Screening of Soybean Genotypes for Waterlogging Stress Tolerance and Understanding the Physiological Mechanisms

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Waterlogging is a common form of abiotic stress that severely impedes global soybean production. Targeting this issue, an experiment was carried out at Sher-e-Bangla Agricultural University during August–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 (CRD) with three replications consisting of 2 water levels (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, and GC-840). On the 15th day after sowing, plants were exposed to waterlogging for 12 days. Waterlogging remarkably declined the growth and yield of all the soybean genotypes compared to control. Reduced plant height, relative water content, above-ground fresh and dry weight, SPAD value, leaf area, number of leaves, branches, pods, seeds pod−1, 100-seed weight, and seed yield plant−1 were observed under waterlogging stress. Conversely, mortality rate and electrolyte leakage were increased under the same condition. The waterlogged plants showed delayed flowering and maturity compared with the control plants. However, among the 12 genotypes, Sohag, BARI Soybean-5, GC-840, BINAsoybean-1, and BINAsoybean-2 showed better waterlogging tolerance. These genotypes showed a greater number of adventitious roots in the base of their stem, which probably helped plants to thrive under waterlogging conditions.

1. Introduction

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 [1], and as a result pose a serious ultimatum to crop production. Waterlogging in cultivated areas is a common abiotic stress which has severe influences on the composition and production of soybean [2, 3] and most crops’ species worldwide [4, 5]. Excess rainfall, tides, floods, storms, and lack of adequate drainage facilities are the causes of waterlogging stress in plants [6].

Gas exchanges between root systems and porous spaces in waterlogged soils are restricted due to diffusion resistance to oxygen as its diffusion is 10,000 times lower in water, relative to air [7]. Primarily, waterlogging stress causes hypoxic (O2 deficient) condition, which affects aerobic respiration. Over the course of time, the stress switched to an anoxic (O2 absent) condition, which causes inhibition of respiration [8], limitation of energy, deposition of noxious compounds (e.g., lactate), and loss of carbon (through the loss of ethanol from the roots) [9]. Oxygen is needed for the division of cells, development of cells, respiration, absorption, and transportation of nutrients in plants. Due to flooding stress, plants experienced chlorosis, necrosis, defoliation, growth reduction, reduced nitrogen (N) fixation, yield loss, and death of plant at both vegetative and reproductive stages [10]. Flooding stress-induced ethanol accumulation imposes adverse impacts on various processes [11]. Voesenek and Bailey-Serres [12] stated that waterlogging stress posed O2 deficiency in the soil, which triggered...
the accumulation of ethylene, phytotoxic mineral nutrients (Mn$^{2+}$ and Fe$^{2+}$) and also the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in plants. Flooding stress-induced production of ROS, which is responsible for oxidative stress as well as cellular damages like lipid peroxidation, damaged nucleic acid, downregulation of enzymes, and activation of programmed cell death [13]. Many countries in Asia experience flooding stress during the rainy season. In Bangladesh, the waterlogging condition is common in kharif season due to flash floods and/or heavy rainfall. Soybean crops are usually intolerant to waterlogging stress [14]. In different flooding durations, soybean plants reacted significantly different to flooding stresses [3]. In soybean, waterlogging stress switched aerobic respiration towards anaerobic. Anaerobic respiration triggers alcoholic fermentation by generating waterlogged-inducible alcohols (C$_2$H$_5$OH) fermentation by generating waterlogged-inducible enzymes, and activation of programmed cell death [13]. Many 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.

2. Materials and Methods

2.1. Climatic Condition, Treatments, and Design. The experiment was conducted in kharif-II season when a temperature around 30°C was prevailed in the months of July to September and started falling from October to November 2019. There were 12 genotypes of soybean and 2 water level conditions as control and waterlogging. Each treatment was compared to its corresponding control. There were two sets of pots in the experiment: one set was for taking growth and physiological data and another was for measuring yield data. Waterlogging treatments were started at 15 days after sowing (DAS). The root zone was submerged and water level was maintained at 5 cm above the soil surface in the pot. After 12 days of waterlogging, the water was removed and allowed to come to field capacity. The experiment was laid out in a completely randomized design with three replications.

