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ARTICLE

Extricating short-duration and bold-grained high-yielding soybean genotypes under waterlogging conditions

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ABSTRACT Two experiments were conducted at BSMRAU, Gazipur, during 2021 and 2022 to analyze the effects of waterlogging (WL) on the performance of short-duration, bold-seeded soybean genotypes and assess the impact on seed quality. In 2021, twelve genotypes—BD2334, G00113, G00164, G00064, G00221, BD2331, G00138, G00321, G00058, G00060, G00025, and BU Soybean-1—were evaluated under control and WL conditions. In 2022, five selected genotypes (BD2334, G00164, BD2331, G00060, and BU Soybean-1) were assessed. Plants were subjected to WL stress for seven days in 2021 and five days in 2022 during the pod formation stage. A split-plot design with three replications was employed. Waterlogging stress significantly reduced yield, yield attributes, SPAD value, photosynthesis rate, photosynthetic pigments, transpiration rate, stomatal conductance, germination rate, and early seedling growth. However, it increased electrolyte leakage in seeds as well as proline and malondialdehyde content in leaves. Waterlogged plants matured earlier than their respective control plants. Genotypic differences in WL tolerance were evident, with BD2334, G00164, G00221, and BD2331 exhibiting better yield performance under WL stress. Genotype G00060 demonstrated higher tolerance to WL based on specific physiological parameters. These findings suggest that these genotypes may be suitable for further field evaluation to identify WL-tolerant soybean varieties.
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Introduction

Soybean (*Glycine max* L.) is one of the most significant legume crops belonging to the family *Leguminosae*. It plays a crucial role in global agriculture, contributing approximately 25% of the world's edible oil supply and serving as a primary protein source for livestock, constituting nearly two-thirds of global animal feed. In addition to its oil content, soybeans are a rich source of essential phospholipids, vitamins, and minerals. It also contains biologically active minor compounds such as trypsin inhibitors, phytates, and oligosaccharides. Isoflavones present in soybean exhibit strong anticancer and disease-preventive properties (Messina et al. 2006). Soybean is widely used not only for household consumption but also in industrial applications, particularly in the production of cooking oil and livestock feed. The poultry industry alone accounts for approximately 75% of global soybean utilization (Twum et al. 2021). Due to its dual purpose as both an oilseed and pulse crop, its high protein content (30-50%), and its well-balanced amino acid profile, soybean is considered an excellent alternative protein source, making it a key commodity in international trade (Sun et al. 2020).

Soybean cultivation spans approximately 6% of the world's arable land, and its production area has expanded significantly since the 1970s, surpassing many other major crops in terms of growth rate (Hartman et al. 2011). In Bangladesh, soybean cultivation began in the early 1970s when the Mennonite Central Committee initiated programs to improve rural nutrition in the greater Noakhali district. Initially, the cultivated area was restricted to 5,000 hectares, primarily in Noakhali (Satter et al. 2005). Over time, this area has expanded significantly, reaching 82,000 hectares, with an annual production of 162,000 metric tons. Major soybean-producing regions in Bangladesh now include Lakshmipur, Noakhali, Barishal, Bhola, Chandpur, Patuakhali, Faridpur, and parts of northern Bangladesh (USDA 2022). However, the national average yield of soybean (1.54 t ha^{-1}) remains significantly lower than the global average (2.79 t ha^{-1}) (FAO STAT 2022), highlighting the need for improved cultivation strategies and resilient varieties.

Over the past few decades, climate change has posed significant challenges to food, fiber, and energy production (Asseng et al. 2009), increasing the frequency and severity of extreme weather events, including excessive rainfall. In this context, waterlogging (WL), caused by heavy rainfall and poor soil drainage, has emerged as a major constraint to agricultural productivity, affecting an estimated 1,700 million hectares worldwide (Konnerup et al. 2018). WL is characterized by prolonged soil saturation, where water content exceeds 20% of field capacity (Aggarwal et al. 2006). This condition leads to a low-oxygen environment (hypoxia), which severely restricts root respiration and nutrient uptake, thereby limiting crop growth and yield. The adverse effects of WL are particularly pronounced in humid regions with flat topography, high water tables, and inadequate drainage infrastructure (Collaku and Harrison 2002; Jitsuyama 2017). WL is estimated to affect 10-12% of global agricultural soils, causing annual grain losses of approximately six million tons and resulting in economic damages of around $1.5 billion (Wu et al. 2020).

In Bangladesh, extreme weather events have frequently disrupted soybean cultivation. In 2016, heavy rainfall from Cyclone NADA delayed soybean sowing, whichwas scheduled immediately after the aman rice harvest (Aman rice is a monsoon-season rice variety sown in July-August and harvested in November-January.) (Mamun et al. 2013). Similarly, in 2020, Cyclone Amphan caused extensive damage to soybean crops at the late pod development stage (Mamun et al. 2022). These extreme rainfall events have significantly reduced soybean productivity, particularly in low-lying regions such as greater Noakhali and Bhola. Additionally, WL negatively affects soybean seed viability and germination due to the hygroscopic nature of soybean seed coats, which readily absorb excess moisture. Consequently, prolonged WL exposure leads to a decline in seed quality and overall crop productivity. Developing short-duration soybean varieties with enhanced WL tolerance is a viable strategy to mitigate these challenges (Mamun et al. 2022). Hossain et al. (2019) identified BU Soybean-1 and Shohag as promising WL-tolerant varieties, exhibiting resilience for up to four days under flooding conditions at the flowering stage.

Considering these challenges, the present study was undertaken to evaluate the effects of WL on the growth, yield performance, and physiological characteristics of short-duration, bold-seeded soybean genotypes. Additionally, this study aimed to assess the impact of WL on soybean seed quality, with the goal of identifying potential WL-tolerant genotypes suitable for cultivation in waterlogged-prone regions.

Materials and methods

Site

Two experiments were conducted during two consecutive rabi seasons at the experimental field of the Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. The first experiment was carried out from January to May 2021, and the second from February to June 2022. The experimental site has a subtropical climate, characterized by heavy rainfall from June to September and a gradual temperature decline from September onward.

Experimental treatments and design

Twelve high-yielding soybean genotypes were selected for evaluation: BD2334, G00113, G00164, G00067, G00221, BD2331, G00138, G00321, G00058, G00060, G00025, and BU Soybean-1 (Check). All genotypes were collected from the Department of Agronomy, BSMRAU. Based on performance and yield in the first experiment, four genotypes were selected along with one check variety for the second experiment. The selected genotypes were BD2334, G00164, BD2331, and G00060.

The experimental plots were initially prepared using a moldboard plow, followed by deep and cross plowing, harrowing, and leveling. The first experiment consisted of two factors: Factor A, including 12 soybean genotypes, and Factor B, comprising control and waterlogging (WL) treatments for seven days at the R4 stage. In the second experiment, Factor A included the five selected genotypes, and Factor B involved control and WL treatment for five days at the R2 stage. Both experiments followed a split-plot design with three replications. A fertilizer dose of urea (55 kg ha^{-1}), triple super phosphate (150 kg ha^{-1}), muriate of potash (100 kg ha^{-1}), gypsum (100 kg ha^{-1}), and zinc sulfate (5 kg ha^{-1}) was applied according to FRG (2018) recommendations.

Crop culture

Soybean seeds were collected from BSMRAU and subjected to germination testing before sowing. Seeds were treated with Vitavax-200 at a rate of 2.5 g kg^{-1} before manual sowing on January 12, 2021, and February 16, 2022, for the first and second experiments, respectively. Plots were lightly irrigated immediately after sowing to ensure uniform emergence. Seedlings emerged within 5–10 days after sowing (DAS). Intercultural operations, including thinning, gap filling, weeding, mulching, insecticide application, and irrigation, were performed to maintain crop growth.

WL plots were enclosed with polythene sheets extending 30 cm above the ground to retain water. In the first experiment, WL was imposed at the R4 stage (77

DAS) for seven days, while in the second experiment, it was initiated at the R2 stage (46 DAS) for five days. WL stress was induced by flooding the plots to 5 cm above the ground level. Lodging and leaf yellowing symptoms were visually recorded during treatment. Control plots were irrigated twice per week.

At physiological maturity, dry pods were harvested at different DAS depending on genotype. Pods were dried, and seeds were collected and sun-dried until the moisture content reached 12%.

Data collection

Morpho-physiological data recorded included days to emergence, flowering, pod formation, physiological maturity, and harvest. Days to first flowering were noted when at least one flower opened in 50% of plants per genotype. Days to 50% flowering were recorded when more than half of the plants had open flowers. Maturity was determined based on yellowing leaves and brown, hardened pods. Plant height was measured from the ground to the tip of the tallest shoot.

Lodging assessment

Lodging intensity was visually recorded after floodwater recession based on the IRRI (2002) scale.

SPAD (Soil-Plant-Analysis Development) value

The SPAD value represents leaf greenness. Chlorophyll content was measured using a SPAD 502 Plus Chlorophyll Meter (Konica Minolta Sensing, Japan). SPAD values were recorded before WL treatment (76 DAS for the first experiment and 45 DAS for the second), during treatment (78, 80, and 82 DAS), and seven days post-treatment (89 DAS for the first experiment and 52 DAS for the second experiment).

Leaf greenness and yellowing

Leaf greenness and yellowing were visually estimated based on the scale established by Akter et al. (2021).

Estimation of proline and malondialdehyde

Proline and malondialdehyde (MDA) content in the leaves of all soybean varieties grown under two water regimes was estimated at 58 DAS in the second experiment. Leaf samples were collected from each plot, immediately placed in ice bags, and transported to the laboratory for analysis.

