. They also reported that waterlogging at R5 stage caused more reduction in yield than waterlogging at R2 stage

VanToai *et al.* (2010) conducted both screen house (SP05 and SU05) and field tests (FD06) with 21 soybean genotypes to analyze waterlogging tolerance. Seed yield were reduced in both tests for two weeks of waterlogging. In SP05 experiment, grain yield ranged from 8 to 18 g plant^{-1} in control plants of 21 genotypes when waterlogging decreased the seed yield on an average of 53%. In SU05 experiment, seed yield ranged from 10 to 19 g plant^{-1} in control plants of 21 genotypes when waterlogging reduced the seed yield on an average of 62%. In FD06 experiment, seed yield ranged from 265 g plot^{-1} to 898 g plot^{-1} in control plants of 21 genotypes when waterlogging reduced the seed yield on an average of 74%. In all three experiments, VND2, NamVang and ATF15-1 genotypes respond tolerant against flooding stress. Two weeks of waterlogging in SP05, SU05 and FD06 experiments decreased grain yield by 80, 75 and 92%, respectively.

Soybean plants showed reduced yield for about 17-40% and 40-57% at vegetative stage and reproductive stage, respectively under waterlogging stressed condition than the non-stressed condition (Nguyen *et al.,* 2012).

VanToai *et al.* (2012) analyzed the change in seed composition of 5 soybean plant introductions (PIs) (PI086449-0, PI398395, PI416753, PI423838, and PI567251) which were tolerant to waterlogging stress and a cultivar (Williams) which were sensitive to waterlogging stress. Under waterlogging stress linoleic and linolenic acids, daidzein, genistein and glycitein contents were decreased in all genotypes. A composite indicator- seed quality index (SQI) was increased by 4% in the PIs, but SQI were decreased by 5% in the check cultivar.

Mustafa and Komatsu (2014) reported that the yield of soybean crops reduced up to 25% in Asia, North America and other regions of the world under waterlogging damages.

Kuswantoro (2015) conducted an experiment with 16 soybean genotypes, including two check varieties (Lawit and Sinabung), exposing waterlogging to plants at 21 days after planting till harvesting. In his study, he observed days to flowering and days to maturing of the tested genotypes were longer in the flooding condition than the control condition. Genotype MLGG 0096 showed the highest yield, which was equivalent to check varieties.

While working on 40 soybean genotypes under waterlogging stress, Wu *et al.* (2017) reported that flood-sensitive genotypes had more reduction of seed yield than the flood-tolerant genotypes.

2.7.6 Oxidative stress and antioxidant defense

In soybean, waterlogging stress switched aerobic respiration towards anaerobic. Anaerobic respiration triggers alcoholic (C_2H_5OH) fermentation by producing flood inducible proteins that help in generation of NAD^+ and conveyed sharp enhancement of ADH activity in soybean plants (Komatsu *et al.,* 2011a). In another experiment, Komatsu *et al.* (2011b) used proteomics and metabolomics in combination to analyze the effects of waterlogging on cells mitochondria. As waterlogging initially damages the mitochondrial electron transport chain (ETC), which triggers ROS generation.

While working on two soybean lines, PI408105A (waterlogging tolerant line (WTL)) and S99-2281 (waterlogging susceptible line (WSL)) in waterlogging stress Kim *et al.* (2015) noticed that formation of ethylene was considerably elevated in WTL (9 fold and 4 fold increase) in comparison with WSL (2 fold and 3 fold) at 5 days of treatment (DAT) and 10 DAT, respectively. A reduction of 61% and 68% methionine content, 38 and 49% abscisic acid (ABA) in WTL at 5 DAT and 10 DAT, respectively but methionine content and ABA were reduced by 31%, 41% and 16, 26% in WSL. At 5 DAT, gibberellic acid (GA) was notably high in WTL, but at 10 DAT it was not that high. Jasmonic acid (JA) content was that different than the control at 5 DAT in both WTL and WSL. Salicylic acid (SA) was higher in WTL at 5 DAT and 10 DAT in waterlogged stressed plants than the control. However, in WSL, SA were not significantly different between waterlogged and control plants. Later, Kim *et al*. (2018) observed that GST, DHAR2 protein was downregulated in the WL only treated soybean plants, but that was it was revived by an application of ethylene (ETP). In shoots, GR activity was reduced in the WL only and WL with ETP treated plants compared with the control. In shoots, GSH activity was increased in WL with ETP treated plants compared to control and WL only treated soybean plants.

Andrade *et al.* (2018) analyzed the role of pretreatment of soybean seeds with hydrogen peroxide (70 mM H_2O_2 solution) for stimulating the tolerance of soybean seedlings under flooding stress. 12 days aged soybean seedlings were exposed to waterlogging (0, 16 and 32 days). Since 16 days of waterlogging improved the antioxidant system in plants pretreated with H_2O_2. They stated that SOD, CAT and APX activity were seen increased and ROS decreased in leaf and root of pretreated plants than the control at 16 days of hypoxia. They also observed a lower level of H_2O_2, $O_2^{\cdot-}$ and lipid peroxidation in leaf and root under the same condition.

2.8 Genotypic variation of waterlogging tolerance in soybean

VanToai *et al.* (2010) conducted both screen house (SP05 and SU05) and field tests (FD06) with 21 soybean genotypes to analyze waterlogging tolerance. Plants height were affected in both tests. In all the three experiments, VND2, NamVang and ATF15-1 genotypes respond tolerant against flooding stress.

Rhine *et al.* (2010) carried out a 3-year cultivar screening trial to assess the tolerance of soybean genotypes to flooded conditions. Each year there performed screening of about 360 soybean cultivars. In this study, the authors found 5 cultivars that showed a range of tolerances under flooding stress. The cultivars are Manokin, P94B73, Mersch- Denver, Desloy 4710 and DK4868.

While working on 40 soybean genotypes under different levels of waterlogging stress (3, 6, 9, 12 days) Wu *et al.* (2017) observed that R1 stage was more sensitive than V5 stage in plants. They used plant survival rate (PSR) and foliar damage score (FDS) as indicators to waterlogging tolerance. These workers also stated some optimum flooding duration, which may be used for screening out of tolerant genotypes. Flooding for about 9 and 6 days in V5 and R1 stages, respectively, were found to be distinguishable for the screening of soybean genotypes.

Rajendran *et al.* (2019) carried out an experiment to assess the germination of 128 soybean genotypes under different waterlogging levels (3, 5, 7, 9 and 11 days). 2 days delay were observed in coleoptiles emergence of genotypes for 3 days flooding. The increase rose to 11 days when waterlogged for 9.7 days where only two genotypes showed 70% or more germination. These workers found two genotypes: WT3 and WT8 that had more than 70% germination and less than 5 days delayed coleoptiles emergence when compared with control. At 9 days of waterlogging treatment, 78 genotypes showed 50% germination and 5.7 days delayed coleoptiles emergence, whereas 73 genotypes showed no germination. At 11 days of waterlogging treatment, almost inhibited germination of all the 128 genotypes except 10 that showed 50-59% germination and delayed in final emergence.

Garcia *et al.* (2020) worked on three soybean genotypes (PELBR10-6000, PELBR11-6028 and PELBR11-6042) and two cultivars (TEC IRGA 6070 and BMX Potência) under flooding stress. They observed that all the genotypes vanquished waterlogging stress following discrete mechanisms. Flooded PELBR10-6000 exceeded control plant levels CO_2 assimilation rate by triggering fermentative enzymes and alanine aminotransferase. Cultivars BMX Potência showed similar mechanisms and restored metabolic activities to control levels till the end of the recovery period. PELBR11-6028 and PELBR11-6042 triggered antioxidant defenses and TEC IRGA 6070 didn’t delay in flowering.

These literature urge for improving genetic resources of soybean and developing waterlogged tolerant genotypes.

Chapter III

MATERIALS AND METHODS

This chapter includes a detailed description of the experimental time, location, environment, seed or planting materials, care, design and layout of the experiment, cultivation process, application of fertilizers, intercultural operations, data collection and statistical analysis of the experiment.

3.1 Location

The experiment was carried out at Sher-e-Bangla Agricultural University, Dhaka (90º 77′ E longitude and 23° 77′ N latitude), Bangladesh from the period of July 2019 to November 2019.

3.2 Climatic condition of the experimental site

The area of the experimental site was under the subtropical climate. The experiment was conducted in *kharif-II* season. During the experiment, a temperature around 30° C prevailed in the month of July to September 2019. The temperature started falling during the month of October to November 2019. Detailed information of monthly air temperature, relative humidity, rainfall, daylight have been presented in Appendix I.

3.3. Plant materials

The plant material used in this study was soybean (*Glycine max* L. Merill) and there were twelve genotypes of soybean. They were Sohag (PB-1), BARI Soybean-5, BINAsoybean-1, BINAsoybean-2, BINAsoybean-3, BINAsoybean-5, BINAsoybean-6, SGB-1, SGB-3, SGB-4, SGB-5, GC-840.

3.4 Treatments

There were 12 genotypes and 2 water level conditions as

1. Control (C)
2. Waterlogging (W)

Each treatment were compared to its corresponding control. Waterlogging treatments were started at 15 days after sowing (DAS). The waterlogging treatment applied for 12 days and then the plants were allowed to recover. The waterlogging condition was created by applying standing water 2 inches above the soil surface.

3.5 Design and layout of the experiment

The experiment was laid out in a completely randomized design with three replications. There were two sets of pot in the experiment. One set was for measuring the growth and biochemical parameters (Destructive data) and another one was for measuring yield parameters. Comparative pictures of control and waterlogged plants were taken at 25 and 75 DAS.

3.6 Seed collection

The seeds of varieties Sohag (PB-1), BARI Soybean-5 were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur; BINAsoybean-1, BINAsoybean-2, BINAsoybean-3, BINAsoybean-5, BINAsoybean-6 from Bangladesh Institute of Nuclear Agriculture (BINA) and the lines SGB-1, SGB-3, SGB-4, SGB-5, GC-840 were collected from Bangladesh Agricultural Development Corporation (BADC).

3.7 Soil preparation

The collected soil was sun-dried and then crushed to break the clods. After that cowdung and fertilizers were mixed well with the soil, then all the pots were filled with the prepared soil and placed at the experimental shed house. Plastic pots (16-L) were used for the experiment.

3.8 Fertilizer application

Cowdung, urea, triple superphosphate, muriate of potash, gypsum and boric acid were used in the experiment. The total amount of fertilizers were mixed with the soil at final pot preparation. After allowing recovery to the waterlogged plants some amounts of urea and TSP were incorporated with the soil. At the reproductive stage, there were observed some nutrient deficiencies in the plants. So, liquid fertilizer was applied by foliar spraying (1 ml liquid fertilizer (Hyponix Japan) in 1 liter of water). Liquid fertilizer was applied by foliar spraying as the plants were already irrigated with water before spraying. Fertilizers were applied on the required rate.

Fertilizer doses are as follows:

Fertilizers	Dose (kg ha^{-1})
Cowdung	5000
Urea	30
Triple superphosphate	70
Muriate of potash	40
Gypsum	45

3.9 Intercultural operations

3.9.1 Gap filling and thinning

The continuous observation of crop was made after sowing seeds. It was observed that some seeds of soybean lines failed to germinate. So, gap-filling performed for them. Thinning was done to maintain 3 seedlings for yield set.

3.9.2 Weeding and mulching

Sometimes there were some weeds observed in pots which were uprooted manually. Mulching was done very often to keep soil moisten.

3.9.3 Irrigation

Irrigation was given to maintain field capacity moisture level to the control plants

3.9.4 Plant protection measure

There were the attack of several insects to the plants throughout the experiment. Caterpillar, leaf roller, stem fly etc insects were seen to harm the crop. For controlling the insects, insecticides of several groups were used with an interval throughout the experiment. Diazinon® 60 EC, Actara® 25 WG, Ripcord® 10 EC insecticides were applied to manage specific insects either singly or in a coctail form with an interval of 15 days. Insecticides were applied when crop was infested by insects.

3.10 General observation of the experimental pots

Observations were made regularly and the control plants looked normal green. The stressed plants showed less greenish leaves, wilting and then died during the waterlogging period.

3.11 Data collection

Growth and physiological parameters were collected at 20 DAS and 25 DAS as there were observed visible symptoms. Leaf area and SPAD values were taken at different interval until the fruiting stage. The yield parameters were recorded at harvest. Data were collected on the following parameters:

3.11.1 Crop growth parameters

- Mortality rate
- Plant height
- Number of leaves plant^{-1}
- Number of branches plant^{-1}
- Leaf area
- Above-ground fresh weight plant^{-1}
- Above- ground dry matter weight plant^{-1}

3.11.2 Physiological parameters

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

3.11.3 Root phenotype

Pictures of adventitious roots of waterlogged plants were taken with the help of a digital camera.