2.2. Crop Husbandry. Ten seeds were sown in each 16 L plastic pots. Gap-filling was performed in some pots when some seeds of soybean failed to germinate. Thinning was done to maintain 3 seedlings for yield set. Mulching was done by loosening the soil very often to maintain moisture. Irrigation was given in control plants maintaining field capacity. Other intercultural operations were performed as required.

2.3. Data Collection

2.3.1. Crop Growth Parameters. Before starting the treatment, the total number of plants per pot was counted, which was denoted as Ni, and again after the completion of treatment duration, the total number of plants per pot was counted, which was denoted as Np. The mortality rate was calculated using the following formula of Anee [17]:

\[
\text{mortality rate} (\%) = \frac{N_i - N_p}{N_i} \times 100. \quad (1)
\]

Plant height was recorded from the ground level to the highest tip of the leaf by a measuring scale at 25 DAS. The numbers of trifoliate leaves and branches plant$^{-1}$ were counted once after the completion of the vegetative growth of plants at 50 DAS. For measuring leaf area, firstly, leaf images were taken by a digital camera at 32 DAS, and then the area was calculated using ImageJ software (version 1.53n).

For plant fresh weight (FW), three sample plants were uprooted from each pot randomly and thoroughly washed under running tap water then weighed in an electric balance. After recording the FW, the samples were dried in an electric oven maintaining 80°C for 48 h then weighed. Both the FW and DW data were taken at 20 DAS. Data of three plants were averaged for all the parameters.

2.3.2. Physiological Parameters. Five leaves were randomly selected from each pot. Each leaflet was measured with atLEAF (FT Green LLC, USA) as atLEAF value at 30 DAS. The total chlorophyll (chl) content was then averaged and calculated by translating the atLEAF value into SPAD units. Relative water content (RWC) was recorded at 20 DAS following the procedure of Barrs and Weatherley [18]; leaf laminas of fully developed leaves were separated from randomly selected plants. Whole leaf discs were weighed as FW and then floated in Petri dishes with distilled water and kept in a dark place. After 24 h, the leaf discs were weighed again by 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:

\[
\text{RWC} (\%) = \frac{FW - DW}{TW - DW} \times 100. \quad (2)
\]

Electrolyte leakage (EL) was measured at 20 DAS following the method of Zhang et al. [19]. To measure EL, 0.5 g leaf samples was 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$_c$) was recorded with an electrical conductivity meter. Samples were again incubated in an Autoclave machine for...
about 1 h and electrical conductivity (EC₂) were measured with an EC meter (HI98301, Hanna, USA) after cooling the samples. Electrolyte leakage was calculated using the following formula as described by Anee [17]:

\[
EL (\%) = \frac{EC_1}{EC_2} \times 100. 
\]

2.3.3. Observation of Phenotypes of Adventitious Root and Plants. Adventitious roots of waterlogged plants were taken at 25 DAS, and pictures were taken with a digital camera. Comparative pictures of plants were taken two times: one at the vegetative phase (25 DAS) and another at the reproductive phase (75 DAS).

2.3.4. Yield and Yield-Contributing Parameters. The total number of pods plant⁻¹ was counted from the three plants and then averaged. Ten pods from each pot were selected, and seeds were counted from each individual pods and then averaged as number of seeds pod⁻¹. Harvested seeds were sundried, and then clean 100 seeds were weighed with an electric balance. After separating seeds from stover, that was weighed as seed yield plant⁻¹.

2.4. Statistical Analysis. Data accumulated from different parameters were subjected to analysis using CoStat v.6.400 [20] 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’s correlation analysis was done using SPSS v.27 [21].

3. Results and Discussion

3.1. Crop Growth Parameters

3.1.1. Mortality Rate. Mortality rates varied in several genotypes. Among all the genotypes, the lowest mortality rate was observed in BARI Soybean-5 (9%), whereas the highest was observed in the genotype 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). All the control plants survived in this experiment. In this experiment, death of soybean genotypes due to imposition of waterlogging was observed. Few genotypes showed severe death, and few showed some tolerance. Survival rate varied in soybean plants at genotypes and duration-dependent manner [3]. While working on 40 soybean genotypes under different levels of waterlogging stress (3, 6, 9, 12, and 15 days), Wu et al. [3] 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.