For proline estimation, 0.5 g of fresh leaf tissue was homogenized in 5 mL of 6% aqueous sulfosalicylic acid and centrifuged at 4000 rpm for 20 min. A 2 mL aliquot of the supernatant was transferred to a test tube containing 2 mL of acid ninhydrin and 2 mL of glacial acetic acid, which was tightly covered with aluminum foil. The test tube was heated at 100 °C for 30 min, and the reaction was terminated by placing it in an ice bath for 15 min. The reaction mixture was then mixed with 4 mL of toluene and shaken vigorously for 15-20 sec. After allowing the mixture to stand at room temperature for 10 min, the toluene layer was separated, and absorbance was measured at 520 nm using a toluene blank. The proline concentration was determined from a standard curve and calculated following the method of Bates et al. (1973) on a fresh weight (FW) basis using the equation:

$$\text{Proline } (\mu g\ g^{-1}\ FW) = (\mu g\ mL^{-1}\ \text{proline} \times \text{vol. of toluene} \times \text{vol. of sulfosalicylic acid}) / 0.5\ g\ \text{sample} \times 115.13\ \mu g\ \text{mole}^{-1}$$

For MDA estimation, 0.5 g of fresh leaves were homogenized in 3 mL of 5% trichloroacetic acid solution. The homogenate was centrifuged at 15 500 g for 15 min at 4 °C. Then, 1 mL of the supernatant was mixed with 4 mL of the reaction mixture in a test tube and heated at 95 °C for 30 min in a water bath. After cooling, the solution was centrifuged again at 15 500 g for 10 min. The absorbance of the colored supernatant was measured at 532 nm and 600 nm (Heath and Packer 1968). The MDA content was calculated on a fresh weight basis using the equation:

$$\text{MDA (nano moles } g^{-1}FW) = \{(A_{532} - A_{600})/155\} \times 10^3 \times \text{dilution factor} / 0.5$$

Where A_{532} = Absorbance reading at 532 nm, A_{600} = Absorbance reading at 600 nm. The MDA concentration is calculated using the Lambert-Beer law with an extinction coefficient $\varepsilon M = 155\ mM^{-1}\ cm^{-1}$.

Determination of chlorophyll (Chl) content

At 60 DAS, chlorophyll (Chl) content was determined on a fresh weight (FW) basis using 80% acetone extraction and a double-beam spectrophotometer in the second experiment. According to Lichtenthaler (1987), the following equations were used to compute Chl a, Chl b, and total Chl:

$$\text{Chl a } (mg\ g^{-1}\ FW) = [12.7(D_{663}) - 2.69(D_{646})] \times [V/1000 \times W]$$
$$\text{Chl b } (mg\ g^{-1}\ FW) = [22.9(D_{646}) - 4.68(D_{663})] \times [V/1000 \times W]$$
$$\text{Total Chl } (mg\ g^{-1}\ FW) = [20.2(D_{646}) + 8.02(D_{663})] \times [V/1000 \times W]$$

Where D (663, 646) = Optical density of the Chl extract at a wavelength of 663 and 646 nm, respectively. V = Final volume (mL) of the 80% acetone with Chl extract and W = Weight of fresh leaf sample in g.

Measurement of photosynthetic traits

Photosynthetic traits, including net photosynthesis (Pn), transpiration rate (Tr), stomatal conductance (Gs), and leaf temperature, were measured in young, fully expanded leaves at identical positions on 45 DAS (before flooding) and 60 DAS (after flooding) under full sunshine during the second experiment. Measurements were taken using a Li-COR-LI-6400 Portable Photosynthesis System with an integrated infrared gas analyzer (Li-COR-LI-6250).

Yield attributes

The average number of pods and seeds per plant was determined by counting five randomly selected plants in the initial experiment and ten randomly selected plants in the subsequent experiment. Seed weight was measured using a precision balance, and pod wall dry weight was recorded after drying the separated pod walls in an oven at 72 °C for 72 h. The final grain yield, adjusted to 12% moisture content, was recorded in tons per hectare based on the total harvested seed plot.

Determination of germination of seeds

Germination of seed is the most important criterion of seed quality. Fifty pure seeds from each sample were placed in a 9 cm plastic tray containing filter paper soaked with distilled water. For each replication one tray was used. The trays were kept in 20 ºC for 7 days for germination. Seedlings were counted every day up to the completion of germination on the seventh day. A seed was germinated as seed coat ruptured and plumule radicle came out up to 2 mm in length. The final germination count was made according to ISTA (ISTA 2019) Germination percentage was calculated following formula:

$$\text{Germination} = (\text{No. of seeds germinated} / \text{No. of seeds incubated for germination}) \times 100$$

The simplest method is to make preliminary germination counts at a standard time before germination is completed. The seed sample that produces the largest number of germinated seeds at the preliminary count will produce the fastest growing seedlings and the fastest stand establishment. The speed of germination of the seed sample was monitored by counting the germinated seedling at an interval of 24 h and counting until germination was completed. An index of the speed of germination was then calculated by adding the quotients of the daily counts divided by the number of days of germination. Thereafter, a germination index (GI) was computed by using the following formula to know the seed vigor (Agrawal, 2005).

$$GI = n/d$$

Where n = number of seedlings emerging on the day 'd', d = day after planting. Seed vigor index (SVI) was calculated by using the following formula:

$$SVI = \text{Seedling length (cm)} \times \text{Germination (\%)} / 100$$

The shoot length, root length, and weight of soybean seedlings were also measured. For shoot and root length, 3 seedlings were collected and measured using a 30 cm scale, while for weight, 5 seedlings of each genotype were dried in an oven for 72 h and weighed using an electrical balance. The mean values for each parameter were recorded.

Determination of seed coat leakage in seeds

The electrical conductivity (EC) of soybean seeds was evaluated to assess seed quality. Twenty seeds from each sample were weighed, immersed in 50 mL of deionized water, and incubated at 20 ºC for 24 h. The electrical conductivity of the seed leachate was measured using a conductivity meter (Model-CM-30ET), and the results were normalized by dividing by the mass of twenty seeds, expressed as $\mu Scm^{-1}g^{-1}$.

Statistical analysis

The data were analyzed by using CropStat 7.2 and R studio software to examine the significant variation of different treatments. The treatment means were compared using the DMRT test at 5% level of significance (Gomez and Gomez 1984).

Results and discussion

Experiment I

This experiment aims to evaluate and compare 12 different soybean genotypes with respect to various plant features to identify and select the ones that have desirable characteristics for developing and improving WL tolerance varieties. In this portion we will focus on the following areas: phenotypic, morphological and physiological traits, yield attributes and germination performances.

Days to maturity of soybeans

Most plants are sensitive to WL, as the diffusion rates of O_2 and CO_2 in roots and stems of plants decrease significantly during WL, and Pn and respiration are significantly inhibited. The difference of days to maturity among the genotypes was significant in different genotypes (Fig. 1).

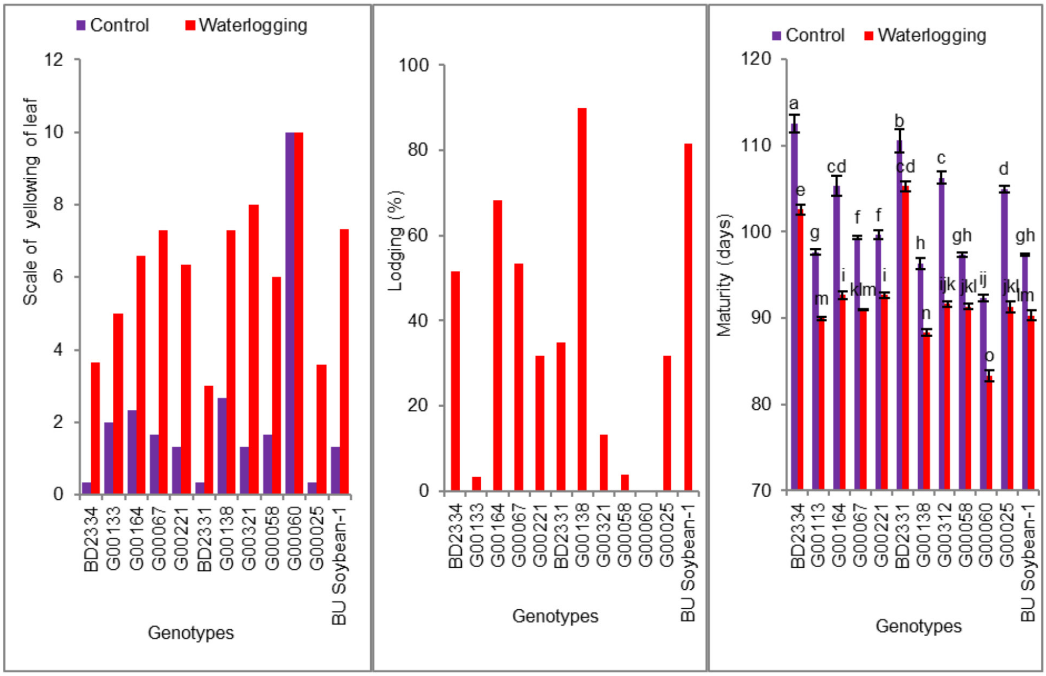

Figure 1. Leaf yellowing, lodging, and days to maturity of soybean genotypes under waterlogging stress in 2021.

WL treatment was applied at the R4 stage, which affected the maturity of the genotypes.

Surprisingly, the genotype G00060 required the minimum number of days to mature in both waterlogged and controlled plots. In the waterlogged plot, the maturity period was 83.33 days, while in the controlled plot, it was 92.33 days. Despite the expectation of delayed maturity due to WL, the opposite occurred as the plant growth stopped, and the leaves turned yellow, leading to chlorosis and senescence, which eventually resulted in early maturation. The other genotypes, including G00138, G00133, BU Soybean-1, G00067, G00058, G00321, G00025, G00164, and G00221, matured in a chronological order, with a requirement of 7 to 9 days more for maturity in the controlled plot compared to the waterlogged plot. However, the genotypes BD2331 and BD2334 took the longest time to mature, with 105.3 and 102.6 days in the waterlogged plot, respectively, compared to 110.6 and 112.6 days in the control plot.