3.11.4 Phenotypic comparative pictures

3.11.5 Yield contributing parameters

- Number of pods plant^{-1}
- Number of seeds pod^{-1}
- 100-seed weight
- Seed yield plant^{-1}
- Stover yield
- Biological yield

3.12 Sampling procedure for growth study during the crop growth period

3.12.1 Mortality rate

Before starting the treatment total number of plants per pot were counted which may be denoted as Ni and again after the completion of treatment duration total number of plants per pot were counted which may be denoted as Np. The mortality rate was calculated using the following formula (Anee, 2016):

$$\text{Mortality rate (\%)} = \{(Ni - Np) \times 100\} / Ni$$

3.12.2 Plant height

Soybean plant height was recorded at different dates. From the ground level to the highest tip of the leaf was measured by a measuring scale and counted as the plant height. The average height of three plants was considered as the height of the plant for each pot.

3.12.3 Number of leaves plant^{-1}

The number of leaves plant^{-1} were counted at 20, 25, 50 DAS. The average number of trifoliate leaves of three plants were considered as the total leaves plant^{-1}.

3.12.4 Number of branches plant^{-1}

The number of branches plant^{-1} were counted once after the completion of the vegetative growth of plants. Branches of three plants were counted and their mean values were taken.

3.12.5 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.

3.12.6 Fresh weight plant^{-1}

Plant fresh weight was recorded during the stress treatment period. Data was taken at 20 and 25 DAS. Three sample plants were uprooted from each pot randomly and thoroughly washed in running tap water. Then the plants were weighed in an electric balance and averaged them to have fresh weight plant^{-1}.

3.12.7 Dry weight plant^{-1}

After recording the fresh weight, the samples were dried in an electric oven maintaining 80 °C for 48 h. Then they were weighed in an electric balance and finally averaged to derive the dry weight plant^{-1}.

3.13 Sampling procedure for the physiological parameters

3.13.1 SPAD value

Five leaves were randomly selected from each pot. Each leaflet were measured with atLEAF (FT Green LLC, USA) as atLEAF value. The total *chl* content was then averaged and calculated by translating the atLEAF value into SPAD units and then the total *chl* content was measured. SPAD value of leaves were taken at different dates.

3.13.2 Relative water content

In the experiment, RWC was recorded during the stress treatment period. According to Barrs and Weatherly (1962), leaf laminas of fully developed leaves were separated from randomly selected plants to measure the Leaf RWC. Whole leaf discs were weighted like FW and then floated in Petri dishes on distilled water and kept in dark place. After 24 h, the leaf discs were weighed again after removing excess surface water and considered as turgid weight (TW). Dry weights (DW) of leaves were measured after drying at 80°C for 48 h finally, Using the following formula, RWC was calculated:

$$\text{RWC}\ (\%) = [(\text{FW} - \text{DW}) / (\text{TW} - \text{DW})] \times 100$$

3.13.3 Electrolyte leakage

Electrolyte leakage (%) was recorded at 20 DAS. Electrolyte leakage was measured according to the method of Zhang *et al.* (2006). To measure EL, 0.5 g leaf samples were put in a Falcon tube with 15 ml distilled water. The Falcon tubes were then incubated in a water bath at 40 °C for about 1 h. After cooling, electrical conductivity (EC_1) was recorded with an electrical conductivity meter. Samples were again incubated in an Autoclave machine for about 1 h and electrical conductivity (EC_2) were measured after cooling the samples. Electrolyte leakage was calculated using the following formula:

$$\text{EL}\ \% = (EC_1 / EC_2) \times 100$$

3.14 Observation of phenotypes of adventitious root

Roots of waterlogged plants were taken and pictures were taken with a digital camera.

3.15 Observation of plant phenotypes

Control and waterlogged plants were arranged and pictures were taken by a digital camera to compare the phenotypes of plants. Comparative pictures were taken two times, one at the vegetative phase (25 DAS) and another at the reproductive phase (75 DAS). Waterlogged plants showed delayed maturity than the control.

3.16 Procedure of measuring yield and yield contributing parameters

3.16.1 Number of pods plant^{-1}

The total number of pods plant^{-1} were counted from the three plants and then averaged.

3.16.2 Number of seeds pod^{-1}

Ten pods from each pot were selected and seeds were counted from each individual pods and then averaged.

3.16.4 100-seed weight

Harvested seeds were sundried and then clean 100 seeds were counted and weighed with an electric balance.

3.16.5 Seed yield plant^{-1}

The seeds were separated from stover and weighed.

3.16.6 Stover yield

The above-ground plant without the pods were weighed and data was taken.

3.16.7 Biological yield

The above-ground plant with the pods were weighed and data was taken.

3.17 Statistical analysis

Data accumulated from different parameters were subjected to analysis using CoStat v.6.400 (CoStat, 2008) and one-way analysis of variance (ANOVA). For finding out mean differences among the replications, Fisher's least significant difference (LSD) test at the 5% level of significance was applied. Pearson correlation analysis was done using SPSS v.27 (SPSS, 2020).

Chapter IV

RESULTS AND DISCUSSIONS

4.1 Crop growth parameters

4.1.1 Mortality rate

Mortality rates vary upon in several genotypes. Among all of the genotypes, the lowest mortality rate was observed in BARI Soybean-5 (9%). The mortality rate was the highest in the genotypes of SGB-3 (43%) followed by SGB-4 (36%), BINAsoybean-2 (35%), BINAsoybean-5 (32%), BINAsoybean-6 (28%), BINAsoybean-1 (28%), BINAsoybean-3 (28%), SGB-5 (21%), SGB-1 (17%), GC-840 (15%) and Sohag (11%) (Figure 1). The genotypes BINAsoybean-2, SGB-4 and BINAsoybean-1, BINAsoybean-3 showed no significant difference. All the control plants survived in this experiment.

Waterlogging for 2 weeks decreased plant survival to 52%, 69% and 60% in the SP05, SU05 and FD06 experiments, respectively (VanToai *et al.,* 2010). Survival rate varied in different soybean genotypes in duration-dependent manner (Wu *et al.,* 2017). While working on 40 soybean genotypes under different levels of waterlogging stress (3, 6, 9, 12 and 15days) Wu *et al.* (2017) observed the survival rate of plants. They reported 96.1% plant survival rate (PSR) in all the genotypes at 3 days of waterlogging at V5 stage of plants. At 6 days of waterlogging, 31 genotypes exhibited tolerant response with 70% PSR, at 9 days, 12 genotypes exhibited tolerant response with 46.7% PSR, at 12 days, only 3 genotypes exhibited tolerant response with 34.9% PSR and at 15 days, all genotypes exhibited sensitive responses and PSR was 21% at V5 stage.

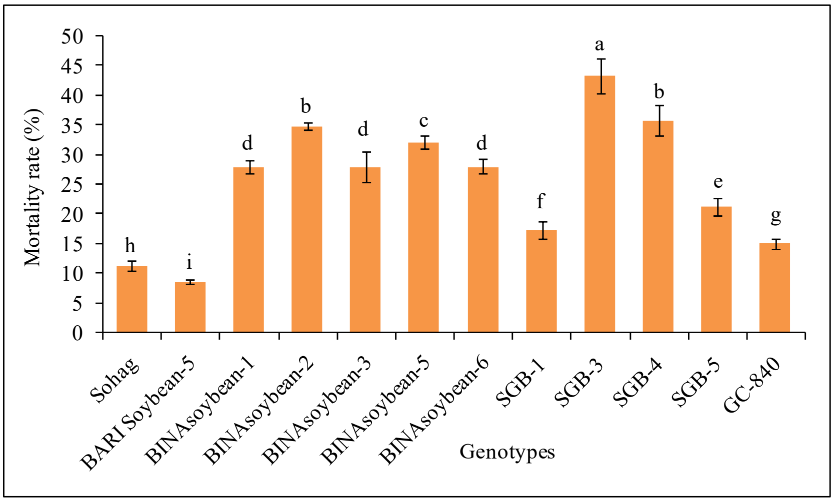

Figure 1. Effect of waterlogging stress on the mortality rate of different soybean genotypes at 12 days after treatment. For each treatment, the mean (±SD) was determined from three replicates. Different alphabets on the bars indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.1.2 Plant height

A sharp reduction of plant height was observed at 20, 25, 30 and 50 DAS upon exposure to waterlogging stress in comparison to the control condition. The lowest reduction in plant height was observed in waterlogged BINAsoybean-2 that was 2, 2, 1 and 4% at 20, 25, 30 and 50 DAS, respectively, when compared to control condition (Table 1). The highest reduction in plant height was observed in waterlogged SGB-1, which was 34, 34, 25 and 26% at 20, 25, 30 and 50 DAS, respectively, in comparison with the control condition. Upon exposure to waterlogging, the reduction in plant height ranged between 3-30% at 20 DAS, 2-33% at 25 DAS, 4-24% at 30 DAS and 9-25% at 50 DAS, respectively in other genotypes when compared with their respective control plants. At 20 DAS, there were observed significant differences in plant height to the genotypes Sohag, BINAsoybean-3, BINAsoybean-6, SGB-1, SGB-3, SGB-4, SGB-5 and GC-840 under waterlogging in contrast to control plants. The genotypes BARI Soybean-5, BINAsoybean-1, BINAsoybean-2, BINAsoybean-5 showed no significant difference in plant height under waterlogging when compared to their respective control. At 25 DAS, the genotypes Sohag, BARI Soybean-5, BINAsoybean-3, BINAsoybean-6, SGB-1, SGB-3, SGB-4, SGB-5 and GC-840 showed significant difference and BINAsoybean-1, BINAsoybean-2, BINAsoybean-5 showed no significant difference in plant height under waterlogging when compared to their respective control. At 30 DAS, BINAsoybean-1, SGB-1, SGB-3, SGB-4, SGB-5 showed significant difference and Sohag, BARI Soybean-5, BINAsoybean-2, BINAsoybean-3, BINAsoybean-5, BINAsoybean-6, GC-840 showed no significant difference in plant height under waterlogging when compared to their respective control. At 50 DAS, Sohag, BARI Soybean-5, BINAsoybean-1, BINAsoybean-3, BINAsoybean-5, BINAsoybean-6, SGB-1, SGB-3, SGB-4, SGB-5, GC-840 showed significant difference and BINAsoybean-2 showed no significant difference in plant height under waterlogging when compared to their respective control.

In this experiment, plant height was declined due to waterlogging at any ages of plants (Table 1). The waterlogging-induced decrease in plant height was noticed in soybean (Kim *et al.,* 2018). Similar outcomes were noted in some other crops like mungbean (Ahmed *et al.,* 2002), sesame (Anee *et al*., 2019; Wei *et al.,* 2013*;* Saha *et al*., 2016). The effect of waterlogging on shoot length was more prominent at the vegetative stage than at the reproductive stage (Ahmed *et al.,* 2002). Excess water lead to a hypoxic situation, which caused damage to the roots as there prevailed insufficient water, minerals, nutrients and hormones. This inadequacy of nutrient and water uptake lead to shoot damage and finally, a reduction in plant height was observed (Jackson and Ricard, 2003). Hypoxic conditions substantially reduced biological nitrogen fixation and accelerated the CO_2 concentration in water as a consequence, the elongation of soybean inhibited (Boru *et al*., 2003). Carbohydrate supply is drastically reduced to the growing cells, which hamper the growth of meristematic tissues under waterlogging stress conditions. Many research works on the waterlogging respondent mechanisms in soybeans showed that most of the proteins manipulated glucose degradation under waterlogging. Whereas proteins related to energy production increased, proteins involved in the maintenance of the structure of the cells (Nanjo *et al*., 2013). These might result in a decrease in plant height in this study. Besides, the plant can not uptake the proper amount of nutrient N, P and K and which result in nutrient deficiency symptoms and finally, reduction of shoot length (Rhine *et al.,* 2010).