3.1.2. Plant Height. A sharp reduction of plant height was observed upon exposure to waterlogging stress in comparison to the control condition (Table 1). The lowest reduction in plant height was observed in waterlogged BINAsoybean-2 that was 2% when compared to the control condition. The highest reduction in plant height was observed in waterlogged SGB-1, which was 34% in comparison with the control condition. Upon exposure to waterlogging, the reduction in plant height ranged between 2 and 33% in other genotypes when compared with their respective control plants. The waterlogging-induced decrease in plant height was noticed in soybean [22]. Waterlogging leads to a hypoxic situation, which caused damage to the roots as there prevailed insufficient water, minerals, nutrients, and hormones. This inadequacy of nutrients and water uptake leads to shoot damage, and finally, a reduction in plant height was observed [23]. Waterlogging can restrict the ability of plant to assimilate carbon and nitrogen by inhibiting carbon and nitrogen metabolism. Reduction of CO₂ assimilation: photosynthesis rate significantly decreases upon exposure to waterlogging; eventually, plant showed stunted growth. These might result in a decrease in plant’s height in this study. Besides, the plant cannot uptake the proper amount of nutrients N, P, and K, which results in nutrient deficiency symptoms and, finally, reduction of shoot length [24].

3.1.3. Number of Leaves Plant⁻¹. When exposed to waterlogging, the number of leaves plant⁻¹ showed a decreasing manner when compared to the control plants (Table 1). The lowest reduction in leaf number was observed in waterlogged SGB-1 (3%) when compared to the control condition. The highest reduction in leaf number was observed in waterlogged BINAsoybean-6 (57%) in comparison with the control condition. Upon exposure to waterlogging, the reduction in leaves number ranged between 4 and 42% in other genotypes when compared with their respective control plants. Flooding leads to a decline in crop growth, net assimilation rate, and leaf expansion, and the ultimate outcome was a reduction of leaf number and leaf area in soybean crops [25].

3.1.4. Number of Branches Plant⁻¹. When exposed to waterlogging, the number of branches plant⁻¹ showed a decreasing manner when compared to the control plants (Figure 2). The lowest reduction in branches number was observed in waterlogged BINAsoybean-1 (19%) followed by BARI Soybean-5 (20%), GC-840 (20%), and Sohag (24%) when compared to the control condition. The highest reduction in branch number was observed in waterlogged BINAsoybean-6 (50%) and SGB-3 (50%) in comparison with the control condition. Upon exposure to waterlogging, the reduction in branch numbers ranged between 28 and 45% in other genotypes when compared with their respective control plants. The stunted plant leads to a lower number of branches. Miura et al. [26] also reported waterlogging for
21 days in soybean, which resulted in a significant reduction in number of branches of soybean plants. Decrease in branch number in response to several stresses in soybean was also reported by several studies [27–29].

3.1.5. Leaf Area. A sharp reduction of leaf area was observed upon exposure to waterlogging stress in comparison with the control condition (Table 1). The lowest reduction of leaf area was observed in waterlogged BARI Soybean-5 (2%) followed by Sohag (3%), BINAsoybean-2 (4%), and BINAsoybean-1 (9%). The highest reduction was observed in SGB-5 (16%). Upon exposure to waterlogging, the reduction of leaf area ranged between 10 and 14% in other genotypes when compared with their respective control plants. Leaf area of different soybean genotypes decreased due to waterlogging stress (Table 1), which is evidenced by many authors [30, 31]. Similar outcomes were observed in some other crops like mungbean [32], barley [33], sesame [34, 35], and green gram [36].

3.1.6. Above-Ground Fresh and Dry Weight Plant$^{-1}$. Upon exposure to waterlogging, above-ground FW plant$^{-1}$ reduced when compared to their control plants. The lowest reduction in plant FW was observed in waterlogged BARI Soybean-5 (6%) followed by Sohag (8%) and BINAsoybean-2 (13%) in comparison with control. The highest decline in plant FW was observed in waterlogged SGB-4 (43%) in comparison with the control condition (Figure 3(a)). Upon exposure to waterlogging, the reduction in plant FW ranged between 17 and 40% in other genotypes when compared with their respective control plants.