Wu and Yang (2016) reported that, under prolonged WL condition, the enzyme activities related to Pn were inhibited; the Chl synthesis ability of leaves decreased, leading to leaf senescence, yellowing, and peeling; the formation of new leaves was blocked, and then the photosynthetic rate decreased, finally leading to early maturity of the plants.

Effect of waterlogging on yellowing and greenness of leaves

Yellowing of leaves (chlorosis) can occur due to WL for several reasons. When soil is waterlogged, oxygen supply to the roots is reduced, which affects the plant's ability to take up and transport nutrients, particularly nitrogen. Nitrogen is a vital nutrient for plant growth and is required to produce Chl, which gives leaves their green color. Without sufficient nitrogen, the plant cannot produce enough Chl, and the leaves start to turn yellow. Additionally, WL can lead to the accumulation of toxins and salts in the soil, which can also impact nutrient uptake and contribute to chlorosis. As the leaves turn yellow, they may also begin to wilt and die, further affecting the plant's overall health and growth.

The severity and duration of WL can affect the extent of yellowing, with prolonged WL leading to more severe symptoms. Leaf greenness and yellowing are interrelated and assessed through visual estimation of the extent of yellowing in the top-most fully expanded leaf and adult-basal leaves. The inverse relationship between leaf greenness and yellowing indicates that higher greenness levels correspond to lower yellowing levels. This parameter is useful in evaluating the impact of WL on leaf greenness, nitrogen remobilization, and senescence across different genotypes. Under 7 days of waterlogged conditions at R4 stage, the genotype with the highest greenness and the lowest leaf yellowing occurred was G00025 (3%), followed by BD2334 (3.66%) and BD2331 (3.60%). On the other hand, the genotype with the lowest greenness or the highest amount of yellowing was G00060 (9%), fol-

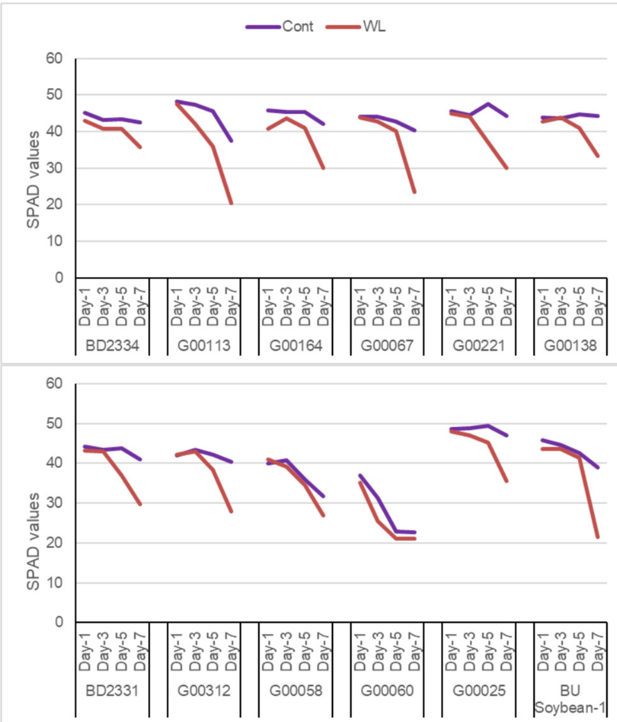

Figure 2. Effect of waterlogging on SPAD (Soil Plant Analysis Development) values of soybean genotypes in 2021.

lowed by G00321 (8%). Though rice plants can tolerate WL but soybean (Mamun et al. 2015, Mamun et al. 2018).

The greenness of adult leaves was significantly lower in waterlogged plants while yellowing of leaf is significantly higher leading to a detrimental carbon fixation performance at the plant level and contributing to such a poorer growth (Peng et al. 2007; Zeng et al. 2017).

Effect of waterlogging on lodging susceptibility of soybean genotypes

The WL can have significant negative effects on soybean crops, including increased susceptibility to lodging. Lodging refers to the bending or breaking of the plant stems, and it is caused by a variety of factors, including weather events like heavy rain or wind. When soybean plants are subjected to WL, their roots become starved of oxygen, which can lead to reduced growth and vigor. This can result in weaker stems that are more susceptible to lodging.

Additionally, waterlogged soils become compacted, which further reduces soil aeration and can limit root development. The WL can also increase disease pressure on soybean plants, particularly from fungal pathogens that thrive in wet conditions. These diseases can weaken plant stems and increase the likelihood of lodging.

In addition to lodging susceptibility, WL also has other negative effects on soybean crops, including reduced nutrient uptake, decreased Pn, and increased plant stress. The WL has a negative impact on the standing nature of soybean genotypes, with lodging being observed only in waterlogged genotypes. Each bar represents a different soybean genotype, with the height of the bar indicating the percentage of lodging observed for that genotype under waterlogged conditions based on the scale from IRRI (2002).

The extent of lodging varied among the different

Table 1. Effect of waterlogging on pod and seed grain production of soybean genotypes (2021)

Genotype	Pod production (no. plant^{-1})		Grain production (no. plant^{-1})	
	Control	WL	Control	WL
BD2334	25.0 def	21.8 ef (87.2)	55.3 e-h	44.3 ghi (80.1)
G00113	23.2 ef	22.0 ef (94.8)	44.8 ghi	38.9 hi (86.8)
G00164	42.2 ab	29.7 cde (70.4)	96.3 a	62.4 def (64.8)
G00067	26.0 def	23.4 ef (90)	54.1 fgh	44.4 ghi (82.1)
G00221	37.7 bc	31.8 cde (84.4)	69.1 b-f	58.8 d-g (85.1)
BD2331	48.1 a	33.5 bcd (69.7)	73.4 bcd	56.1d-h (76.4)
G00138	36.3 bc	33.0 bcd (90.9)	81.1 abc	70.8 b-f (87.3)
G00321	33.3 bcd	34.4 bcd (103.3)	70.2 b-f	70.4 b-f (100.3)
G00058	17.7 f	16.1 f (90.9)	34.5 i	33.2 i (96.2)
G00060	30.4 cde	31.9 cde (104.9)	54.5 fgh	62.4 def (114.5)
G00025	28.6 cde	23.7 ef (82.9)	64.1 c-f	53.2 fgh (83.0)
BU Soybean-1	42.6 ab	35.9 bcd (118.6)	87.4 ab	85.4 ab (117.8)
CV (%).	19.6		17.2	

WL = waterlogging; CV = coefficient of variation. Values in parentheses indicate percent relative to the control.. Means followed by different letters in the same column are significantly different at the 0.05 level according to the LSD test.

genotypes, with G00138 showing the highest lodging percentage of 90%, followed by BU Soybean-1 with 81%, and G00164 with 68.33%. On the other hand, the genotypes G00113 and G00058 showed the lowest lodging percentages, with 3.33% and 4%, respectively (Fig. 1). The genotype G00060 didn't show any lodging under these 7 days of WL condition.

Sun et al. (2022) also found that lodging may occur if the maximum bending moment at the base exceeds soil strength, which is the function of soil parameters and soil moisture contents and affects the yield of soybean.

Effect of waterlogging on SPAD (Soil-Plant-Analysis Development) value:

The soybean plants exhibited a sensitive and immediate response to WL stress, with a noticeable change in leaf color from green to yellow within 24 hours of exposure. This change in color was strongly linked to Chl content, which was assessed using SPAD measurements taken four times to track changes in Chl levels.

The findings showed that the Chl content in soybean leaves decreased significantly under WL, leading to the observed leaf color variation. The SPAD values generally decreased throughout the measurement period, particularly during the 7-day WL treatment. On the 82nd day after sowing, the rate of decreasing was higher.

The genotypes BD2334 and G00025 had the highest SPAD value (35.66), while G00060 had the lowest (2.11), with the latter being less affected by the stress due to early maturation. The trend of low SPAD values in adult and young leaves during the stress was evident in WL plants (Fig. 2).

Rocío et al. (2018) also observed a progressive decline in SPAD values of adult leaves was observed one week after WL was applied and joined by decreases in SPAD values of young leaves at the end of the stress It was also in agreement with previous statements that leaf Chl contents decreased under WL stress (Manzur et al. 2009).

Effect of waterlogging on pod and seed grain production

The WL led to a decrease in Pn and respiration rates, which caused plant stress and damage to reproductive structures. The reduced availability of oxygen to plant roots may have also limited nutrient uptake, resulting in lower pod and seed grain production. The WL had a notable impact on the number of pods per plant in different soybean genotypes, with varying degrees of reduction observed (Table 1). All tested genotypes experienced a significant decline in pod numbers, likely attributable to the shedding of flowers and developing pods caused by WL. The extent of reduction ranged from 2% in G00321 to 30% in G00164 after 7 days of WL. In the case of other genotypes 18% in G00025 and BU Soybean-1; 15% in G00221, 12% in BD2334 and G00058, G00138, G00113 and G0060 these genotypes range from 4-9% reduction of pod formation. A similar outcome was reported by Jin-Woong et al. (2006) in their study on soybean. The WL had a relatively moderate impact on the number of grains per pod in all the tested soybean genotypes, but the reduction was still noteworthy. Every genotype experienced a decline in the number of seed grains per plant, with the lowest reduction recorded in G00058 (3%) and the highest in G00164 (35%) after 7 days of WL. The highest seed per plant has been seen in G00164 (96.3) in controlled condition and BU Soybean-1 (85.4) in waterlogged condition (Table 1). The reduction rate was 23% in BD2331 which was followed by BD2334 at 19%. In G00025, G00067 the reduction rate was 17%. Other genotypes ranged from 15% to 12%. Although there were no instances where no reduction was recorded, the extent of reduction varied between genotypes. WL induced reduction in seeds per pod was higher at later stage WL.