Table 1. Effect of waterlogging stress on plant height of different soybean genotypes

Treatments	**Plant height (cm)**							
	20 DAS		**25 DAS**		**30 DAS**		**50 DAS**	
	Control	**Waterlogged**	**Control**	**Waterlogged**	**Control**	**Waterlogged**	**Control**	**Waterlogged**
Sohag	21.76 ±1.05ab	19.58 ± 1.12c-f	28.37 ±1.21a-d	25.21 ±1.10efg	35.72 ±0.55b-h	33.83 ±2.17e-i	47.67 ±1.40ij	41.33 ±0.88k
BARI Soybean-5	21.20 ±1.21bcd	19.50 ± 1.86def	27.30 ±1.51cde	24.70 ±1.67fgh	33.28 ±1.25c-i	31.68 ±0.98d-i	59.98 ±2.03e	54.29 ±2.10fg
BINAsoybean-1	21.30 ±0.18bc	19.50 ± 0.35c-f	26.24 ±1.05def	25.19 ±1.23efg	39.68 ±0.98abc	30.08 ±3.15d-i	69.69 ±2.70ab	60.60 ±5.17e
BINAsoybean-2	17.10 ±0.84hi	16.70 ± 0.61hi	23.50 ±0.93ghi	23.00 ±0.50ghi	33.38 ±0.83c-i	33.17 ±2.38c-i	60.84 ±4.26de	58.40 ±1.71ef
BINAsoybean-3	17.60 ±0.39gh	15.50 ± 1.47ij	23.90 ±0.82ghi	19.60 ±1.17l	27.47 ±1.03hij	26.37 ±0.87g-j	69.56 ±2.35ab	51.82 ±1.58ghi
BINAsoybean-5	17.02 ±0.91hi	16.57 ± 1.27hi	21.23 ± 1.27jkl	20.73 ±1.57kl	26.33 ±0.33ij	24.28 ±1.38j	61.29 ±1.54de	48.80 ±1.34hi
BINAsoybean-6	22.10 ±0.41ab	19.28±1.18efg	27.88 ±1.63bcd	25.27 ±1.31efg	43.50 ±3.17a	38.83 ±1.77a-f	71.47 ±2.32a	54.50 ±3.30fg
SGB-1	21.07 ±1.25bcd	13.98 ± 0.60j	28.79 ±1.99abc	19.10 ±1.27kl	36.00 ±2.33a-e	27.10 ±1.40hij	72.93 ±1.60a	54.20 ±2.33fg
SGB-3	22.42 ±1.94ab	18.22 ± 0.54fgh	30.19 ±0.59a	25.27 ±1.28efg	38.50 ±1.50a-d	31.53 ±2.20f-i	58.00 ±1.73ef	48.93 ±2.90hi
SGB-4	23.50 ±1.08a	16.56 ± 1.00hi	29.86 ±0.90ab	20.09 ±1.17kl	39.17 ±1.83a-e	30.68 ±0.58g-j	52.44 ±2.51gh	44.60 ±2.96jk
SGB-5	23.31 ±1.14a	19.22 ±1.35efg	28.18 ±1.61a-d	24.37 ±1.58fgh	41.58 ±0.05ab	31.60 ±0.03g-j	52.27 ±2.01gh	42.67 ±1.29k
GC-840	21.00 ±0.82b-e	17.36 ±1.21h	28.42 ±1.79abc	22.88 ±0.90hij	37.95 ±1.72abc	35.83 ±2.13b-g	73.64 ±1.36a	64.98 ±4.48cd
LSD$_{(0.05)}$	1.78		2.14		6.43		4.19	
CV (%)	5.2		5.1		4.2		4.1	

For each treatment, the mean (±SD) was determined from three replicates. Different alphabets indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.1.3 Number of leaves plant^{-1}

When exposed to waterlogging, the number of leaves plant^{-1} showed a decreasing manner than the control plants. The lowest reduction in leaves number were observed in waterlogged GC-840 (2%), BINAsoybean-1 (0.3%) and SGB-1 (3%) at 20, 25 and 50 DAS, respectively when compared to the control condition (Table 2). The highest reduction in leaves number were observed in waterlogged BINAsoybean-1 (26%), BINAsoybean-6 (41%) and BINAsoybean-6 (57%) at 20, 25 and 50 DAS, respectively in comparison with the control condition. Upon exposure to waterlogging, the reduction in leaves number ranged between 2-18% at 20 DAS, 2-38% at 25 DAS and 4-42% at 30 DAS, respectively, in other genotypes when compared with their respective control plants. At 20 DAS, there were observed noticeable differences in the number of leaves plant^{-1} to the genotypes BINAsoybean-1, BINAsoybean-2, BINAsoybean-6, SGB-1 and SGB-3 under waterlogging in contrast to control plants. The genotypes Sohag, BARI Soybean-5, BINAsoybean-3, BINAsoybean-5, SGB-4, SGB-5 and GC-840 showed insignificant differences in the number of leaves plant^{-1} under waterlogging when compared to their respective control. At 25 DAS, there were observed significant differences in the number of leaves plant^{-1} to the genotypes Sohag, BARI Soybean-5, BINAsoybean-2, BINAsoybean-3, BINAsoybean-6, SGB-1, SGB-3 and SGB-4 under waterlogging in contrast to control plants. The genotypes BINAsoybean-1, BINAsoybean-5, SGB-5 and GC-840 showed no significant difference in plant height under waterlogging when compared to their respective control. At 50 DAS, there were observed a significant difference in all the genotypes.

The number of leaves plant^{-1} was decreased due to waterlogging at any age of plants (Table 2). Flooding lead to a decline in crop growth, net assimilation rate, leaf expansion and the ultimate outcome was a reduction of leaf number and leaf area in soybean crops (Ezin *et al*., 2010). Prasanna and Rao (2014) stated that the number of leaves plant^{-1} decreased owing to waterlogging stress in green gram plants. The number of leaves plant^{-1} during and after flooding treatment was also decreased drastically at the vegetative stage of mungbean (Ahmed *et al.,* 2002).

Table 2. Effect of waterlogging stress on number of leaves plant^{-1} of different soybean genotypes

Treatments	**Number of leaves plant^{-1}**					
	20 DAS		**25 DAS**		**50 DAS**	
	Control	**Waterlogged**	**Control**	**Waterlogged**	**Control**	**Waterlogged**
Sohag	4.50±0.14efg	4.40±0.17efg	5.27±0.10c	4.34±0.16d	23.15±0.34jk	19.66±0.82l
BARI Soybean-5	4.70±0.13efg	4.30±022fg	5.53±0.37c	4.36±0.19d	28.57±0.27e	18.38±1.19mn
BINAsoybean-1	5.80±0.21d	4.30±0.26g	5.27±0.10c	5.26±0.12c	33.75±0.40a	26.15±0.62h
BINAsoybean-2	5.90±0.39cd	4.90±0.28e	6.40±0.12b	5.30v0.09c	28.50±0.27ef	27.50±0.32g
BINAsoybean-3	5.80±0.40cd	5.40±0.29d	5.29±0.08c	4.21±0.09d	27.63±0.31fg	16.71±0.44o
BINAsoybean-5	4.82±0.34ef	4.68±0.22efg	5.34±0.08c	5.26±0.12c	30.58±0.37cd	19.99±0.29l
BINAsoybean-6	6.58±0.46ab	5.41±0.49d	7.36±0.17a	4.34±0.16d	29.74±0.32d	12.70±0.12p
SGB-1	6.91±0.38a	5.79±0.29cd	5.29±0.09c	4.37±0.21d	19.28±0.41lm	18.71±0.63m
SGB-3	5.74±0.25d	4.80±0.31ef	5.40±0.16c	4.36±0.19d	30.85±0.38bc	17.77±0.44n
SGB-4	6.30±0.28bc	5.90±0.40cd	5.43±0.22c	3.36±0.30e	25.30±0.86i	22.30±0.36k
SGB-5	6.00±0.28cd	5.88±0.33cd	5.50±0.11c	5.38±0.13c	31.50±0.87b	23.90±0.42j
GC-840	5.90±0.29cd	5.80±0.28cd	5.53±0.38c	5.41±0.18c	27.40±0.81g	18.67±0.39mn
LSD$_{(0.05)}$	0.508		0.299		0.895	
CV (%)	5.41		3.29		2.18	

For each treatment, the mean (±SD) was determined from three replicates. Different alphabets indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.1.4 Number of branches plant^{-1}

When exposed to waterlogging, the number of branches plant^{-1} showed a decreasing manner than the control plants. The lowest reduction in branches number was observed in waterlogged BINAsoybean-1 (19%) at 50 DAS when compared to the control condition (Figure 2). The highest reduction in branches number were observed in waterlogged BINAsoybean-6 (50%) and SGB-3 (50%) at 50 DAS in comparison with the control condition (Figure 2). Upon exposure to waterlogging, the reduction in branches numbers ranged between 20-45% at 50 DAS (Figure 2) in other genotypes when compared with their respective control plants.

Branch number plant^{-1} decreased upon exposure to waterlogging stress (Figure 2). The number of branches plant^{-1} also reduced in a genotype-dependent manner in soybean plants. Kuswantoro (2015) reported that some genotypes of soybean showed significant differences, whilst many of the genotypes showed insignificant differences among them. Cho and Yamakawa (2006a) showed the number of leaves, branches number, nodulation significantly reduced due to waterlogging in soybean. Miura *et al.* (2012) also reported waterlogging for 21 days in soybean, resulting in significant reduction in number of branches of soybean plants. Branch number substantially decreased 7 days after waterlogging at both the vegetative and reproductive stages of soybean (Linkemer *et al*., 1998). At the vegetative stage, prolonged waterlogging greatly reduced branches number in mungbean (Ahmed *et al.,* 2002; Koyama *et al*., 2019) and branches number declined by 50% in chickpea (Paltaa *et al.,* 2010). The N fixation also declined as a result of the reduction of total biomass. Waterlogging can restrict the ability of plant to assimilate carbon and nitrogen by inhibiting carbon and nitrogen metabolism. Reduction of CO_2 assimilation, photosynthesis rate significantly decrease upon exposure to waterlogging; eventually, plant showed stunted growth. The stunted plant leads to a lower number of branches. Decreased in branches number in respondents to several stresses in soybean were also reported by several studies (Akram *et al*., 2017; El-Sabagh *et al.,* 2015; Hamayun *et al.,* 2015). Many studies on the waterlogging responsive mechanisms in soybeans have shown that under waterlogging, many proteins control glucose degradation. As proteins related to energy production increment, proteins have been involved in preserving the structure of cells. Due to lack of energy, plant height declined and eventually reduction of branches number (Nanjo *et al.*, 2013).

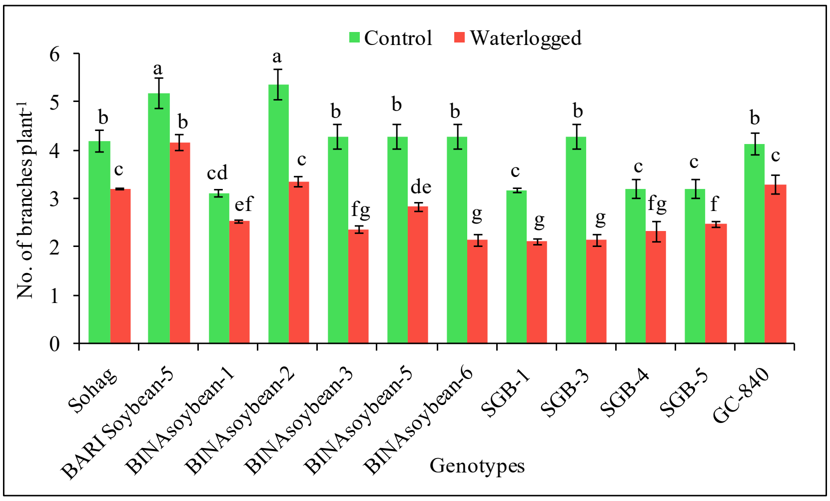

Figure 2. Effect of waterlogging stress on number of branches plant^{-1} of different soybean genotypes. For each treatment, the mean (±SD) was determined from three replicates. Different alphabets on the bars indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.1.5 Leaf area

A sharp reduction of leaf area was observed at 32, 39 and 46 DAS (Table 3) upon exposure to waterlogging stress in comparison with the control condition. The lowest reduction of leaf area was observed in waterlogged BARI Soybean-5 (2%) and the highest was in SGB-5 (16%) at 32 DAS. At 39 and 46 DAS, the lowest reduction was observed in waterlogged genotype Sohag (6% and 5%) and the highest was observed in waterlogged genotype SGB-3 (47 and 17%), respectively when compared to the control condition. Upon exposure to waterlogging, the reduction of leaf area ranged between 3-15% at 32 DAS, 8-30% at 39 DAS and 7-16% at 46 DAS, respectively in other genotypes when compared with their respective control plants. Leaf area of different soybean genotypes decreased due to waterlogging stress (Table 3), which is evidenced by many authors (Pedó *et al.*, 2015; Youn *et al.*, 2008). Similar outcomes were observed in some other crops like mungbean (Kumar *et al.*, 2013), barley (Zhang *et al.*, 2007), sesame (Anee *et al.*, 2019; Saha *et al.*, 2016) and green gram (Prasanna and Rao, 2014).

Table 3. Effect of waterlogging stress on leaf area of different soybean genotypes

Treatments	Leaf area					
	32 DAS		39 DAS		46 DAS	
	Control	Waterlogged	Control	Waterlogged	Control	Waterlogged
Sohag	36.73±0.91ab	35.51±0.88bc	41.02±3.75ab	38.73±3.54a-d	45.49±3.18a-d	43.07±2.71b-f
BARI Soybean-5	33.27±0.82d	32.60±0.81def	40.71±3.72ab	36.73±3.36b-f	49.04±3.42a	43.06±3.01b-f
BINAsoybean-1	35.50±0.88bc	32.27±0.80d-g	38.50±3.52a-e	33.70±3.08d-h	43.06±3.01b-f	38.85±2.71e-i
BINAsoybean-2	29.70±0.74jk	28.47±0.70k	40.08±3.67abc	34.64±3.17d-h	47.70±3.33ab	40.07±2.80e-h
BINAsoybean-3	35.43±1.01bc	30.48±0.75hij	38.09±3.48a-e	34.97±3.20c-g	45.49±3.18a-d	43.06±3.01b-f
BINAsoybean-5	31.49±0.78f-i	28.47±0.70k	36.11±3.30b-f	33.18±3.03e-h	38.41±2.68f-i	33.32±2.33j
BINAsoybean-6	31.71±0.78e-h	31.18±0.77ghi	35.27±2.23c-g	29.32±2.68hi	42.39±2.96c-g	37.74±2.64g-j
SGB-1	30.38±0.75ij	25.79±0.64l	36.31±3.32b-f	25.32±2.32ij	46.16±3.22abc	41.08±2.87d-h
SGB-3	28.80±0.71k	24.67±0.61l	38.83±3.55a-d	20.40±1.87j	39.08±2.73e-h	32.50±2.38j
SGB-4	28.58±0.71k	24.60±0.70l	36.11±3.30b-f	29.97±2.74ghi	41.18±2.88d-h	36.64±2.56hij
SGB-5	35.84±0.89bc	30.26±0.75ij	42.80±3.91a	30.57±2.80ghi	46.60±3.25abc	43.28±3.02b-e
GC-840	37.63±0.93a	32.82±0.81de	43.21±3.95a	32.55±2.98fgh	48.37±3.38a	43.50±3.04b-e
LSD$_{(0.05)}$	1.30		5.36		4.83	
CV (%)	2.49		9.15		6.98	

For each treatment, the mean (±SD) was determined from three replicates. Different alphabets indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.1.6 Above-ground fresh weight plant^{-1}

Upon exposure to waterlogging above-ground fresh weight plant^{-1} reduced when compared to their control plants. The lowest reduction in plant FW were observed in waterlogged BARI Soybean-5 (5.7%) and GC-840 (13.1%) at 20 and 25 DAS, respectively in comparison with control (Figure 3). The highest decline in plant FW were observed in waterlogged SGB-4 (42.6%) and SGB-4 (71.3%) at 20 and 25 DAS, respectively in comparison with the control condition (Figure 3). Upon exposure to waterlogging, the reduction in plant FW ranged between 8-40% at 20 DAS and 13-61% at 25 DAS, respectively, in other genotypes when compared with their respective control plants.