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%, when compared to the control condition. The highest reduction in plant DW was observed in waterlogged SGB-3 (65%) in comparison with the control condition. Upon exposure to waterlogging, the reduction in plant DW ranged between 8 and 62% (Figure 3(b)) in other genotypes when compared with their respective control plants.

Waterlogging-induced reduction in FW and DW was found in soybean in different studies [37, 38]. Research works showed that waterlogging affects chl and reduce the content of chl and resulting reduction of photosynthetic process and the decrease in the rate of photosynthesis that inhibited plant growth and accumulation of biomass [39]. Under waterlogging, the phytopathology and catabolism of plants are disrupted, restricting stomatal conductance, the transition of gases, and metabolism of CO$_2$. Reduction of CO$_2$ entering the leaf reduced transpiration, leading to wilting of the leaves, and decreased chl content as a result lower dry matter accumulation [40], which result greatly in decline of FW and then DW. 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 [41, 42]. Several scientists recorded that flooding stress reduced shoot DW of maize [43], green gram [36], and mungbean [32, 44].

3.2. Physiological Parameters

3.2.1. SPAD Value. SPAD reading showed lower value in the leaves of waterlogged plants when compared with the control plants. The lowest reduction was observed in waterlogged BARI Soybean-5 (12%) in waterlogged plants.
Table 1: Effect of waterlogging stress on plant height, number of leaves plant\(^{-1}\), leaf area, and SPAD value of different soybean genotypes.

| Treatments       | Plant height (cm) | Number of leaves plant\(^{-1}\) | Leaf area | SPAD value |
|------------------|-------------------|---------------------------------|-----------|------------|
|                  | Control           | Waterlogged                     | Control   | Waterlogged | Control | Waterlogged |
| Sohag            | 28.37 ± 1.21a-d   | 25.21 ± 1.10efg                | 23.15 ± 0.34jk | 19.66 ± 0.82l | 36.73 ± 0.91ab | 35.51 ± 0.88bc | 45.63 ± 0.84a-e | 39.67 ± 3.84def |
| BARI Soybean-5   | 27.30 ± 1.51cde   | 24.70 ± 1.67fgih               | 28.57 ± 0.27e  | 18.38 ± 1.19mn | 33.27 ± 0.82d  | 32.60 ± 0.81def | 43.68 ± 3.34b-f  | 38.51 ± 2.01ef  |
| BINAsoybean-1    | 26.24 ± 1.05def   | 25.19 ± 1.23efg                | 33.75 ± 0.40a  | 26.15 ± 0.62h  | 35.50 ± 0.88bc | 32.27 ± 0.80d-g | 45.47 ± 3.83a-e  | 39.25 ± 3.41def |
| BINAsoybean-2    | 23.50 ± 0.93ghi   | 23.00 ± 0.50ghi                | 28.50 ± 0.27ef | 27.50 ± 0.32g  | 29.70 ± 0.74jk | 28.47 ± 0.70k  | 50.14 ± 1.67ab  | 43.23 ± 3.44b-f |
| BINAsoybean-3    | 23.90 ± 0.82ghi   | 19.60 ± 1.17f                  | 27.63 ± 0.31fg | 16.71 ± 0.44" | 35.43 ± 1.01bc | 30.48 ± 0.75hij | 46.47 ± 0.48a-d | 39.90 ± 2.63def |
| BINAsoybean-5    | 21.23 ± 1.27jkl   | 20.73 ± 1.57kl                 | 30.58 ± 0.37cd | 19.99 ± 0.29l  | 31.49 ± 0.78f-i | 28.47 ± 0.70k  | 44.74 ± 0.93a-e  | 36.65 ± 2.91f   |
| BINAsoybean-6    | 27.88 ± 1.63bcd   | 25.27 ± 1.31efg                | 29.74 ± 0.32d  | 12.70 ± 0.12p  | 31.71 ± 0.78e-h | 31.18 ± 0.77ghi | 49.61 ± 2.14ab  | 39.87 ± 3.92def |
| SGB-1            | 28.79 ± 1.99abc   | 19.10 ± 1.27kl                 | 19.28 ± 0.41im | 18.71 ± 0.63m  | 30.38 ± 0.75ij | 25.79 ± 0.64l  | 50.00 ± 0.79ab  | 42.79 ± 2.35b-f |
| SGB-3            | 30.19 ± 0.59a     | 25.27 ± 1.28efg                | 30.85 ± 0.38bc | 17.77 ± 0.44n  | 28.80 ± 0.71k  | 24.67 ± 0.61l  | 48.99 ± 2.87abc | 37.91 ± 3.27g   |
| SGB-4            | 29.86 ± 0.90ab    | 20.09 ± 1.17kl                 | 25.30 ± 0.86i  | 22.30 ± 0.36k  | 28.58 ± 0.71k  | 24.60 ± 0.70l  | 51.73 ± 2.03a  | 42.63 ± 4.41b-f |
| SGB-5            | 28.18 ± 1.61a-d   | 24.37 ± 1.58fgih               | 31.50 ± 0.87b  | 23.90 ± 0.42j  | 35.84 ± 0.89bc | 30.26 ± 0.75ij | 50.32 ± 1.26ab  | 41.72 ± 2.11c-f |
| GC-840           | 28.42 ± 1.79abc   | 22.88 ± 0.90hij                | 28.42 ± 1.79abc | 22.88 ± 0.90hij | 37.63 ± 0.93a  | 32.82 ± 0.81de | 51.75 ± 2.16a  | 40.61 ± 2.34def |