Effect of waterlogging on 100-seed weight and grain yield

The WL has a significant negative impact on yield components, with the highest yield losses occurring at reproductive stages. The WL during early reproductive stages (R1-R3) can result in the highest yield losses, with reductions of 55-60%. Even short durations of WL can result in significant yield reductions. WL at later reproductive stages (R4-R7) results in less yield reduction (35-50%) due to the number of pods and grains already being established (Rhine et al. 2010).

The 100 seed weight of soybean genotypes ranged from 4.17 to 17.2 g and 3.4 to 9.1 g under control and 7 days of WL conditions, respectively. The WL caused a reduction in 100 seed weight, which was likely due to the inhibition of photo assimilate translocation, leading to poor development of the seed and a poor source-sink relationship. Among the genotypes tested, BD2334, G00164 and BD2331 had the highest 100 seed weight under both control and WL conditions. In contrast, BU Soybean-1 and G00321 showed the highest reduction in 100 seed weight compared to their corresponding controls after 7 days of WL. Notably, G00060 showed the lowest reduction in 100 seed-weight under WL conditions compared to the other genotypes.

The finding that WL can cause a reduction in 100 seed weight in soybean is consistent with a previous study by Hossain et al. (2019).

The WL has a significant negative impact on the grain yield plant^{-1} in soybeans, and this effect becomes more pronounced with longer durations of WL. The grain yield

Table 2. Effect of waterlogging on 100-seed weight and grain yield of soybean (2021)

Genotype	100-seed weight (g)		Grain yield (g plant^{-1})	
	Control	WL	Control	WL
BD2334	17.2 a	9.1 d-g (52.9)	17.4 a	9.20 de (52.8)
G00113	6.8 g-j	4.9 ik (72.1)	6.9 efg	5.08 fgh (73.6)
G00164	17.3 a	8.2 e-h (47.4)	17.5 a	8.20 def (46.9)
G00067	6.8 g-j	4.2 jk (61.8)	6.9 efg	4.30 gh (62.3)
G00221	13.2 bc	6.9 g-j (45.4)	13.5 b	7.00 efg (51.9)
BD2331	14.1 b	7.6 f-i (53.9)	14.1 b	7.80 d-g (55.3)
G00138	11.1 b-e	7.5 f-i (67.6)	11.3 bc	7.60 d-g (67.3)
G00312	11.6 bcd	7.2 f-j (62.1)	11.8 bc	7.40 d-g (62.7)
G00058	4.71 ik	3.4 k (72.2)	4.7 gh	3.60 h (76.6)
G00060	5.8 h-k	6.9 g-j (118.9)	5.9 fgh	7.00 efg (118.6)
G00025	10.2 cef	5.6 h-k (54.9)	10.4 bcd	5.80 fgh (55.8)
BU Soybean-1	8.6 d-h	6.1 g-k (70.9)	8.7 def	6.20 e-h (71.3)
CV (%).	20.8		22.01	

WL = waterlogging; CV = coefficient of variation. Values in parentheses indicate percent relative to the control. Means followed by different letters in the same column are significantly different at the 0.05 level according to the LSD test.

plant^{-1} was found to range from 5.9 to 17.4 g plant^{-1} under normal conditions, and from 3.6 to 9.2 g plant^{-1} under 7 days of WL. This reduction in seed yield plant^{-1} under WL is likely due to a decrease in the number of pods per plant, number of seeds per pod, and pod setting. Among the soybean genotypes, G00060 was found to exhibit the lowest reduction in grain yield plant^{-1} under WL, followed by G00113, G00058, and BU Soybean-1. On the other hand, G00164 was found to have the highest reduction in grain yield plant^{-1} under 7 days of WL condition (Table 2).

These findings are consistent with a previous study by Rhine et al. (2010), which also reported a decrease in seed yield in soybean under WL stress.

Germination performances of soybean

The WL is known to have a negative effect on the germination of soybean seeds. When seeds are exposed to WL, the excess water can displace air in the soil, reducing the availability of oxygen that is necessary for the seeds to respire and metabolize stored nutrients. This can lead

Table 3. Effect of waterlogging on germination, and germination index of soybean genotypes (2021)

Genotype	Germination (%)		Germination index	
	Control	WL	Control	WL
BD2334	67.33 de	46.00 fg (68.3)	18.65 de	11.03 fg (59.1)
G00113	80.67 a-d	69.33 cde (85.9)	23.26 a-d	18.58 def (79.8)
G00164	92.67 ab	97.33 a (105.0)	29.11 a	23.80 a-d (81.7)
G00067	92.33 ab	98.00 a (106.1)	26.87 abc	26.47 abc (98.5)
G00221	95.00 ab	88.67 ab (93.3)	22.29 a-e	23.50 a-d (105.4)
BD2331	68.67 cde	80.67 a-d (117.5)	23.90 a-d	21.24 b-e (88.8)
G00138	82.00 a-d	91.33 ab (111.4)	21.24 b-e	28.32 ab (133.3)
G00321	65.33 def	93.00 ab (142.4)	20.29 cde	28.63 a (141.1)
G00058	75.66 b-e	92.67 ab (122.5)	20.73 b-e	29.07 a (140.2)
G00060	94.67 ab	88.00 abc (92.9)	29.12 a	22.41 a-e (76.9)
G00025	97.33 a	28.67 g (29.5)	29.88 a	07.93 g (26.5)
BU Soybean-1	60.00 ef	32.67 g (54.5)	14.96 efg	09.58 g (64.0)
CV (%).	15.2		20.9	

WL = waterlogging; CV = coefficient of variation. Values in parentheses indicate percent relative to the control. Means followed by different letters in the same column are significantly different at the 0.05 level according to the LSD test.

Table 4. Effect of waterlogging on seed vigor index and electrical conductivity (2021)

Genotype	Seed vigor index		EC (µS cm^{-1} g^{-1})	
	Control	WL	Control	WL
BD2334	3.73 g-j	1.10 j (29.5)	82b c	94 ab (114.6)
G00113	8.43 a-d	4.36 f-i (51.7)	70.2 cef	73.2 cde (104.2)
G00164	7.89 cde	6.25 c-g (79.2)	62.3 d-g	64.4 d-g (103.3)
G00067	9.00 abc	8.89 abc (98.7)	92.2 ab	93.6 ab (101.5)
G00221	8.52 a-d	7.19 c-f (84.9)	63.5 d-g	69.4 cef (109.3)
BD2331	6.16 c-g	4.77 e-h (77.4)	62.8 d-g	65.3 d-g (103.9)
G00138	5.05 e-h	6.34 c-g (125.5)	52.5 g	57.1 fg (108.7)
G00321	5.41 d-g	6.99 c-f (129.2)	61.1 efg	63.2 d-g (103.4)
G00058	5.95 c-g	7.99 a-e (134.3)	76.6 cd	80.5 bc (105.4)
G00060	11.50 a	11.23 ab (97.6)	94.5 ab	98.1 a (103.8)
G00025	7.58 c-f	2.31 hij (30.4)	71.2 cef	73.4 ce (103.1)
BU Soybean-1	4.58 fgh	1.37 ij (29.9)	82.1 bc	92.7 ab (112.9)
CV (%).	31.00		27.60	

WL = waterlogging; EC = electrical conductivity; CV = coefficient of variation. Values in parentheses indicate percent relative to the control. Means followed by different letters in the same column are significantly different at the 0.05 level according to the LSD test.

to a buildup of toxic metabolites and a reduction in seed vigor and viability. Several factors can influence the degree of negative impact of WL on soybean germination, including the duration and severity of WL, as well as the genetic makeup and physiological characteristics of the seed. In general, soybean seeds that have a high tolerance to WL are better able to withstand the effects of excess water and maintain germination rates. The highest germination had been shown in the genotype G00025 where BD2334 showed lower germination rate in the case of controlled seed but in case of WL treated seed germination rate is higher. The genotype showed Highest rate of germination is G00067 (98%) which is followed by G00164 (97%) (Table 3).

Germination index (GI) is a measure of the uniformity and speed of seed germination. A lower GI value indicates slower and less uniform germination, while a higher GI indicates faster and more uniform germination. WL can have a negative impact on the GI of soybean seeds and increase the number of abnormal or deformed seedlings. The impact of WL on GI can vary depending on the genotype of soybean seeds.

The soybean genotypes G00025, G00060, and G00164 had a higher range of GI values in the controlled condition (without WL) compared to other genotypes. However, in waterlogged conditions, the soybean genotype G00058 had the highest GI value (29.07), while G00025 had a lower GI value. This indicates that the impact of WL on GI varies significantly depending on the genotype of the soybean seeds being grown. In other words, the negative impact of WL on soybean seed germination is not uniform across all genotypes, and some genotypes may be more resistant to the effects of WL than others. There are several reports that account for the negative correlation between germination percentage and flooding stress (Maryam and Nasreen 2012).

Effect of waterlogging on seed vigor index and electrical conductivity

Seed vigor index (SVI) is a measure of seed quality that considers both seed germination and seedling growth. WL has a negative impact on the SVI of soybean seeds. WL significantly decreased the SVI of soybean seeds compared to the control condition (without WL). This decrease in SVI is likely due to a reduction in seed germination and seedling growth caused by the lack of oxygen and excess water in the soil during WL.

The finding showed a significant effect on seed vigor index of soybean. The highest seed vigor index (11.50) was found in case of G00060 under controlled condition and the lowest one (3.73) in BD2334. In case of WL condition, the same result was observed and SVI decreased significantly (Table 4).