Aboveground FW plant^{-1} significantly decreased upon exposure to waterlogging stress (Figure 3). Waterlogging-induced reduction in aboveground FW was found in soybean in different studies (Kim *et al.,* 2019; Beutler *et al.,* 2014; Miao *et al.,* 2012). Research works showed that waterlogging affects Chl and reduces the content of Chl and resulting reduction of photosynthetic activity and the decrease in the rate of photosynthesis that inhibited plant growth and accumulation of biomass (Ren *et al.,* 2014). Under waterlogging, the phytology and catabolism of plants are disrupted. Restricted stomatal conductance, the transition of gases, 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 can increase cell osmotic pressure and causes in various metabolic enzymes, including carbohydrate synthesis. The waterlogging condition can restrict the ability of plant to assimilate carbon and nitrogen by inhibiting carbon and N metabolism, which result greatly decline of FW (Rhine *et al.,* 2010). Decreased in aboveground FW in response to different stresses also reported by many studies (Ahmed *et al.,* 2002; Mutava *et al*., 2015).

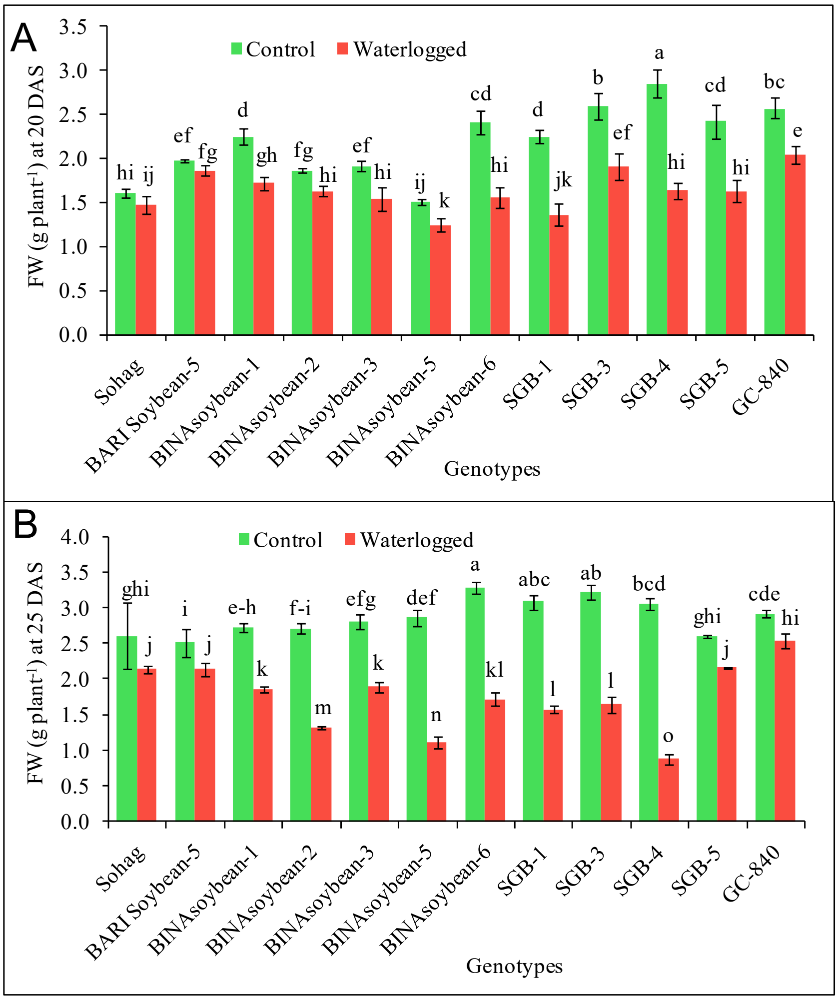

Figure 3. Effect of waterlogging stress on above-ground fresh weight (FW) plant^{-1} of different soybean genotypes. For each treatment, the mean (±SD) was determined from three replicates. Different alphabets on the bars indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.1.7 Above ground dry matter weight plant^{-1}

The remarkable decline was recorded in above-ground dry matter weight plant^{-1} when exposed to waterlogging condition. The lowest reduction in plant DW was observed in waterlogged Sohag, which was 11 and 8% at 20 and 25 DAS, respectively, when compared to the control condition (Figure 4). The highest reduction in plant DW were observed in waterlogged SGB-3 (65%) and SGB-1 (57%) at 20 and 25 DAS, respectively in comparison with the control condition (Figure 4). Upon exposure to waterlogging, the reduction in plant DW ranged between 8-62% at 20 DAS (Figure 4A) and 8-43% at 25 DAS (Figure 4B), respectively in other genotypes when compared with their respective control plants.

Under waterlogging, aboveground dry matter accumulation also decline at any ages of the plants (Figure 4). Similar to fresh weight waterlogging-induced reduction in aboveground DW was noticed in soybean in many studies (Miao *et al.,* 2012; Beutler *et al.,* 2014; Kim *et al.,* 2019). The mechanism by which plants accumulate dry matter is photosynthesis (Ren *et al.,* 2016). Previous researches have shown that waterlogging stresses have hindered plant growth and production and, subsequently, reduced dry matter accumulation. The reduction of dry matter accumulation in the current findings may be due to a decrease in water absorption and inhibition of photosynthetic processing and synthesis of carbohydrates. The decrease in photosynthesis was due to the decrease in available CO_2 through stomatal closure, combined effects of leaf water, osmotic capacity, transpirational rate of stomatal conductance, RWC of leaf and biochemical constituents such as photosynthetic pigments, protein and carbohydrates (Khan *et al.,* 2017; Zhang *et al.,* 2017). Several scientists recorded that flooding stress reduced shoot DW of maize (Tian *et al.,* 2019), greengram (Prasanna and Rao, 2014) and mung bean (Ahmed *et al.,* 2002; Kumar *et al.,* 2013).

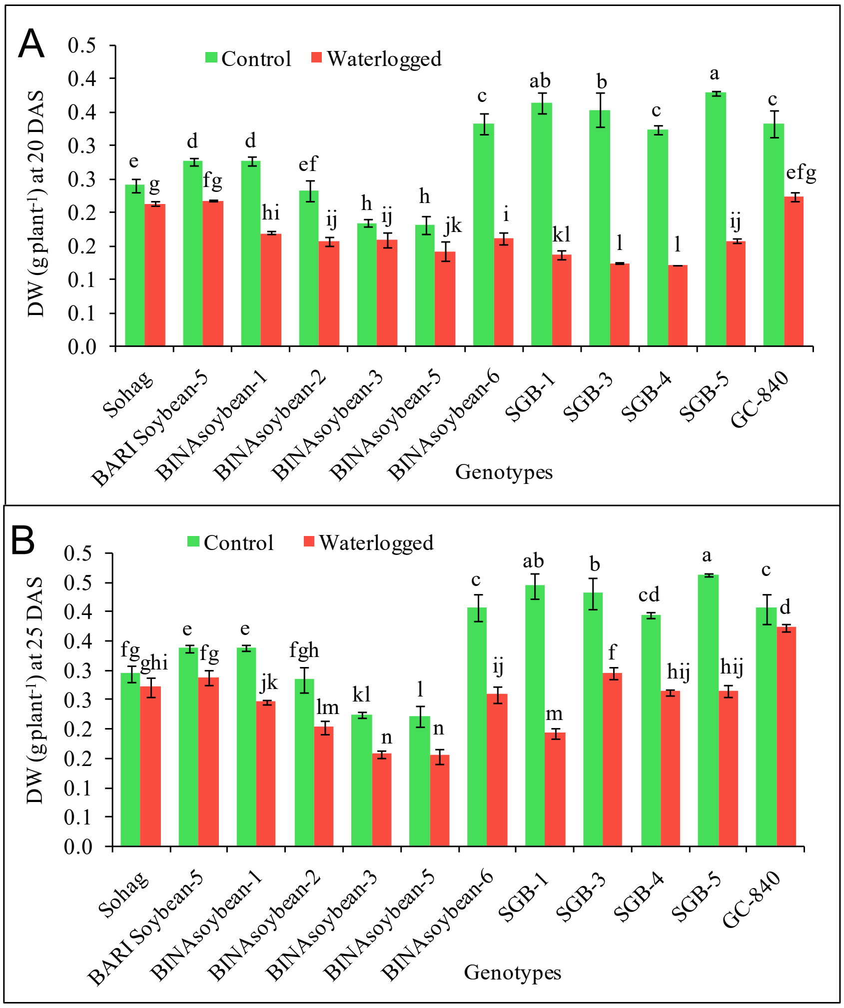

Figure 4. Effect of waterlogging stress on above-ground dry weight (DW) plant^{-1} of different soybean genotypes. For each treatment, the mean (±SD) was determined from three replicates. Different alphabets on the bars indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.2 Physiological parameters

4.2.1 SPAD value

SPAD reading which is the indicator of chl content of leaf showed lower value in the leaves of waterlogged plants when compared with the control plants. The lowest reduction was observed in waterlogged SGB-3 (2%), BINAsoybean-2 (8%), BARI

Soybean-5 (12%) and BINAsoybean-2 (8%) at 20, 25, 30 and 40 DAS, respectively in waterlogged plants compared to control plants (Table 4). The highest reduction was observed in waterlogged SGB-4 (21%), SGB-1 (22%), SGB-3 (23%) and BINAsoybean-1 (21%) at 20, 25, 30 and 40 DAS respectively in waterlogged plants compared to control plants (Table 4). Upon exposure to waterlogging, the reduction in SPAD value ranged between 2-18% at 20 DAS, 10-17% at 25 DAS, 13-22% at 30 DAS and 15-20% at 40 DAS, respectively in other genotypes when compared with their respective control plants.

Soybean responses to waterlogging is sensitive and endeavoring. The soybean leaves color appeared from green to yellow after 24 hr waterlogging stress, which is doubted to be the decrease of chl content in soybean leaf. The author observed leaf color variation after 3-days waterlogging as there observed a significant reduction in chl content (Wu *et al.,* 2017). The most fundamental life activity of plants and one of the most sensitive physiological processes for waterlogging is photosynthesis. In this experiment, SPAD value was decreased due to waterlogging stress (Table 4). However, at an earlier stage (15 DAS) did not show any differences in SPAD value. Waterlogging in soybeans exhibits a decline in activity in photosynthesis (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. Earlier studies stated that hypoxic stress affects Chl and reduces chl content, the resulting decrease in photosynthetic activity and the decrease in the rate of photosynthesis that inhibited plant growth and accumulation of biomass. It was noted that waterlogging significantly reduced N uptake in soybean leaves and branches. Prolonged waterlogging causes a decreased in CO_2 assimilation and leading to a remarkable drop in photosynthesis and chl (Yordanova and Popova, 2007).