CV (%) 5.1 2.18 4.2 4.1

For each treatment, the mean (±SD) was determined from three replicates. Different alphabets indicate significant difference at P ≤ 0.05 after mean comparison by Fisher’s LSD test.
when compared to control plants. The highest reduction was observed in waterlogged SGB-3 (23%) in waterlogged plants when compared to control plants. Upon exposure to waterlogging, the reduction in SPAD value ranged between 13 and 22% in other genotypes when compared with their respective control plants (Table 1). Soybean responses to waterlogging are 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-day waterlogging as there a significant reduction in chl content was observed [3]. SPAD reading, which is the indicator of chl content of leaf, decreased due to waterlogging stress and the percent reduction varied upon genotype to genotype (Table 1). Waterlogging in soybeans exhibits a decline in activity in photosynthesis [45]. Tian et al. [43] showed that SPAD value reduced 10–38% in KY16 variety and 5–30% in DMY1 variety of maize due to waterlogging.

### 3.2.2. Relative Water Content

When exposed to waterlogging, plants exhibited reduction in leaf RWC (Figure 4). The lowest reduction in leaf RWC was observed in waterlogged BARI Soybean-5 (17%) and the highest reduction were in waterlogged BINAsoybean-5 (42%) in comparison with the control condition. The reduction ranged between 18 and 41% in other genotypes when compared with their respective control plants. Relative leaf water content was found to be a crucial factor in assessing plant tolerance to osmotic stress caused by waterlogging. In our study, waterlogging leads to a significantly reduced RWC content in the several genotypes of soybean plants (Figure 4). Reduction in leaf RWC indicates an insufficient supply of water for cell expansion [46]. 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 [40] and as a result, leaf wilting symptoms were found on plants. The corresponding decrease in RWC due to waterlogging was also observed in mungbean [32] and sesame [34].

### 3.2.3. Electrolyte Leakage

Electrolyte leakage was increased upon exposure to waterlogging in several genotypes of soybean plants (Figure 5). Electrolyte leakage was enhanced with increasing stress levels as compared to the control. The highest increase was observed in waterlogged BINAsoybean-1 (260%) and the lowest increase was in waterlogged BINAsoybean-5 (29%) followed by GC-840 (34%) and Sohag (35%) in contrast to control. Upon exposure to waterlogging, the increase in EL ranged between 51 and 111% in other genotypes. Waterlogged BINAsoybean-1 and BINAsoybean-6; waterlogged BINAsoybean-3, SGB-1, and SGB-4; and waterlogged BARI Soybean-5, BINAsoybean-5, and control BINAsoybean-2, SGB-4 showed no significant difference among them. Electrolyte leakage was increased upon exposure to waterlogging in several genotypes of soybean plants (Figure 6). Due to waterlogging stress, cell membrane became disorganized, which increased the generation of ROS and metabolic toxicity [47]. The membrane injury increased with the increasing duration of waterlogging stress in pigeon pea [5, 48].