Wuebker et al. (2001) reported that when seeds were flooded 3days after the start of imbibition, a significant drop in germination percentage occurred and seed injury was observed. The seeds had the lowest germination percentage and the highest electric conductivity with WL for the longest period. Some reports also indicated that a negative correlation was observed between germination percentage and electric conductivity in soybean (Yaklich and Abdul-Baki, 1975).

Genotype G00060 gave the highest amount of EC under WL condition. The second highest EC (12 was

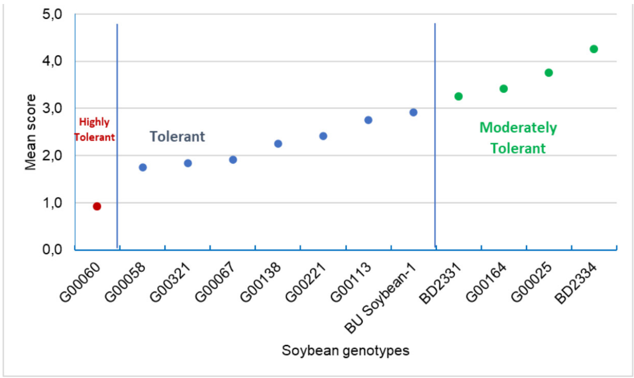

Figure 3. Waterlogging tolerance ranking of 12 soybean genotypes based on mean stress tolerance index in 2021.

obtained from BD2334. The lowest value of EC was found in G00321 in both WL and controlled conditions. WL has a negative correlation with seed germination. There are significant differences in the case of EC between controlled and WL condition. As this result Yaklich and Abdul-Baki, (1975) reported that WL can increase the likelihood of seed damage, and this can result in increased electrical conductivity.

Ranking of WL tolerance

Based on the morpho-physiological changes due to WL, soybean genotypes were classified into five groups viz. highly tolerant (HT) in score ≤ 1, tolerant (T) in 1 < Score ≤ 3, moderately tolerant (MT) in 3 < Score ≤ 5, susceptible (S) in 5 < Score ≤ 7 and very susceptible (VS) in Score > 7 following the IRRI standard evaluation system (SES). Based on mean stress tolerant index (STI) score, this scoring has been done.

Seven days of WL revealed 1 genotype to be highly tolerant (HT) (score ≤ 1), 7 genotypes were tolerant (T) (1 < Score ≤ 3) and 4 genotypes were moderately tolerant (MT) (3 < Score ≤ 5) subjected to WL stress. No genotypes were found susceptible (S) (5 < Score ≤ 7) and very susceptible (VS) (Score > 7) (Table 5).

According to the mean STI score, G00060 was scored in ≤ 1, as highly tolerant the soybean genotype which could completely retain its growth in WL condition. On the other hand, G00113, G00067, G00221, G00138, G00321, G00058, BU Soybean-1 were scored in 1 < Score ≤ 3 as tolerant (T) and G00025, G00164, BD2331, BD2334 were scored in 3 < Score ≤ 5 as moderately tolerant (MT) genotypes subjected to seven days of WL (Fig. 3). Similar classifications were made by (Wu et al. 2017) based on the standard of flooding evaluation.

On the basis morpho-physiological changes due to WL in soybean genotypes showed that among the 12 genotypes evaluated, 1 genotype was highly tolerant (HT), 7 genotypes were tolerant (T), and 4 genotypes were moderately tolerant (MT) to WL stress. No genotypes were found to be susceptible (S) or very susceptible (VS) to WL. G00060 have a lower mean score and lower mean score indicates highly tolerance. The mean score increased chronologically G0058, G00321, G00067, G00138, G00221, G00113 and Bu Soybean-1. The mean score of this genotype ranges in 1-3. This indicates they are WL tolerant. The genotypes BD2331, G00164, G00025, Bd2334 have the score ranges from 3-5 which indicates these genotypes are moderately tolerant.

Analysis of the treatment-traits and genotypes relationship

This graph represents the correlation between various traits and genotypes of soybean in relation to their WL tolerance index. The size, shape, and depth of shading of the ellipses indicate the direction and closeness of Pearson's coefficient of correlation between the different parameters. White boxes represent non-significant associations. The parameters included in the analysis are seed vigor index (SVI), germination (Gn), germination index (GI), soil plant analysis development (SPAD), hundred seed weight (HSW), grain yield (GY), root length (RL), shoot length (SL), plant height (PH), pod per plant (PPP), and seed per plant (SPP).

The correlation coefficient is a measure of the strength and direction of the linear relationship between two variables. A correlation of 1 indicates a perfect positive linear relationship, meaning that as one variable increases, the other variable also increases. On the other hand,

Table 5. Comparative waterlogging tolerance ranking of 12 soybean genotypes according to mean ranking score (2021)

Mean Score	Description	Genotypes
Score ≤ 1	Highly Tolerant (HT)	G00060
1 < Score ≤ 3	Tolerant (T)	G00113, G00067, G00221, G00138, G00321, G00058, BU Soybean-1
3 < Score ≤ 5	Moderately Tolerant (MT)	G00025, G00164, BD2331, BD2334
5 < Score ≤ 7	Susceptible (S)	
Score > 7	Very Susceptible (VS)	

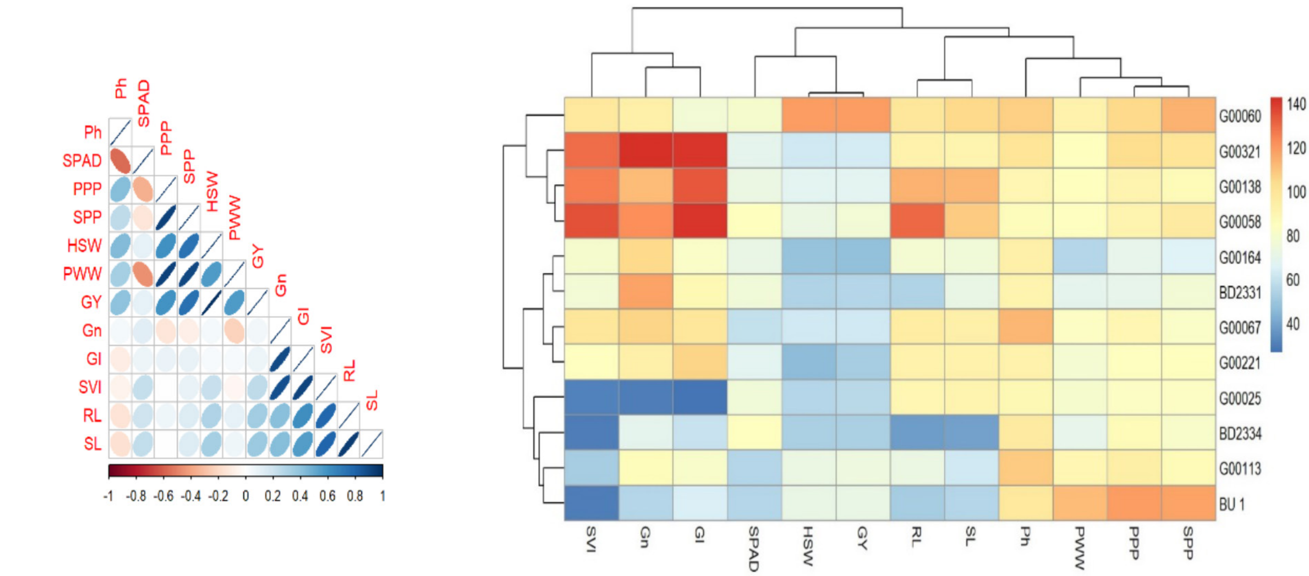

Figure 4. Trait associations of soybean under waterlogging based on Pearson's correlation coefficients in 2021. The direction, size, and shading of ellipses indicate the strength and nature of associations; white boxes denote non-significant correlations. Abbreviations: SVI = seed vigor index, Gn = germination, GI = germination index, SPAD = soil plant analysis development, HSW = 100-seed weight, GY = grain yield, RL = root length, SL = shoot length, PH = plant height, PPP = pods per plant, SPP = seeds per plant.

a correlation of -1 indicates a perfect negative linear relationship, meaning that as one variable increases, the other decreases. The size and depth of shading of the ellipses in the graph represent the magnitude of the correlation coefficient, with larger and darker ellipses indicating stronger correlations. The non-significant associations represented by white boxes indicate that there is no significant relationship between the two variables. Here Pearson's coefficient of correlation represents, SPAD value is negative and weakly correlated with plant height; where, PPP negative and weakly correlated with SPAD but positive and comparatively strongly related with plant height. The results show that plant height has a positive correlation with seed per plant, pod per plant, hundred seed weight, pod wall weight, and grain yield. On the other hand, SPAD values are negatively correlated with plant height and weakly correlated with pod per plant. HSW has a strong positive correlation with PPP and SPP and also a positive correlation with pod wall weight and grain yield. GY has a positive correlation with pod per plant, SPAD, and pod wall weight, but has a stronger correlation with HSW. GI has a strong correlation with Gn and SVI has a strong correlation with Gn and GI. Root length and shoot length have a positive and strong correlation with each other. Maranna et al. (2021) revealed that soybean grain yield per plant had significant positive correlation with 100 seed weight, biomass, pods per plant, branches per plant and days to flowering.

Hierarchical clustering is a method of grouping data points based on their similarity or dissimilarity. In this context, it can be used to identify similarities and differences among the 12 soybean genotypes under the WL condition based on the 13 parameters measured.

The hierarchical clustering heatmap presents the relationship between the WL tolerance index of various soybean genotypes and their corresponding traits. The heatmap shows that there are three distinct clusters at the trait level for all studied genotypes. The color scale indicates the strength of the normalized mean values of different traits, with different colors indicating different levels of expression.