Table 4. Effect of waterlogging stress on SPAD value of different soybean genotypes

Treatments	**SPAD value**							
	20 DAS		**25 DAS**		**30 DAS**		**40 DAS**	
	Control	**Waterlogged**	**Control**	**Waterlogged**	**Control**	**Waterlogged**	**Control**	**Waterlogged**
Sohag	44.81±0.64d-h	39.57±2.45jkl	43.17±0.75efg	37.59±0.34jk	45.63±0.84a-e	39.67±3.84def	49.13±2.23def	41.89±2.22ijk
BARI Soybean-5	47.35±0.40a-e	41.25±2.02ijk	44.47±1.62def	37.09±2.11jk	43.68±3.34b-f	38.51±2.01ef	46.79±2.29fg	39.12±3.72jkl
Binasoybean-1	45.32±0.49d-h	39.11±0.64jkl	45.46±1.34cde	35.49±1.82k	45.47±0.38a-e	39.25±3.41def	49.30±0.61c-f	39.14±3.04jkl
Binasoybean-2	49.82±2.21ab	43.76±2.22f-i	48.46±0.87ab	44.69±1.10de	50.14±1.67ab	43.23±3.44b-f	51.13±0.98cd	47.31±1.48efg
Binasoybean-3	46.99±3.08b-f	38.87±3.81kl	45.32±0.28cde	38.75±0.11ij	46.47±0.48a-d	39.90±2.63def	47.14±2.14efg	38.51±2.67kl
Binasoybean-5	45.18±1.68d-h	40.92±3.35i-l	41.85±2.40fgh	35.71±2.87k	44.74±0.93a-e	36.65±2.91f	45.93±2.49fgh	36.75±3.13l
Binasoybean-6	45.85±3.39c-f	37.69±1.99l	45.71±1.03b-e	39.17±3.92hij	49.61±2.14ab	39.87±3.92def	51.93±0.89bcd	41.55±2.25ijk
SGB-1	50.44±1.93a	42.43±1.66g-j	48.83±1.89a	38.05±2.68ijk	50.00±0.79ab	42.79±2.35b-f	52.96±1.38abc	44.50±2.39ghi
SGB-3	45.41±0.19c-g	44.62±0.65e-h	46.53±1.11a-d	38.94±1.99ij	48.99±2.87abc	37.91±3.27g	50.51±1.12cde	41.13±1.67ijk
SGB-4	50.25±0.56ab	39.49±3.28jkl	47.70±0.20abc	43.01±0.68efg	51.73±2.03a	42.63±4.41b-f	56.52±2.41a	47.21±2.49efg
SGB-5	48.69±0.71abc	42.41±2.21g-j	47.75±1.50abc	43.17±1.25efg	50.32±1.26ab	41.72±2.11c-f	51.95±1.58bcd	42.39±2.08hij
GC-840	47.99±0.80a-d	41.97±1.23h-k	48.89±1.21a	40.80±1.53ghi	51.75±2.16a	40.61±2.34def	55.13±0.87ab	44.36±3.93ghi
LSD$_{(0.05)}$	3.35		2.79		7.84		3.70	
CV (%)	4.03		3.49		5.43		4.71	

For each treatment, the mean (±SD) was determined from three replicates. Different alphabets indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.2.2 Relative water content

When exposed to waterlogging, plants exhibited reduction in leaf RWC at 20 and 25 DAS. The lowest reduction in leaf RWC was observed in waterlogged BARI Soybean-5 (17%) and BINAsoybean-1 (4%) at 20 and 25 DAS, respectively, when compared to control condition (Figure 5). The highest reduction in leaf RWC were observed in waterlogged BINAsoybean-5 (42%) and SGB-4 (37%) at 20 and 25 DAS, respectively, in comparison with the control condition (Figure 5). Upon exposure to waterlogging, the reduction in leaf RWC ranged between 18-41% at 20 DAS (Figure 5A) and 7-29% at 25 DAS (Figure 5B), respectively, in other genotypes when compared with their respective control plants.

Relative leaf water content was found to be a crucial factor in assessing plants' tolerance to osmotic stress caused by waterlogging. In our study, waterlogging lead to a significantly reduced RWC content in the several genotypes of soybean plants (Figure 5). Reduction in leaf relative water content indicates an insufficient supply of water for cell expansion (Katerji *et al.,* 1997). Despite the excess quantity of water available under waterlogged conditions, RWC leaves were reduced by soybean plants. This may be due to the prevalence of hypoxia or anoxia that inhibited the permeability of the root (Asharf, 2012) and as a result, leaf wilting symptoms were found on plants. The corresponding decrease in RWC due to waterlogging was also observed in sesame (Anee *et al.,* 2019) and mungbean (Kumar *et al.,* 2013).

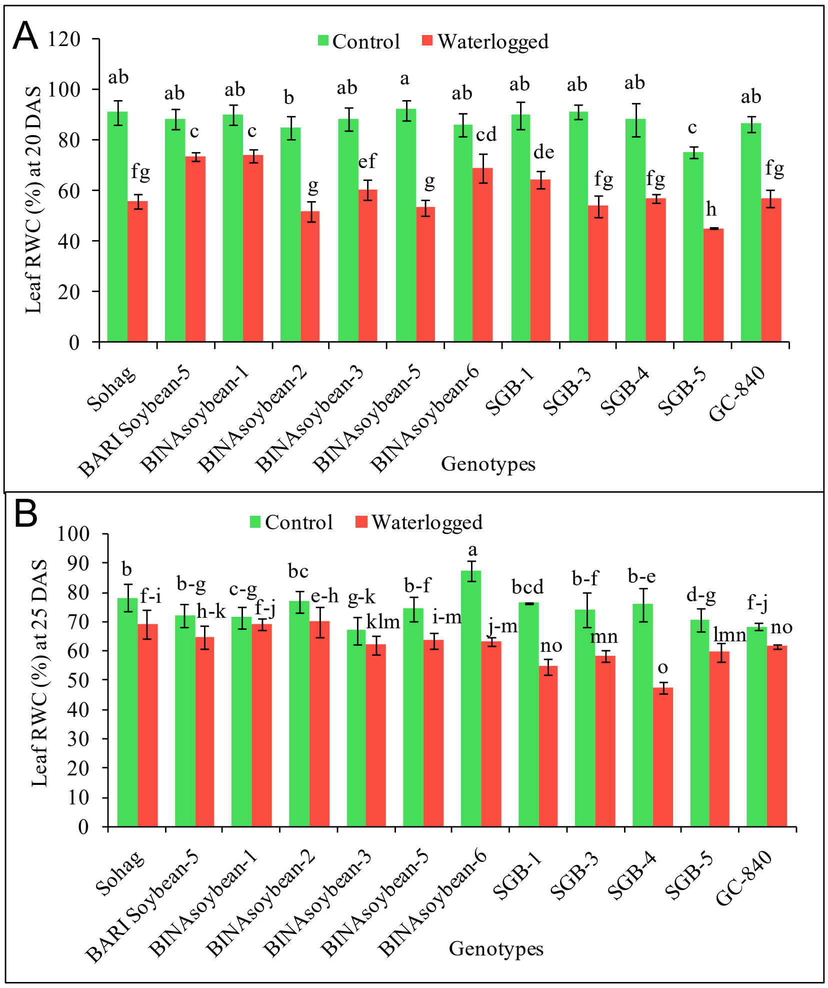

Figure 5. Effect of waterlogging stress on leaf relative water content (RWC) of different soybean genotypes. For each treatment, the mean (±SD) was determined from three replicates. Different alphabets on the bars indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.2.3 Electrolyte leakage

Due to the imposition of waterlogging, electrolyte leakage increased significantly in waterlogged plants than the control plants. The highest increase was observed in waterlogged BINAsoybean-1 (260%) and the lowest increase was observed in waterlogged BINAsoybean-5 (29%) at 20 DAS in contrast to control. Upon exposure to waterlogging, the increase in EL ranged between 34-111% at 20 DAS (Figure 6), respectively, in other genotypes when compared with their respective control plants. Waterlogged BINAsoybean-1, BINAsoybean-6; waterlogged BINAsoybean-3, SGB-1, SGB-4; waterlogged BARI Soybean-5, BINAsoybean-5 and control BINAsoybean-2, SGB-4 showed no significant difference among them (Figure 6).

Electrolyte leakage was increased upon exposure to waterlogging in several genotypes of soybean plants (Figure 6). Electrolyte leakage was enhanced with increasing stress levels as compared to the control. Due to Waterlogging stress, cell membrane became disorganized which increased the generation of ROS and metabolic toxicity (Jaleel *et al.,* 2007). The membrane injury increased with the increasing duration of waterlogging stress in pideonpea (Duhan *et al.,* 2018; Kumutha *et al.,* 2009).

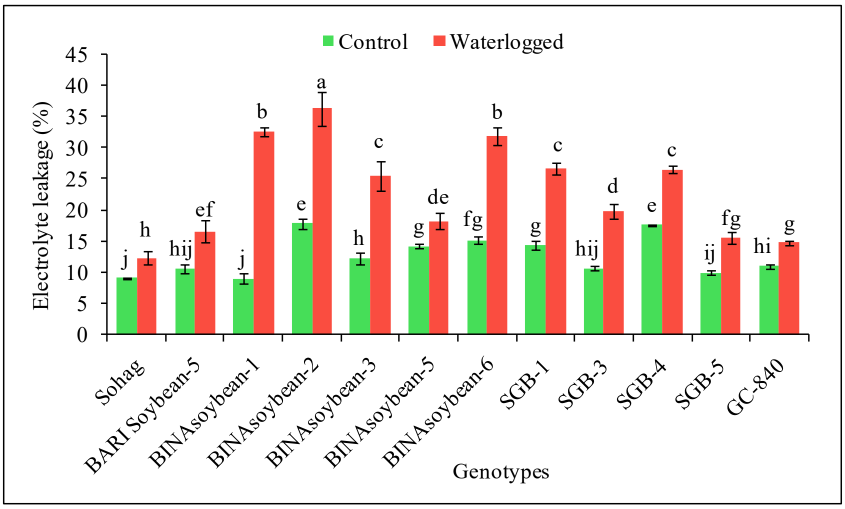

Figure 6. Effect of waterlogging stress on electrolyte leakage of different soybean genotypes. For each treatment, the mean (±SD) was determined from three replicates. Different alphabets on the bars indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.3 Root phenotypes

Waterlogging caused injury to roots owing to cellular anoxia; furthermore root meristems exhibited susceptibility (Valliyodan *et al.,* 2014, 2017). Uptake of water and nutrients failed due to damaged roots. Bacanamwo and Purcell (1999) stated that soybean plants showed morphological acclimatization under waterlogging stress to avoid water loss by declining area of leaf and inducing adventitious root formation. They also stated that leaf expansion was not occupied with the accumulation of carbohydrates in the leaf of waterlogged plants. Usually, the carbohydrate used for leaf expansion may be translocated to the roots to generate adventitious roots. One of the main adaptation responses under waterlogging stress is adventitious root formation (Ahmed *et al.,* 2002; Yin *et al.,* 2009;). Under flooding, soybean adventitious roots showed elevated cortex cell breakup, generating aerenchyma used as pores for the transfer of O_2 to roots, becoming a plant sustenance technique under unfavorable conditions (Beutler *et al.,* 2014). Adventitious roots of soybean under waterlogging stress exploited rupture of cortex cells, creating aerenchyma used as pores for O_2 transferring to roots, being a plant adaptive mechanism under these stress conditions (Beutler *et al.,* 2014). Adventitious roots are formed near the surface of water where the stem generates aerenchyma to obtain oxygen (Suralta and Yamauchi, 2008). Adventitious roots have not been found in soybean control plants (Kim *et al.,* 2018).

Our experiment also supported the hypothesis. Morphological acclimation to waterlogging in soybean emerges to adventitious root formation. There formed no adventitious roots under control condition. The lowest adventitious roots number were recorded in SGB-4 genotype and the highest was observed in GC-840 genotype at 20 DAS (Figure 7). The least number of adventitious roots were observed in SGB-4, SGB-3, SGB-1 and the rest of the genotypes showed a quite better number of adventitious roots. Among them, GC-840 showed a higher number of adventitious roots (Figure 8).

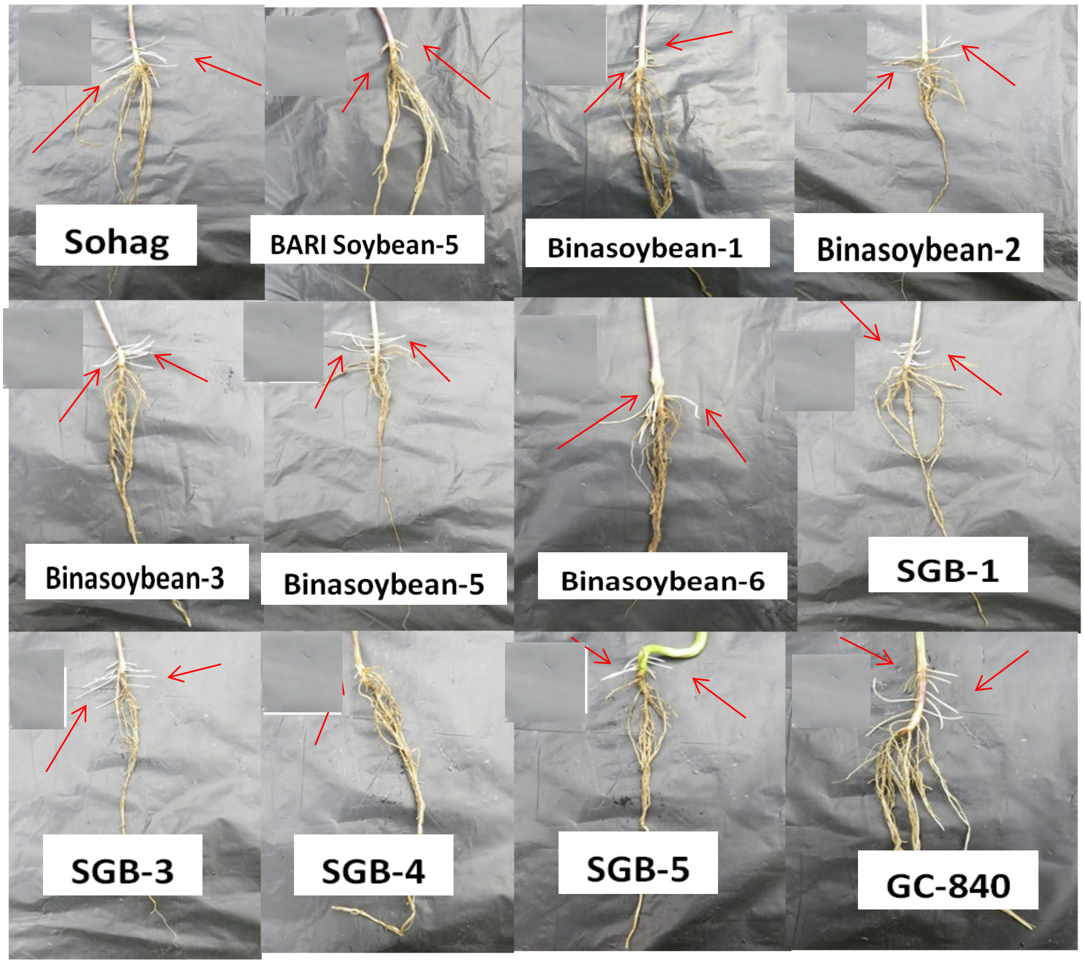

Figure 7. Adventitious root formation of different soybean genotypes under waterlogging condition at 20 DAS. Here, the arrows indicating the adventitious roots