### 3.3. Root Phenotypes

Morphological acclimation to waterlogging in soybean emerges to adventitious root formation. The least number of adventitious roots was observed in SGB-4, SGB-3, and SGB-1, and the rest of the genotypes showed a quite better number of adventitious roots. Among them, GC-
showed a higher number of adventitious roots (Figure 6). Waterlogging caused injury to roots owing to cellular anoxia; furthermore, root meristems exhibited susceptibility [49, 50]. Uptake of water and nutrients failed due to damaged roots. Bacanamwo and Purcel [51] 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 [44, 52]. Adventitious roots of soybean under waterlogging stress exploited rupture of cortex cells, creating aerenchyma used as pores for O2 transferring to roots, being a plant adaptive mechanism under these stress conditions [38]. Our experiment also supported that waterlogging resulted in higher number of adventitious roots in intolerant genotypes. Adventitious roots have not been found in soybean control plants, which is supported by other studies [22].
3.4. Phenotypic Comparative Observation. In this study, a visible appearance was observed under control and waterlogged plants. Plants displayed a decrease in height compared to their control plants. Death of plants was also observed due to waterlogging stress (Figure 7). Waterlogging for 12 days at the vegetative stage caused delayed flowering and maturity in all the genotypes when compared with their control plants (Figure 8), which is supported by the study of Kuswantoro [53]. 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 that 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 [54]. Kuswantoro [53] observed that days to flowering and days to maturity were delayed by 46, 49 days and 98, 100 days, respectively in Sinabung and Lawit variety. The lengthier days to flowering are supposedly due to the plant...
always try to thrive against waterlogging stress by reno-
vating 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
to maturing of plants. Moreover, plants faced nu-
trient deficiency to uptake by the roots as well as ad-
ventitious roots from soil and water, which hindered
plants growth as well delayed days to maturity of soybean
plants. Khaireulina and Tikhonchuk [55] found some
dissimilar results in soybean under waterlogging stress.
Waterlogged plants took a shorter interstage period than
the control plants.

3.5. Yield and Yield-Contributing Parameters. Due to the
imposition of waterlogging, the number of pods plant$^{-1}$
was sharply reduced compared to control (Figure 9(a)). The
lowest decline in the number of pods plant$^{-1}$ was observed in
waterlogged Sohag (5%) when compared to the control
condition. The highest decline in the number of pods plant$^{-1}$
was observed in waterlogged SGB-1 (37%) in comparison
with the control condition. Upon exposure to waterlogging,
the reduction in the number of pods plant$^{-1}$ ranged between
10 and 35% in other genotypes in comparison with the
control plants.

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

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%) followed by BARI
Soybean-5 (8%) and Sohag (9%). The highest reduction was

![Figure 6: Adventitious root formation of different soybean genotypes under waterlogging condition at 25 DAS. Here, the arrows indicate the adventitious roots.](image-url)
Figure 7: Phenotypic variations of soybean under waterlogged and control condition at 25 DAS. Here, C denotes control and WL waterlogged plants.

Figure 8: Phenotypic variations of soybean under waterlogged and control condition at 75 DAS. Here, C denotes control and WL waterlogged plants.
in waterlogged BINAsoybean-5 (29%) in comparison with the control condition. The reduction ranged between 11 and 25% (Figure 9(c)) in other genotypes when compared with their respective control plants.

Upon exposure to waterlogging, stress seed yield plant\(^{-1}\) decreased in comparison to the control condition (Figure 9(d)). The lowest reduction in seed yield was observed in waterlogged GC-840 (11%) followed by SGB-5 (15%), BARI Soybean-5 (21%), and Sohag (23%). The highest reduction in seed yield was observed in waterlogged SGB-4 (51%) in comparison with the control condition. Upon exposure to waterlogging, the reduction in seed yield ranged between 28 and 40% in other genotypes when compared with their respective control plants. 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 [56]. Primarily under waterlogging conditions, seed yield decreased due to the reduction of number of pods plant\(^{-1}\) and pod setting. Kuswantoro [53] observed that sensitive genotypes of soybean bear the least number of pods plant\(^{-1}\) than the tolerant one under waterlogging stress. 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 accomplished well under flooding stress. Least number of pods plant\(^{-1}\) lead to least number of seeds pod\(^{-1}\) which ultimately reduced 100-seed weight and seed yield plant\(^{-1}\). Branch numbers were correlated to pod number increase [57]. A similar decrease in plant yield has been reported in soybean [24, 38, 58, 59]. In addition, yield reduced significantly, and the reduction was greater in waterlogged-sensitive genotypes than the waterlogged-tolerant genotypes of soybean [3], which exhibits that waterlogging has varied responses towards different genetic backgrounds [60].