The heatmap illustrates the relationships among soybean genotypes and their corresponding traits. The variables are grouped into five distinct clusters, labeled A, B, C, D, and E. Cluster A includes seed vigor index (SVI), germination (Gn), and germination index (GI). Cluster B contains soil plant analysis development (SPAD), hundred seed weight (HSW), and grain yield (GY). Cluster C includes root length (RL) and shoot length (SL). Cluster D contains plant height (PH), and Cluster E includes pod per plant (PPP), seed per plant (SPP), and pod wall weight (PWW).

When examining the soybean genotypes, three distinct clusters are formed. The genotypes in Cluster A, including G00060, G00321, G00138, and G00058, show significant interaction on GI, Gn, and SVI (Figure 4). These same genotypes show significant interaction with all parameters except HSW and GY. G00060 also exhibits

Table 6. List of clusters of 12 soybean genotypes classified on plant characters (2021)

Cluster	No of genotypes	Genotype
I	04	BD2334, G00113, G00025, BU Soybean-1
II	04	G00164, G00067, G00221, BD2331
III	03	G00138, G00321, G00058
IV	1	G00060

significant interaction with HSW and GY, while the other three genotypes show non-significant interaction.

The genotypes in Cluster B, including G00164, BD2331, G0067, and G00221, have significant interaction with Gn, GI, and SVI. However, these same genotypes exhibit weaker interaction in terms of GY, HSW, and SPAD. G00164 and BD2331 also exhibit weaker interaction in terms of RL, SL, PWW, PPP, and SPP. All genotypes exhibit significant interaction with PH.

The genotypes in Cluster C, including G00025, BD2334, G00113, and BU Soybean-1, have significant interaction with SPP, PPP, and PWW. BD2334 exhibits weaker interaction in comparison to the other genotypes. BD2334, G00113, and BU Soybean-1 show weaker interaction with RL, SL, GY, and HSW. G00025 exhibits strong interaction with RL and SL. BD2334 has strong positive interaction with SPAD, while G00113 has this interaction with GI and Gn. The remaining genotypes exhibit negative, weaker interaction.

Biplot analysis is frequently employed to determine which factors have the greatest influence on genotypic variation. A visual comparison tool for genotypes based on several traits is also included (Al-Naggar et al. 2020). A biplot represents variables in a principal component analysis as vectors overlaid on a plot, with the relative length of 52 the vectors signifying the relative level of variability in each variable shown on the biplot.

In a biplot, each variable is represented as a vector, and the length and direction of the vector reflect the relative importance of that variable in explaining the variation in the data. The position of each observation in the biplot is determined by its scores on the principal components that capture the most variation in the data. The angle between two vectors in the biplot indicates the degree of correlation between the two variables. If the angle is small, the two variables are positively correlated, meaning that they tend to vary together. If the angle is large, the two variables are negatively correlated, meaning that they tend to vary in opposite directions. If the angle is 90 degrees, there is no correlation between the two variables.

Based on the biplot analysis, it appears that certain genotypes are more strongly correlated with certain traits than others. For example, genotypes G00138, G00058, and G00321 are strongly positively correlated with germination (Gn), germination index (GI), seed vigor index (SVI), and root length (RL). This is indicated by the acute angle

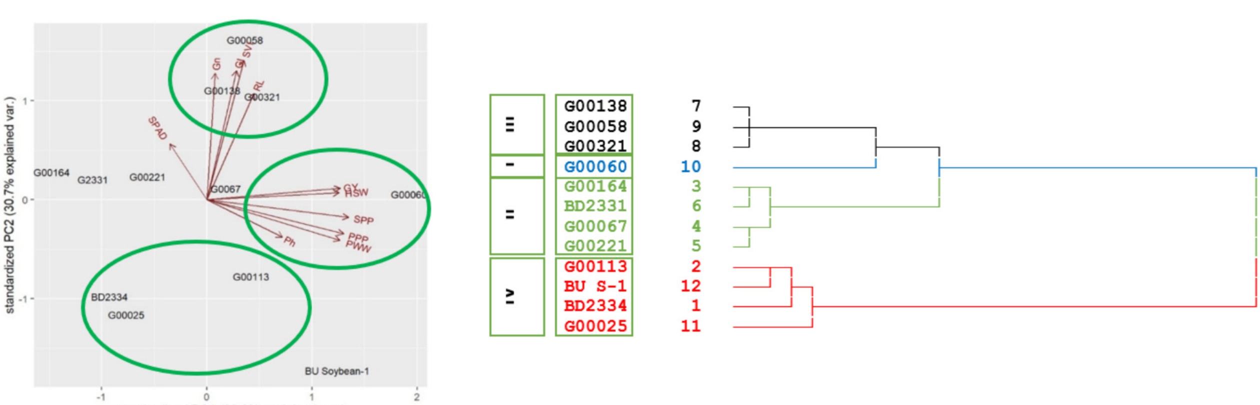

Figure 5. PCA biplot and hierarchical clustering (dendrogram) of 12 soybean genotypes based on growth and yield-related traits under 7-day waterlogging in 2021. Trait vectors indicate their contribution to principal components (PCs); angles reflect trait correlations. Abbreviations: SVI = seed vigor index, Gn = germination (%), GI = germination index, SPAD = soil plant analysis development, HSW = 100-seed weight (g), GY = grain yield (g), RL = root length (cm), SL = shoot length (cm), PH = plant height (cm), PPP = pods per plant, SPP = seeds per plant, PWW = pod wall weight (g).

formed between the vectors for these genotypes and the vectors for these traits in the biplot (Figure 5).

On the other hand, the genotype G00060 shows a weak positive correlation with 100-seed weight (HSW), as indicated by the obtuse angle formed between the vectors for this genotype and HSW. Similarly, G00060 shows weak negative correlations with seed per plant (SPP), pod per plant (PPP), pod wall weight (PWW), and plant height (PH), as indicated by the obtuse angles formed between the vectors for G00060 and these traits. In addition, the genotypes G00164, BD2331, and G00221 appear to have negative interactions, as indicated by their positions in the biplot. This suggests that these genotypes may not be well-suited for stress environments. Finally, the genotypes G00113, BD2334, G00025, and BU Soybean-1 show negative interactions with SPAD value, which is an indicator of plant health and vigor. This suggests that these genotypes may be less resistant to environmental stresses or disease.

Based on multivariate analysis, four significant groupings among 12 soybean genotypes were found. There was 1 genotype in Cluster-I, which was separated into one sub-cluster. Cluster-II, which had two sub clusters included four genotypes, whereas Cluster-III, which had two sub clusters and three genotypes. Three sub-clusters of Cluster-IV included four genotypes.

Grouping of genotypes through cluster analysis

To categorize the genotypes and find the desirable genotypes and plant features, we employed multivariate analysis. Based on the variables that were assessed, a statistically homogeneous grouping was required in this situation.) (Table 6). Maranna et al. (2021) showed in a cluster analysis and grouped the 75 genotypes into five clusters, with cluster V showing the highest yield and desirable means for several characters, indicating its potential contribution to genetic diversity.

The overall effect of waterlogging on soybean genotypes

WL is a type of abiotic stress that can negatively impact soybean growth and yield. The Effect of waterlogging on soybean genotypes vary depending on the cultivar, as some genotypes may be more tolerant to WL stress than others. Studies have shown that WL can cause changes in gene expression, root morphology, and hormonal regulation in soybean plants, which can ultimately lead to reduced growth and yield.

WL causes plant lodging in several ways. The reduction of oxygen availability in the soil due to the saturation of water, leads to root damage or death, and a reduction in the ability of the plant to anchor itself in the soil. As a result, the plants become more susceptible to bending or breaking during strong winds or heavy rainfall. This leads to limiting the plant's ability to take up water and nutrients and causes a buildup of toxic metabolites such as ethanol and acetaldehyde. In turn, there is a reduction in Chl content and photosynthetic activity, as well as an accumulation of oxidative stresses that damage the plant's cell membranes and other structures. Which affects the balance of plant hormones, including ethylene, which promotes leaf senescence and abscission. This leads to a premature shedding of leaves and a reduction in the plant's overall ability to carry out Pn. One of the potential effects of WL on soybeans is early maturity. This is thought to occur because of changes in plant hormone levels, particularly ethylene, which promotes flower and pod abortion. In addition, WL reduces Pn and nutrient uptake, which can limit plant growth and development. As a result, soybean plants mature earlier to complete their life cycle before the stress becomes too severe.

Overall, the effects of WL on soybean genotypes and early maturity are complex and depend on many factors including the duration and severity of the stress, the specific genotype, and environmental conditions. However, WL has a significant impact on soybean growth and yield, and understanding the underlying mechanisms aid in the development of more stress-tolerant soybean cultivars.

Experiment II

Out of the 12 genotypes tested, G00060 exhibited high tolerance to WL stress, along with being the earliest-maturing and shortest-duration genotype. While genotypes BD2334, G00164, and BD2331 displayed high grain yield under both controlled and waterlogged conditions, they were only moderately tolerant to WL stress according to the correlation-based ranking. These genotypes were further selected to study the physiological changes contributing to their superior performance. Additionally, for comparison, a highly tolerant genotype (G00060) and a tolerant genotype (BU Soybean-1) were chosen, considering their characteristics and the limitations of other genotypes.

Effect of waterlogging on photosynthetic pigments

WL has a significant impact on the photosynthetic pigments Chl a and Chl b in soybean plants. WL refers to a condition where the soil is saturated with water, which reduces the availability of oxygen to plant roots. This lack of oxygen causes stress to the plant, leading to a reduction in photosynthetic activity and a decrease in the production of photosynthetic pigments. Several studies have shown that WL causes a decrease in the concentration of Chl a and Chl b in soybean plants (Fig. 6).