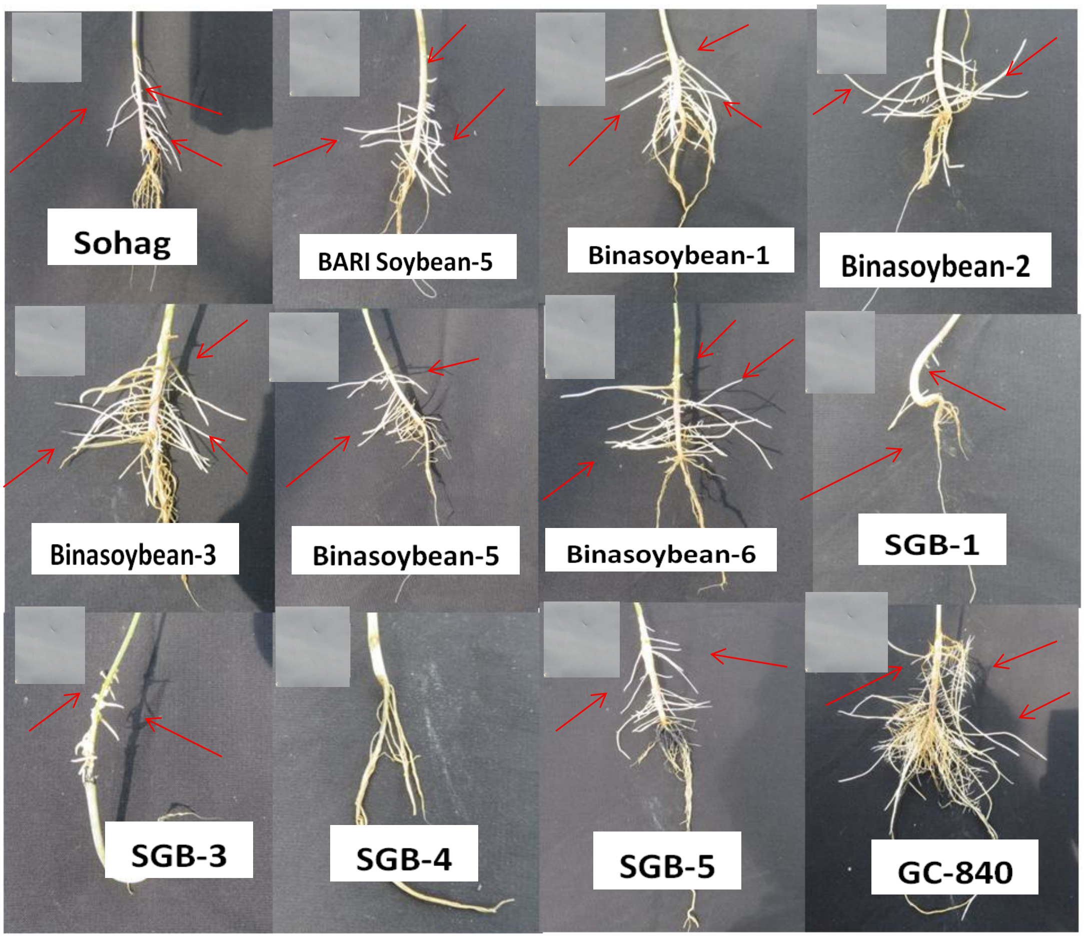

Figure 8. Adventitious root formation of different soybean genotypes under waterlogging condition at 25 DAS. Here, the arrows indicating the adventitious roots

4.4 Phenotypic comparative observation

In this study, a visible appearance observed under control and waterlogged plants. Plants displayed a decrease in height compared to their control plants. Death of plants also observed due to waterlogging stress (Figure 9).

Figure 9. Phenotypic variations of soybean under waterlogged and control condition at 25 DAS. Here, C is denoted for Control and WL for waterlogged plants

Waterlogging for 12 days at the vegetative stage caused delayed flowering and maturity in all the genotypes when compared with their control plants (Figure 10), which is supported by the study of Kuswantoro (2015). This author carried out an experiment with 16 soybean lines, including 2 check varieties (Lawit and Sinabung), exposing waterlogging to plants after 21 days of planting till harvesting. In his study, he observed days to flowering and days to maturing of the genotypes were longer in the flooding condition than the control condition. The variety Sinabung and Lawit generally bore flower by 35 and 40 days and became mature by 88 and 84 days, respectively (Balitkabi, 2012). Kuswantoro (2015) observed that days to flowering and days to maturity delayed by 46, 49 days and 98, 100 days, respectively in Sinabung and Lawit variety. The lengthier days to flowering supposedly due to the plant always try to thrive against waterlogging stress by renovating their vegetative growth, for instance, adventitious root formation. The formation of adventitious root needed high energy used by the plants. Furthermore, plants declined energy for flowering initiation. The delay in days to flowering initiation is a consequence of delay in days to maturing of plants. Moreover, plants faced nutrient deficiency to uptake by the roots as well as adventitious roots from soil and water. Which hindered the plants growth as well delayed days to maturity of soybean plants.

Khairulina and Tikhonchuk (2012) found some dissimilar result in soybean under waterlogging stress. Waterlogged plants took shorter interstage period than the control plants.

Our study also showed extending of interstage period in the waterlogged plants than the control plants.

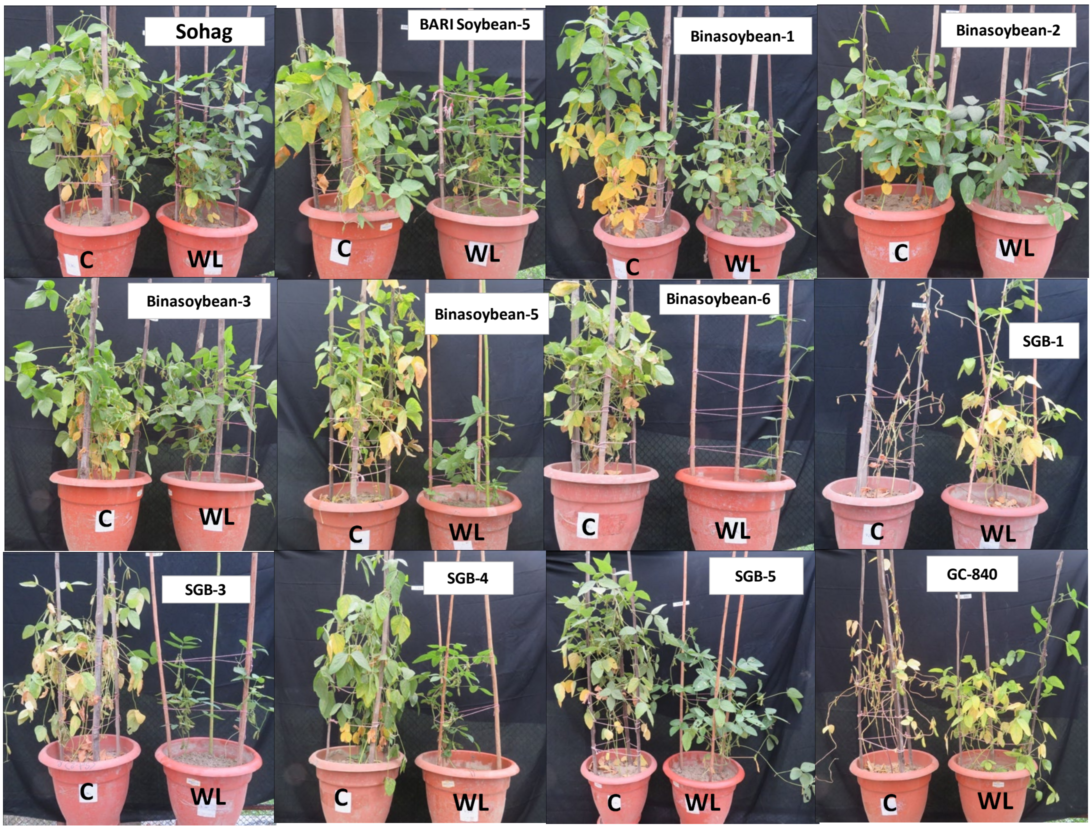

Figure 10. Phenotypic variations of soybean under waterlogged and control condition at 75 DAS. Here, C is denoted for Control and WL for waterlogged plants

4.5 Yield contributing parameters

4.5.1 Number of pods plant^{-1}

In response to exposure upon waterlogging stress, the number of pods plant^{-1} was sharply reduced compared to control. The lowest decline in number of pods plant^{-1} was observed in waterlogged Sohag (5%) when compared to the control condition (Figure 11). The highest decline in number of pods plant^{-1} was observed in waterlogged SGB-1 (37%) in comparison with the control condition (Figure 11). Upon exposure to waterlogging, the reduction in number of pods plant^{-1} ranged between 10-35% (Figure 11) in other genotypes in comparison with the control plants.

Primarily under waterlogging conditions, seed yield decreased due to the reduction of number of pods plant^{-1} and pod setting.

Kuswantoro (2015) observed that sensitive genotypes of soybean bore the least number of pods plant^{-1} than the tolerant one under waterlogging stress. The lowest no. of filled pods presumably owing to the least uptake of nutrients by roots because they have poor nutrients in the water region instead of rich nutrients in the soil. Branch numbers correlated to pod numbers increasing (Koyama *et al.,* 2019). A similar decrease in plant yield has been reported in soybean (Beutler *et al.,* 2014; Miao *et al.,* 2012; Mustafa and Komastsu, 2014; Rhine *et al.,* 2010). Waterlogging also reduces the pod number other crops were observed in green gram (Kumar *et al.,* 2013), green gram (Rao, 2014).

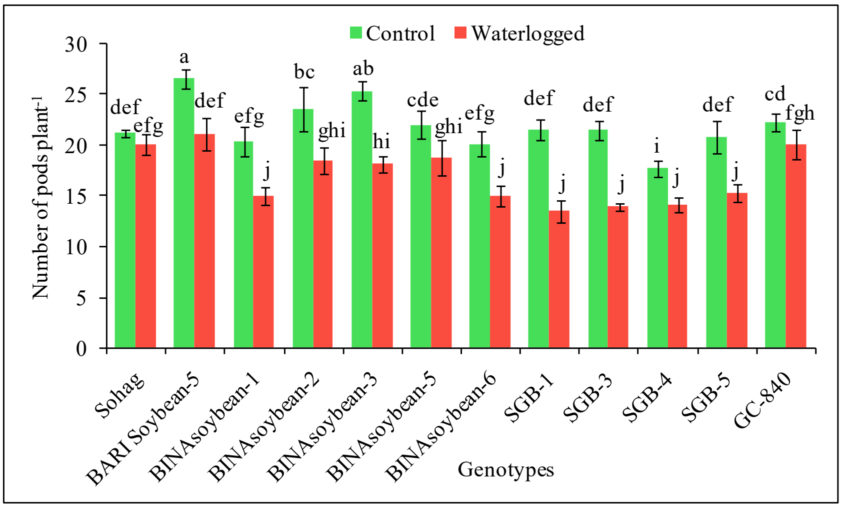

Figure 11. Effect of waterlogging stress on number of pods plant^{-1} of different soybean genotypes. For each treatment, the mean (±SD) was determined from three replicates. Different alphabets on the bars indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.5.2 Number of seeds pod^{-1}

When subjected to waterlogging stress, in response plants showed a reduction of the number of seeds pod^{-1} in contrast to control. The lowest reduction in the number seeds pod^{-1} was observed in waterlogged Sohag (1%) when compared to the control condition (Figure 12). The highest reduction in number of seeds pod^{-1} was observed in waterlogged BINAsoybean-5 (9%) in comparison with the control condition (Figure 12). Upon exposure to waterlogging, the reduction in number of seeds pod^{-1} ranged between 3-8% (Figure 12) in other genotypes in comparison with their control plants.