3.6. 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 2). The stress markers like MDA, $H_2O_2$, and EL were negatively correlated with the growth, physiological, yield, and yield contributing parameters [1, 34].

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**Table 2: Correlation Matrix Study**

| Genotypes | Control | Waterlogged |
|-----------|---------|-------------|
| Sohag     |         |             |
| BARI       |         |             |
| BINAsoybean-1 |     |             |
| BINAsoybean-2 |     |             |
| BINAsoybean-3 |     |             |
| BINAsoybean-4 |     |             |
| BINAsoybean-5 |     |             |
| BINAsoybean-6 |     |             |
| SGB-1     |         |             |
| SGB-2     |         |             |
| SGB-3     |         |             |
| SGB-4     |         |             |
| SGB-5     |         |             |
| GC-840    |         |             |

**Figure 9:** Effect of waterlogging stress on the number of pods plant\(^{-1}\) (a), number of seeds pod\(^{-1}\) (b), 100-seed weight, (c) and seed yield plant\(^{-1}\) (d) of different soybean genotypes. For each treatment, the mean ($\pm$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. Conclusion

Soybean crops are usually intolerant 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, and BINAsoybean-2 performed better than other genotypes under waterlogging. The genotypes SGB-1, SGB-3, SGB-4, SGB-5, BINAsoybean-5, and BINAsoybean-6 did not perform well under waterlogged conditions.

Data Availability

All data are included within the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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Table 2: Correlation matrix of different parameters activities observed in different soybean genotypes under waterlogging stress condition.

| Variables | Plant height | No. of leaves | No. of branches | Fresh weight | Dry weight | SPAD value | Relative water content | Electrolyte leakage | Leaf area | Pod plant−1 | Seeds pod−1 | 100-seed weight | Seed yield |
|-----------|--------------|---------------|-----------------|--------------|------------|------------|------------------------|---------------------|------------|-------------|-------------|----------------|-----------|
| Plant height | 1 | 0.219 ** | 0.355 * | 0.574 * | 0.379 * | 0.467 * | 0.296 * | −0.203 ** | −0.046 ** | 0.394 * | 0.417 * | 0.332 * | 0.319 * |
| No. of leaves | 1 | 0.426 * | 0.359 * | 0.179 ns | 0.280 * | 0.278 * | −0.300 * | 0.486 * | 0.321 * | 0.103 ** | 0.348 * | 0.309 * |
| No. of branches | 1 | 0.621 * | 0.311 * | 0.426 * | 0.589 * | −0.542 * | −0.060 ** | 0.859 * | 0.547 * | 0.725 * | 0.785 * |
| Fresh weight | 1 | 0.691 * | 0.634 * | 0.652 * | −0.687 * | 0.168 ** | 0.616 * | 0.632 * | 0.748 * | 0.707 * |
| Dry weight | 1 | 0.688 * | 0.403 * | −0.550 * | 0.148 ** | 0.305 * | 0.538 * | 0.445 * | 0.354 * |
| SPAD value | 1 | 0.483 * | −0.394 * | 0.027 ** | 0.360 * | 0.477 * | 0.518 * | 0.445 * |
| Relative water content | 1 | −0.324 * | 0.142 ** | 0.508 * | 0.249 * | 0.544 * | 0.679 * |
| Electrolyte leakage | 1 | −0.056 ns | −0.633 * | −0.534 * | −0.726 * | −0.632 * |
| Leaf area | 1 | −0.156 ** | 0.012 ** | 0.205 ** | 0.151 ** |
| Pod plant−1 | 1 | 0.590 * | 0.710 * | 0.720 * |
| Seeds pod−1 | 1 | 0.625 * | 0.543 * |
| 100-seed weight | 1 | 0.878 * |
| Seed yield | 1 | |

Here, *significant at P ≤ 0.05; ns = nonsignificant.
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