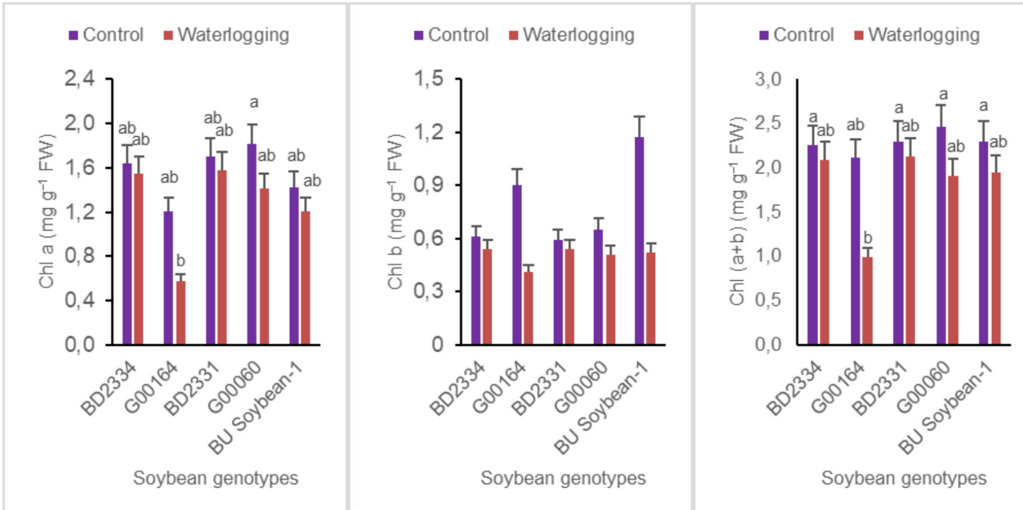

Figure 6. Effect of waterlogging on photosynthetic pigment contents (chlorophyll a, b, and total chlorophyll) of five soybean genotypes in 2022. Means followed by different letters in the same column are significantly different according to the LSD test at the 0.05 level. Values in parentheses represent percentages relative to the control.

Here a slight visible yellowing of leaves was noticed during WL indicating Chl degradation under 5 days of WL condition. Under control condition, the higher Chl a content had been seen in genotype G00060 and after 5 days of WL the amount decreased significantly. G00164 also showed significant differences. Chl b content also decreased due to WL but there were no significant changes seen. In case of total Chl content all the genotype showed significant changes. Total Chl decreased due to WL. Higher amount of total Chl had been seen in G00060, followed by BD2331 and BU Soybean-1. That's mean after 5 days of soil WL, the decrease in the levels of Chl a and Chl b are negatively correlated. Similar results have been recently observed for several genotypes of soybean plants challenged by soil WL (Da-Silva and do Amarante, 2020; Garcia et al. 2020).

The decrease in Chl concentration was attributed to a reduction in the activity of the enzymes involved in Chl biosynthesis, as well as an increase in the breakdown of Chl molecules. Additionally, WL can cause damage to the chloroplast structure, which can also contribute to the decrease in Chl concentration (Table 7).

Effect of waterlogging on Photosynthesis (Pn)

WL have a significant effect on the Pn of soybean plants. When soil becomes waterlogged, the amount of oxygen available to the roots is reduced, which results in plant stress and damage. One of the primary impacts of WL on Pn is that it reduces the efficiency of the plant's chloroplasts. This is because chloroplasts require a constant supply of carbon dioxide and oxygen to function properly, and WL disrupts this supply. This disruption leads to a decrease in the rate of Pn and ultimately reduces the plant's overall growth and yield. Overall, the negative impacts of WL on soybean Pn were significant and ultimately reduce the crop yield. WL was imposed on the 46th day after sowing, which was the early reproductive stage, to investigate the Effect of waterlogging stress on the Pn rate of different soybean genotypes. Before the imposition of WL stress, no significant difference in Pn rate was observed between the controlled and waterlogged conditions (Table 7). However, after WL, a significant difference was found in the Pn rate of the different genotypes. The genotype BD2334 had the highest Pn rate in both waterlogged and controlled conditions, suggesting its potential as a WL-tolerant genotype. Furthermore, the reduction rate of Pn varied among the different genotypes under WL stress. The genotype with the lowest reduction rate was BD2334 (13%), followed by BD2331 (10%) and BU Soybean-1 (16%). In contrast, the genotypes with the highest reduction rates were G00060 (44%) and G00164 (35%). This indicates that some genotypes are more tolerant to WL stress than others. According to Polischuk et al. (2022), it was determined that WL has a significant impact on Pn in C3 species. Pn was reduced by an average of 41% (with a range of 30-55%) due to WL.

Effect of waterlogging on stomatal conductance (Gs)

Gs refers to the ability of the stomata, which are small pores on the surface of leaves, to open and close in response to various environmental factors. When plants are waterlogged, the soil becomes saturated with water and

Table 7. Effect of waterlogging on photosynthesis of soybean genotypes (2022)

Soybean genotypes	Pn		Gs		Tr	
	Control	WL	Control	WL	Control	WL
BD2334	20.40 a	17.60 abc (86.3)	0.56 a	0.14 c (25)	6.46 a	2.41 c (37.3)
G00164	17.53 abc	11.36 d (64.8)	0.50 ab	0.30 abc (60)	5.75 ab	3.92 bc (68.2)
BD2331	13.71 cd	12.32 d (89.9)	0.55 a	0.25 bc (45.5)	6.18 a	3.68 bc (59.5)
G00060	19.47 ab	10.76 d (55.3)	0.58 a	0.15 c (25.9)	5.80 ab	2.51 c (43.3)
BU Soybean-1	17.63 abc	14.80 bcd (83.9)	0.39 abc	0.35 abc (89.7)	4.51 abc	4.41 abc (97.8)
CV (%).	31.00		27.60		21.9	

WL = waterlogging; CV = coefficient of variation; Pn = net photosynthesis rate; Gs = stomatal conductance; Tr = transpiration rate. Values in parentheses indicate percent relative to the control. Means followed by different letters in the same column are significantly different at the 0.05 level according to the LSD test.

there is a lack of oxygen, which can cause several physiological changes in the plants, including changes in Gs.

Before flooding, the genotypes of control and waterlogged soybean plants did not differ significantly. Here in genotype G00164 and G00060 differences are seen.

However, after 5 days when flooding was removed, there was a significant decrease in Gs in all genotypes. The greatest reductions were observed in BD2334 (75%) and G00060 (74%), while the smallest reduction was observed in BU Soybean-1 (10%). The other genotypes, BD2331 and G00164, showed intermediate reductions of 54% and 40%, respectively.The results suggest that in response to WL stress, soybean plants may reduce Gs in order to limit water loss through Tr and conserve water. By reducing Tr, the plant can maintain a higher water potential in the leaves, which can help prevent wilting and maintain turgor pressure (Pereira et al. 2020). The genotypes with the highest rate of reduction in Gs (BD2334 and G00060) may be better adapted to reduce water loss and maintain water balance under WL stress. Lapaz et al. (2020) also observed that three days of WL resulted in a 33% reduction in Gs, compared to the control. Five days of WL led to an even greater reduction of 80% compared to the control.

Effect of waterlogging on transpiration (Tr)

WL reduce the availability of oxygen to plant roots, leading to a decrease in Pn and other metabolic processes. As a result, the rate of Tr decrease, as plants reduce their water loss to compensate for the reduced capacity to produce energy. This can ultimately lead to a decrease in plant growth and yield, particularly if the WL is prolonged. Before flooding, there was very little difference in the Tr rates of the plant varieties being studied. However, after 5 days of flooding, there was a significant decrease in the Tr rate of the plants, ranging from 2% to 62%. Specifically, the genotype BD2334 showed the highest decrease in Tr rate at 62%, followed by G00060 at 56%, BD2331 at 40%, G00164 at 31%, and BU Soybean-1 at 2%.

The decrease in Tr rate is indicative of a lower rate of water loss, with BD2334 exhibiting the highest level of water retention compared to the other plant varieties. This suggests that BD2334 is more effective at conserving water in flooded conditions compared to the other plant varieties. Conversely, BU Soybean-1 exhibited the lowest decrease in Tr rate, indicating a comparatively higher rate of water loss.

It is important to note that the observed variations in Tr rate between the different plant varieties can have significant implications for plant growth and survival in flooded conditions. Yamakawa (2006) also highlighted the variability in tolerance to WL among different genotypes, where WL during the V4-V5 or R2 stage reduced the Tr rate by 28-32% in the tolerant genotype, whereas the sensitive genotype saw a reduction of 55-61% compared to control plants.

Effect of waterlogging on Proline content and MDA

WL have a significant effect on the proline content of soybean plants. Proline is an amino acid that acts as an osmoprotectant, helping plants to maintain their cellular water balance and protect against stressors.WL induce an increase in proline content in soybean plants. All five genotypes exhibited a significant increase in proline content under WL stress compared to their respective control groups. The increase rate of proline content was 35% for BD2334, 116% for G00164, 97% for BD2331, 85% for G00060, and 41% for BU Soybean-1 (Table 8). These results indicate that proline accumulation is a common response to WL stress in soybean plants. Furthermore, we found that there were significant differences in proline accumulation between the genotypes. G00164 had the highest increase in proline content (116%), followed by BD2331 (97%) and G00060 (85%). In contrast, BD2334 had the lowest increase in proline content (35%) under WL stress. These findings suggest that G00164 may have

Table 8. Effect of waterlogging on proline and MDA content of soybean genotypes, 2022

Genotype	Proline		MDA	
	Control	WL	Control	WL
BD2334	0.71 bc	0.96 ab (135.2)	1.19 bc	1.33 abc (111.8)
G00164	0.49 cd	1.06 a (216.3)	1.70 a	1.71 a (100.6)
BD2331	0.43 d	0.85 ab (197.7)	1.19 c	1.22 abc (102.5)
G00060	0.41 d	0.76 abc (185.4)	1.41 abc	1.62 ab (114.9)
BU Soybean-1	0.36 d	0.51 cd (141.7)	1.06 c	1.28 abc (120.8)
CV (%).	26.7		21.3	

WL = waterlogging; CV = coefficient of variation; Values in parentheses indicate percent relative to the control. Means followed by different letters in the same column are significantly different at the 0.05 level according to the LSD test.

a higher protective nature in the context of WL stress compared to BD2334.