The number of pods plant^{-1}, seeds pod^{-1} and the weight of single seed are the main determinants of seed yield in legumes as well as in soybean. Kuswantoro (2015) reported that under waterlogging stress soybean plants could not produce seeds perfectly due to the lack of nutrients uptake by the roots. They noticed less number of seeds while comparing the ratio of seeds/pods. Generally, two or three grains are filled in a pod. Whereas, this author found that the pods were filled with 1 or 2 seeds, which specified the production of seeds were not accomplish well under flooding stress.

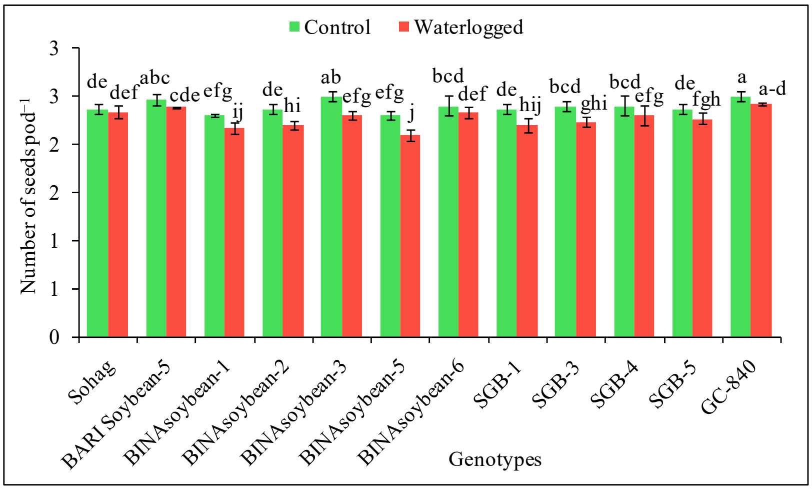

Figure 12. Effect of waterlogging stress on number of seeds pod^{-1} of different soybean genotypes. For each treatment, the mean (±SD) was determined from three replicates. Different alphabets on the bars indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.5.3 100-seed weight

When the plants were subjected to waterlogging stress, 100-seed weight decreased in comparison to the control condition. The lowest reduction in 100-seed weight was observed in waterlogged GC-840 (5%) when compared to the control condition (Figure 13). The highest reduction in 100-seed weight was observed in waterlogged BINAsoybean-5 (29%) in comparison with the control condition (Figure 13). Upon exposure to waterlogging, the reduction in 100-seed weight ranged between 8-25% (Figure 13) in other genotypes when compared with their respective control plants.

Our findings also showed that the reduction in seed yield was in line with the decrease in the weight of 100 seeds. In this study, 100-seed weight decline due to waterlogging stress (Figure 13). In response to a rise in the length of waterlogging in soybeans, the 100-seed weight showed a decreasing trend (Beutler *et al.,* 2014; Miao *et al.,* 2012).

Similar results were observed in some other crops like maize (Tian *et al.,* 2019), wheat and barley (de San Celedonio *et al.,* 2014). Ahmed *et al.* (2002) reported waterlogging at both vegetative and reproductive stages had significantly decreased 100 seed weight in mungbean. Waterlogging caused many physiological disruptions that resulted in low yield, including a decrease in growth, dry matter accumulation, photosynthesis and pod formation.

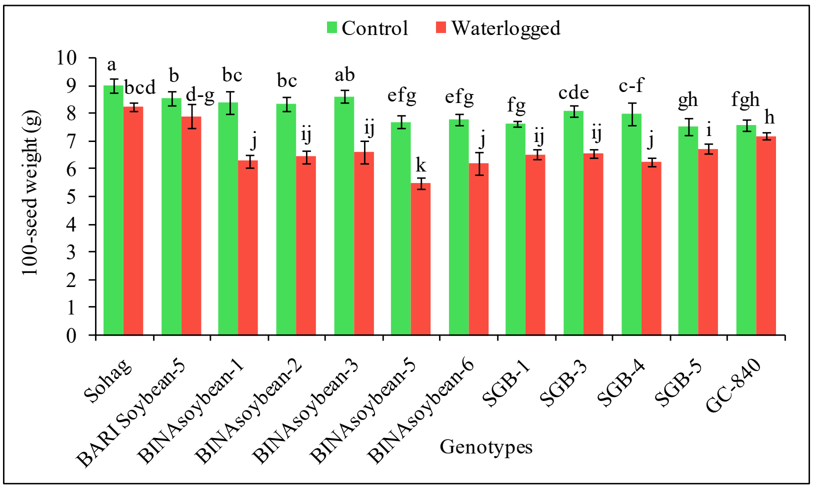

Figure 13. Effect of waterlogging stress on 100-seed weight of different soybean genotypes. For each treatment, the mean (±SD) was determined from three replicates. Different alphabets on the bars indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.5.4 Seed yield plant^{-1}

Upon exposure to waterlogging, stress seed yield plant^{-1} decreased in comparison to the control condition. The lowest reduction in seed yield was observed in waterlogged GC-840 (11%) when compared to the control condition (Figure 14). The highest reduction in seed yield was observed in waterlogged SGB-4 (51%) in comparison with the control condition (Figure 14). Upon exposure to waterlogging, the reduction in seed yield ranged between 15-40% (Figure 14) in other genotypes when compared with their respective control plants.

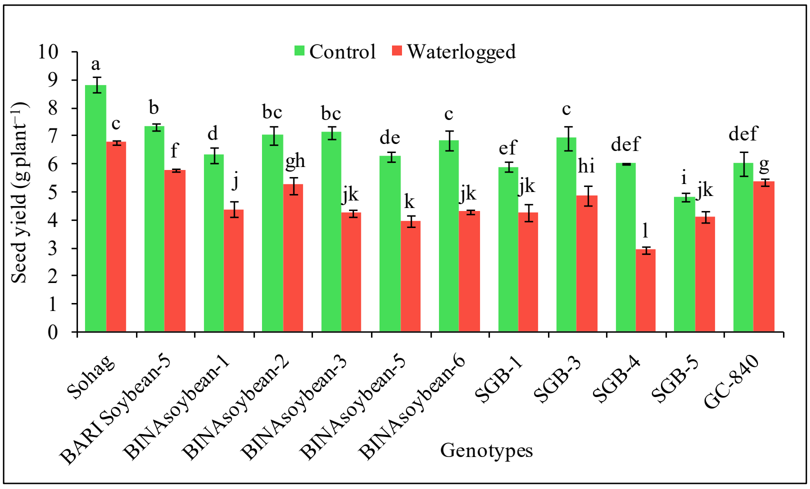

Figure 14. Effect of waterlogging stress on seed yield plant^{-1} of different soybean genotypes. For each treatment, the mean (±SD) was determined from three replicates. Different alphabets on the bars indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

A seed is the most crucial element as it is intimately related to seed yield plant^{-1}, where seed yield plant^{-1} is affected due to variation of yield in soybean (Kobraee and Shamsi, 2011). Kuswantoro (2015) reported that under waterlogging stress plants could not produce grains perfectly due to the lack of nutrients uptake by the roots. They noticed less number of grains while comparing the ratio of seeds/pods. Generally, two or three grains are filled in a pod. Whereas, this author found that the pods were filled with 1 or 2 seeds that specified the production of seeds were not accomplish well. In addition, yield reduced significantly and the reduction was greater in waterlogged-sensitive genotypes than the waterlogged-tolerant genotypes of soybean (Wu *et al.,* 2017). VanToai *et al.* (2010) observed a considerable relation between seed yield with no. of branches, no. of nodes and no. of total seed in waterlogging. Howsoever, this compatibility is not coherent amongst the 3 tested soybean genotypes. Which exhibits that waterlogging has varied responses towards different genetic backgrounds (Jitsuyama, 2013). With the increment in waterlogging length, yield losses increased. In this experiment, seed yield decreased due to waterlogging stress (Fig 21). 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.,* 2019), barley and wheat (de San Celedonio *et al.,* 2014), green gram (Kumar *et al.,* 2013). In addition, with an increase in the length of waterlogging, the rate of transpiration, stomatal conductance and concentration of intercellular CO_2 decreased, which caused the total weight of dry matter to decrease and ultimately resulted in a significant reduction in maize grain yield (Tian *et al.,* 2019).

4.5.5 Stover yield

The imposition of waterlogging caused a marked decline in the stover yield compared to control condition. The lowest reduction in stover yield was observed in waterlogged BARI Soybean-5 (1%) when compared to the control condition (Figure 15). The highest reduction in stover yield was observed in waterlogged SGB-5 (22%) in comparison with the control condition (Figure 15). Upon exposure to waterlogging, the reduction in stover yield ranged between 1-21% (Figure 15) in other genotypes when compared with their respective control plants.

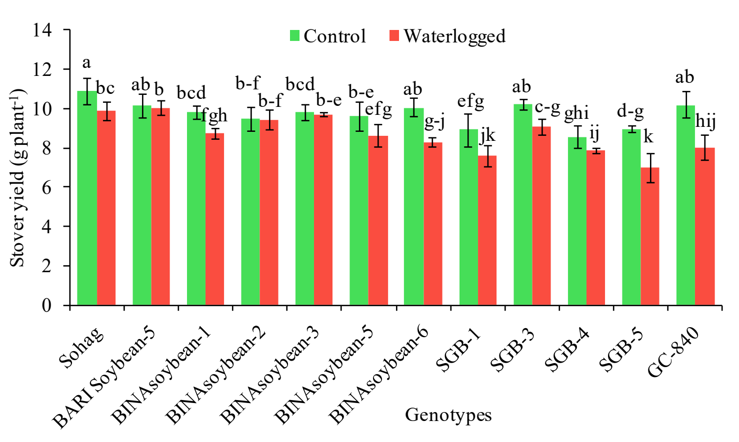

Figure 15. Effect of waterlogging stress on stover yield of different soybean genotypes. For each treatment, the mean (±SD) was determined from three replicates. Different alphabets on the bars indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

4.5.6 Biological yield

Upon exposure to waterlogging, biological yield decreased in comparison to the control condition. The lowest reduction in biological yield was observed in waterlogged BINAsoybean-1 (6%) when compared to the control condition (Figure 16). The highest reduction in biological yield was observed in waterlogged SGB-4 (26%) in comparison with the control condition (Figure 16). Upon exposure to waterlogging, the reduction in biological yield ranged between 8-26% (Figure 16) in other genotypes when compared with their respective control plants.

The total dry matter decreased in waterlogged plants which reduced the biological yield of plants. Similar results were also found in pigeonpea (Duhan *et al.,* 2018; Kumutha *et al.,* 2009).

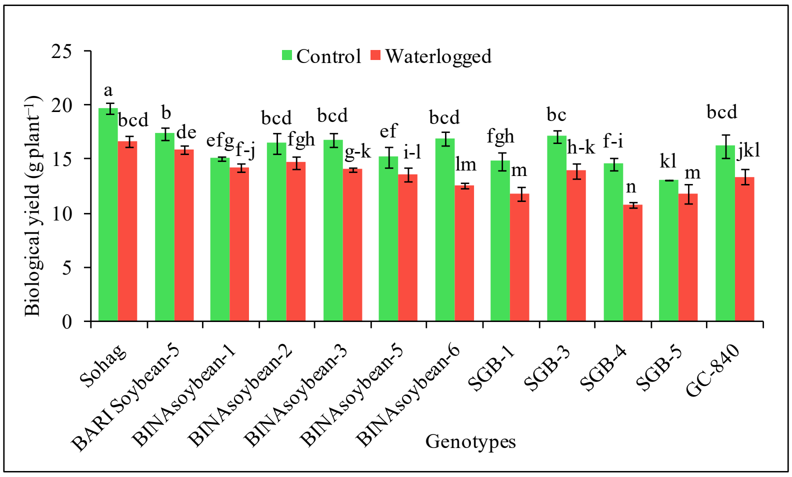

Figure 16. Effect of waterlogging stress on biological yield of different soybean genotypes. For each treatment, the mean (±SD) was determined from three replicates. Different alphabets on the bars indicate significant difference at $P \leq 0.05$ after mean comparison by Fisher's LSD test

Yield is a result of the integration of metabolic reactions in plants. Any factor that influences the metabolic activity at any stage of the duration of plant growth can affect the yield. Waterlogging stress has shown mostly negative effects on yield attributes (number of pod plant^{-1}, number of seed pod^{-1}, seed yield plant^{-1}, stover yield plant^{-1}, biological yield) of soybean plants at different phases and durations. The damaging effects increased with increasing periods of waterlogging. There are some authors who suggested the waterlogging, which can be tolerated to soybean plants was approximately 24-30 hours from the starting to the end of waterlogging (Griffin *et al*., 1985; Heatherly and Pringle, 1991). Such negative effects of waterlogging stress on the yield of soybean were also proved in earlier works (Beutler *et al.,* 2014; Miao *et al.,* 2012).

4.6 Correlation among the Parameters

From the correlation matrix study, it is clear that the mortality rate and electrolyte leakage were negatively correlated ($P \leq 0.05$) with most of the parameters (Table 5).

The stress markers like MDA, H_2O_2, EL were negatively correlated with the growth, physiological, yield and yield contributing parameters (Anee *et al.,* 2019; Hasanuzzaman *et al.,* 2018).

Table 5. Correlation matrix of different parameters activities observed in different soybean genotypes under waterlogging stress condition

VR	PHT	LN 20	LN 50	BN	SFW	SDW	SPAD	RWC	EL	LA	Pod	Seed	100 SW	SY	StY	BY
PHT	1	0.263*	0.219^{ns}	0.355*	0.574*	0.379*	0.467*	0.296*	-0.203^{ns}	-0.046^{ns}	0.394*	0.417*	0.332*	0.319*	0.016^{ns}	0.214^{ns}
LN 20		1	-0.033^{ns}	-0.248*	0.094^{ns}	0.291*	0.284*	-0.059^{ns}	0.281*	0.001^{ns}	-0.262*	0.027 ^{ns}	-0.319*	-0.371*	-0.312*	-0.392*
LN 50			1	0.426*	0.359*	0.179 ^{ns}	0.280*	0.278*	-0.300*	0.486*	0.321*	0.103^{ns}	0.348*	0.309*	0.053^{ns}	0.226^{ns}
BN				1	0.621*	0.311*	0.426*	0.589*	-0.542*	-0.060^{ns}	0.859*	0.547*	0.725*	0.785*	0.473*	0.738*
SFW					1	0.691*	0.634*	0.652*	-0.687*	0.168^{ns}	0.616*	0.632*	0.748*	0.707*	0.242*	0.575*
SDW						1	0.688*	0.403*	-0.550*	0.148^{ns}	0.305*	0.538*	0.445*	0.354*	-0.072^{ns}	0.194^{ns}
SPAD							1	0.483*	-0.394*	0.027^{ns}	0.360*	0.477*	0.518*	0.445*	-0.051^{ns}	0.263*
RWC								1	-0.324*	0.142 ^{ns}	0.508*	0.249*	0.544*	0.679*	0.443*	0.655*
EL									1	-0.056 ^{ns}	-0.633*	-0.534*	-0.726*	-0.632*	-0.195 ^{ns}	-0.504*
LA										1	-0.156^{ns}	0.012 ^{ns}	0.205 ^{ns}	0.151 ^{ns}	-0.005 ^{ns}	0.095 ^{ns}
Pod											1	0.590*	0.710*	0.720*	0.372*	0.647*
Seed												1	0.625*	0.543*	0.128 ^{ns}	0.413*
100 SW													1	0.878*	0.345*	0.736*
GY														1	0.548*	0.914*
SY															1	0.841*
BY																1

Here, *significant at $P \leq 0.05$, ns= Non significant, VR= variables, PHT= plant height 50, LN 20= Leaves no. 20, LN 50= Leaves No. 50, BN= Branch No., SFW= Shoot FW 25, SDW= Shoot DW 25, SPAD= SPAD 40, RWC= relative water content 25, EL= Electrolyte leakage, LA= leaf area, pod= Pod plant^{-1}, Seed= Seed pod $^{-1}$, 100 SW= 100 seed wt, SY= grain yield, StY= stover yield, BY= biological yield

Chapter V

SUMMARY AND CONCLUSION

The experiment was conducted to screen out the genotypes of soybean under waterlogging stress conditions at the seedling stage. Morphological, physiological, phenotypic and yield attributes of different soybean genotypes were studied.

The experiment was laid out in a completely randomized design with three replications. Plastic pots were used in the experimental shed to maintain uniformity in waterlogging stress throughout the period. The experiment consisted of 2 water level conditions (control and waterlogging) and 12 genotypes (Sohag, BARI Soybean-5, BINAsoybean-1, BINAsoybean-2, BINAsoybean-3, BINAsoybean-5, BINAsoybean-6, SGB-1, SGB-3, SGB-4, SGB-5, GC-840). After germination and seedling establishment 7 plants were allowed to grow in set-1 of each pot for taking vegetative and destructive data and 3 plants in set-2 for taking yield data. In the experiment, data were taken during stress period and recovery stage for each treatment. Yield parameters were measured at the time of harvesting.

For measuring the growth of plants, mortality rate, plant height, number of leaves plant^{-1}, number of branches plant^{-1}, leaf area, above-ground fresh weight and above-ground dry weight were measured. SPAD value of leaf, relative water content (RWC), electrolyte leakage were measured as physiological parameters. Number of pods plant^{-1}, number of seeds pod^{-1}, seed yield plant^{-1}, 100-seed weight, stover yield, biological yield were measured at harvest time to observe the effects on yield of soybean.

Mortality rate (%) of seedlings was the highest in the genotypes of SGB-3 (43%) and the lowest in BARI Soybean-5 (9%) when waterlogging continued for 12 days.
The sharp reduction of plant height was observed at 20, 25. 30 and 50 DAS upon exposure to waterlogging stress, in comparison to the control condition. The lowest reduction in plant height was observed in waterlogged BINAsoybean-2 that was 2, 2,

1 and 4% at 20, 25, 30 and 50 DAS, respectively and the highest reduction was observed in waterlogged SGB-1 which was 34, 34, 25 and 26% at 20, 25, 30 and 50 DAS, respectively in comparison with the control condition

The lowest reduction in leaves number were observed in waterlogged GC-840 (2%), BINAsoybean-1 (0.3%) and SGB-1 (3%) at 20, 25 and 50 DAS, respectively when compared to control condition. The highest reduction in leaves number were observed in waterlogged BINAsoybean-1 (26%), BINAsoybean-6 (41%) and BINAsoybean-6 (57%) at 20, 25 and 50 DAS, respectively in comparison with the control condition.

Upon exposure to waterlogging, BINAsoybean-1 showed the lowest reduction in branches number plant$^{-1,}$ which was 19% in comparison with the control condition. The highest reduction was observed in waterlogged BINAsoybean-6 (50%) and SGB-3 (50%) in comparison with the control condition.

The lowest reduction of leaf area was observed in waterlogged BARI Soybean-5 (2%) and the highest was in SGB-5 (16%) at 32 DAS. At 39 and 46 DAS, the lowest reduction was observed in waterlogged genotype Sohag (6% and 5%) and the highest was observed in waterlogged genotype SGB-3 (47 and 17%) in comparison with their control plants.

Plant exhibited the highest reduction of above-ground FW in waterlogged SGB-4 (42.6%) and SGB-4 (71.3%) at 20 and 25 DAS, respectively and The lowest reduction was observed in waterlogged BARI Soybean-5 (5.7%) and GC-840 (13.1%) at 20 and 25 DAS, respectively when compared to control condition.

The lowest reduction in plant DW was observed in waterlogged Sohag, which was 11 and 8% at 20 and 25 DAS, respectively, when compared to the control condition. The highest reduction in plant DW were observed in waterlogged SGB-3 (65%) and SGB-1 (57%) at 20 and 25 DAS, respectively, in comparison with the control condition.

In comparison with the control condition, the lowest reduction in SPAD value was observed in waterlogged SGB-3 (2%), BINAsoybean-2 (8%), BARI Soybean-5 (12%) and BINAsoybean-2 (8%) at 20, 25, 30 and 40 DAS, respectively in waterlogged plants and the highest reduction was observed in waterlogged SGB-4 (21%), SGB-1 (22%), SGB-3 (23%) and BINAsoybean-1 (21%) at 20, 25, 30 and 40 DAS respectively in waterlogged plants.

The lowest reduction in leaf RWC was observed in waterlogged BARI Soybean-5 (17%) and BINAsoybean-1 (4%) at 20 and 25 DAS, respectively and the highest reduction was observed in waterlogged BINAsoybean-5 (42%) and SGB-4 (37%) at 20 and 25 DAS, respectively in comparison with the control condition.

Due to the imposition of waterlogging, electrolyte leakage increased significantly in waterlogged plants than the control plants. The highest increase was observed in waterlogged BINAsoybean-1 (260%) and the lowest increase was observed in waterlogged BINAsoybean-5 (29%) at 20 DAS in contrast to control.

Root phenotypic pictures exhibited that the lowest number of adventitious roots were developed in the waterlogged SGB-4 and the highest number of adventitious roots were developed in the waterlogged GC-840 genotypes at both 20 and 25 DAS, respectively.

The lowest reduction in the number of pods plant^{-1} was observed in waterlogged Sohag (5%) and the highest reduction was in waterlogged SGB-1 (37%) when compared to the control plants.

In comparison with the control condition, the lowest reduction in the number of seeds pod^{-1} was observed in waterlogged Sohag (1%) and the highest reduction in waterlogged BINAsoybean-5 (9%).

Due to the imposition of waterlogging, 100-seed weight decreased significantly when compared to control. The lowest reduction in 100-seed weight was observed in waterlogged GC-840 (5%) and the highest reduction in waterlogged BINAsoybean-5 (29%) in comparison with the control condition.

The lowest reduction in seed yield was observed in waterlogged GC-840 (11%) and the highest reduction in waterlogged SGB-4 (51%) in comparison with the control condition.

The lowest reduction in stover yield was observed in waterlogged BARI Soybean-5 (1%) and the highest reduction in stover yield was observed in waterlogged SGB-5 (22%) in comparison with the control condition.

Plant exhibited the lowest reduction in biological yield in waterlogged BINAsoybean-1 (6%) and the highest reduction in waterlogged SGB-4 (26%) when compared to control.

The lowest reduction in harvest index (%) was observed in waterlogged GC-840 (7%) and the highest reduction in harvest index (%) was observed in waterlogged SGB-4 (34%) in comparison with the control condition.

Soybean crops are usually sensitive to waterlogging stress. By considering all the above-mentioned results, it can be concluded that waterlogging remarkably reduced the growth and yield of all the soybean genotypes when compared with their respective control plants. Among the 12 genotypes Sohag, BARI Soybean-5, GC-840, BINAsoybean-1, BINAsoybean-2 performed better than the other genotypes under waterlogging stress. The genotypes SGB-1, SGB-3, SGB-4, SGB-5, BINAsoybean-5, BINAsoybean-6 performed worst than the other genotypes under waterlogging stress condition. This experiment was conducted with the application of waterlogging treatment for about 12 days period at the seedling stage. However, further research work might be conducted for identifying tolerance mechanisms of soybean genotypes under prolonged waterlogging stress and also waterlogging at reproductive stages.

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APPENDICES

Appendix I: Monthly average air temperature, relative humidity, rainfall and daylight of the experimental site during the time from July 2019 to November 2019

Months	Air temperature (°C)		Relative humidity (%)	Total rainfall (mm)	Daylight (h)
	Maximum	Minimum			
July	26.2	31.4	72	373.1	13.4
August	26.3	31.6	74	316.5	12.9
September	25.9	31.6	71	300.4	12.3
October	23.8	31.6	65	172.3	11.6
November	19.2	29.6	53	34.4	11

Appendix II: Mean square values and degrees of freedom (DF) of mortality rate, plant height (20, 25, 30 and 50 DAS), number of branches plant^{-1} of different soybean genotypes under waterlogging

Sources of variation	DF	Mean square values of					
		Mortality rate	Plant height				Number of branches plant^{-1}
			20 DAS	25 DAS	30 DAS	50 DAS	
Treatment	23	666.106	19.950	31.506	63.669	308.812	2.769
Error	48	1.372	1.175	1.702	15.337	6.541	0.035

Appendix III: Mean square values and degrees of freedom (DF) of number of leaves plant-1 (20, 25 and 50 DAS), leaf area (32, 39 and 46 DAS) of different soybean genotypes under waterlogging

Sources of variation	DF	Mean square values of					
		Number of leaves plant^{-1}			Leaf area		
		20 DAS	25 DAS	50 DAS	32 DAS	39 DAS	46 DAS
Treatment	23	1.614	1.875	94.742	45.830	87.886	55.337
Error	48	0.096	0.033	0.297	0.627	10.656	8.662

Appendix IV: Mean square values and degrees of freedom (DF) of shoot FW (20 and 25 DAS), shoot DW (20 and 25 DAS), RWC (20 and 25 DAS) of different soybean genotypes under waterlogging

Sources of variation	DF	Mean square values of					
		Shoot FW		Shoot DW		RWC	RWC
		20 DAS	25 DAS	20 DAS	25 DAS	20 DAS	25 DAS
Treatment	23	0.558	1.395	0.021	0.024	756.366	232.141
Error	48	0.011	0.016	0.0001	0.0001	15.378	13.285

Appendix V: Mean square values and degrees of freedom (DF) of SPAD value (20, 25, 30 and 40 DAS), electrolyte leakage of different soybean genotypes under waterlogging

Sources of variation	DF	Mean square values of				
		SPAD value				electrolyte leakage (20 DAS)
		20 DAS	25 DAS	30 DAS	40 DAS	
Treatment	23	44.041	56.339	104.790	90.284	189.048
Error	48	4.172	2.888	22.825	5.084	1.146

Appendix VI: Mean square values and degrees of freedom (DF) of number of pods plant^{-1}, number seeds pod^{-1}, 100-seed weight, seed yield plant^{-1}, stover yield and biological yield of different soybean genotypes under waterlogging

Sources of variation	DF	Mean square values of					
		Number of pods plant^{-1}	Number of seeds pod^{-1}	100-seed weight	Seed yield plant^{-1}	Stover yield (tha^{-1})	Biological yield (tha^{-1})
Treatment	23	38.182	0.031	267.73	5.716	2.754	13.155
Error	48	1.393	0.004	6.857	0.059	0.262	0.382