The higher increase in proline content observed in G00164, BD2331, and G00060 genotypes under WL stress may indicate that these genotypes have higher levels of stress tolerance. In contrast, the lower increase in proline content observed in BD2334 under WL stress may indicate that this cultivar is more susceptible to WL stress at early reproductive stage (R2 stage). Hasanuzzaman et al. (2022) also observed that the concentration of proline increased by 58, 80, and 108% in plants that were subjected to WL for 3, 6, and 9 days, respectively, as compared to the control group that was not subjected to WL. Which completely supports our results. Malondialdehyde (MDA) is a byproduct of lipid peroxidation that is often utilized as a marker for oxidative stress in plants. Lipid peroxidation is a common response to oxidative stress in plants, and the observed increase in MDA content in response to WL stress is consistent with these findings. The fact that there were significant differences in MDA content between the soybean genotypes indicates that some genotypes may be more vulnerable to oxidative damage under WL stress than others.Specifically, the cultivar BU Soybean-1 had the highest increase in MDA content at 20%, which suggests that it may be more susceptible to oxidative stress and membrane damage. On the other hand, G00164 had the lowest increase in MDA content at 0.5%, followed by BD2331 (2.5%), BD2334 (11%), and G00060 (14%). These findings suggest that these genotypes may have a higher tolerance to WL stress and reduced susceptibility to oxidative damage. Lapaz et al. (2020) observed in his study that, regardless of Fe levels, H_2O_2 and MDA concentrations increased by 94 and 23%, respectively, in plants in waterlogged soil. Therefore, the use of MDA content as a biomarker for oxidative stress in plants can provide valuable insights into the response of different genotypes to stress.

Conclusions

From the findings of this study, it may be concluded that waterlogging during reproductive stage showed a detrimental effect on plant height, SPAD value, pods and seeds plant^{-1}, 100-seed weight and grain yield of soybean. On the other hand, flood affected seeds of genotype BD2334, G00164, G00067, G00321, G0060 and G00058 genotypes higher germination percent, seed vigor index, electrical conductivity. Waterlogging also showed detrimental effects on photosynthetic pigments, photosynthesis, stomatal conductance, and transpiration rate. However, the plants had higher proline and MDA content to survive in oxidative stress.

The soybean genotype G00060 was highly tolerance of waterlogging. On the other hand, G00113, G00067, G00221, G00138, G00321, G00058, BU Soybean-1 exhibited tolerance and G00025, G00164, BD2331, BD2334 exhibited moderate tolerance under 7 days of waterlogging.

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References

Aggarwal PK, Kalra N, Chander S, Pathak H (2006) InfoCrop: a dynamic simulation model for the assessment of crop yields, losses due to pests, and environmental impact of agro-ecosystems in tropical environments. I. Model description. Agril Sys 89(1):1-25.

Agrawal RL (2005) Seed Technology. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India. pp. 565-590.

Akter M, Akter M, Mamun MAA, Karim MA (2021) Waterlogging effect on short duration soybean genotypes: phenology and greenness. Ecology J 3(2):169-173.

Al-Naggar A, Lee MH, Hur J, Lee YH, Igalavithana AD, Shaheen SM, Ryu C, Rinklebe J, Tsang DCW, Ok YS (2020) Biochar-induced metal immobilization and soil biogeochemical process: an integrated mechanistic approach. Sci. Total Environ 698:134112.

Andales AA, Batchelor WD, Anderson CE, Farnham DE, Whigham DK (2000) Incorporating tillage effects into a soybean model. Agril Sys 66(2):69-98.

Asseng S, Cao W, Zhang W, Ludwig F (2009) Crop physiology, modelling and climate change: impact and adaptation strategies. In Crop Physiology: Applications for Genetic Improvement and Agronomy. Elsevier, pp. 511-543.

Bates LS, Waldren RA, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205-207.

Collaku A, Harrison SA (2002) Losses in wheat due to waterlogging. Crop Sci 42(2):444-450.

Da-Silva CJ, do Amarante L (2020) Short-term nitrate supply decreases fermentation and oxidative stress caused by waterlogging in soybean plants. Environ Exp Bot 176:104078.

FAOSTAT (2022) Food and Agriculture Organization (www.fao.org/faostat/en/#data/qc).

FRG (2018) Fertilizer Recommendation Guide-2018. Bangladesh Agricultural Research Council, Dhaka.

Garcia N, da-Silva CJ, Cocco KLT, Pomagualli D, de Oliveira FK, da Silva JVL, de Oliveira ACB, do Amarante L (2020) Waterlogging tolerance of five soybean genotypes through different physiological and biochemical mechanisms. Environ Exp Bot 172:103975.

Gómez E, Gomez-Viilegas MA, Marín JM (1998) A multivariate generalization of the power exponential family of distributions. Comm Stat Theory Meth 27(3):589-600.

Hartman GL, West ED, Herman TK (2011) Crops that feed the World 2. Soybean worldwide production, use, and constraints caused by pathogens and pests. Food Sec 3:5-17.

Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125(1):189-198.

Hossain MS, Mamun MAA, Khaliq QA, Higuchi H, Nawata E, Karim MA (2019) Waterlogging induced changes in morpho-physiology of soybean. Trop Agril Res 1:53-54.

IRRI (2002) International Rice Research Institute, Manila, Philippines, Standard Evaluation System, for Rice.

ISTA (2019) International Seed Testing Association. Available online: https://www.seedtest.org/en/home.html (accessed on 31 January 2019).

Jin-Woong CHO, Ji HC, Yamakawa T (2006) Comparison of photosynthetic response of two soybean cultivars to soil flooding. J Fac Agril Kyushu Univ 51(2):227-232.

Jitsuyama Y (2017) Hypoxia-responsive root hydraulic conductivity influences soybean cultivar-specific waterlogging tolerance. Am J Plant Sci 8(04):770.

Kaur G, Singh G, Motavalli PP, Nelson KA, Orlowski JM, Golden BR (2020) Impacts and management strategies for crop production in waterlogged or flooded soils: A review. Agron J 112(3):1475-1501.

Konnerup D, Toro G, Pedersen O, Colmer TD (2018) Waterlogging tolerance, tissue nitrogen and oxygen transport in the forage legume *Melilotus siculus*: a comparison of nodulated and nitrate-fed plants. Ann Bot 121(4):699-709.

Lapaz ADM, de Camargos LS, Yoshida CHP, Firmino AC, de Figueiredo PAM, Aguilar JV, Nicolai AB, de Paiva WDS, Cruz VH, Tomaz RS (2020) Response of soybean to soil waterlogging associated with iron excess in the reproductive stage. Physiol Mol Biol Plants 26:1635-1648.

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic bio membranes. Methods Enzymol 148:350-382.

Mamun MAA, Shultana R, Rana MM, Mridha AJ (2013) Economic threshold density of multi species weed for direct seeded rice, Asian J Agri Rural Dev 3(8):523-531.

Mamun MAA, Haque MM, Khaliq QA, Karim MA, Karim AJMS, Mridha AJ, Saleque MA (2015) Assessment of soil chemical properties and rice yield in tidal submergence ecosystem. Bangladesh Agron J 18(2):79–87.

Mamun MAA, Haque MM, Saleque MA, Khaliq QA, Karim AJMS, Karim MA (2018) Evaluation of different fertilizer management guidelines for boro rice cultivation in south central coastal region of Bangladesh. Ann Agra Sci 16(4):466–475.

Mamun MAA, Julekha, Sarker U, Mannan MA, Rahman MM, Karim MA, Ercisli S, Marc RA, Golokhvast KS (2022) Application of potassium after waterlogging improves quality and productivity of soybean seeds. Life 12:1816.

Manzur ME, Grimoldi AA, Insausti P, Striker GG (2009) Escape from water or remain quiescent *Lotus tenuis* changes its strategy depending on depth of submergence. Ann Bot 104(6):1163-1169.

Maranna S, Nataraj V, Kumawat G, Chandra S, Rajesh V, Ramteke R, Patel RM, Ratnaparkhe BM, Hussain SM, Gupta S, Khandekar N (2021) Breeding for higher yield, early maturity, wider adaptability and waterlogging tolerance in soybean (*Glycine max* L.): A case study. Sci Rep 11(1):22853.

Maryam A, Nasreen S (2012) A review: water logging effects on morphological, anatomical, physiological and biochemical attributes of food and cash crops. Int J Water Res Env Sci 1(4):113-120.

Messina CD, Jones JW, Boote KJ, Vallejos CE (2006) A gene-based model to simulate soybean development and yield responses to environment. Crop Sci 46(1):456-466.

Peng S, Chen L, Qin JG, Hou J, Yu N, Long Z, Ye J, Sun X (2007) Effects of replacement of dietary fish oil by soybean oil on growth performance and liver biochemical composition in juvenile black seabream, *Acanthopagrus schlegeli*. Aquacult 276(1-4):154-161.

Polischuk RA, Miralles DJ, Striker GG (2022) A quantitative review of soybean responses to waterlogging: Agronomical, morpho-physiological and anatomical traits of tolerance. Plant Soil 475(1-2):237-252.

Rhine MD, Stevens G, Shannon G, Wrather A, Sleper D (2010) Yield and nutritional responses to waterlogging of soybean cultivars. Irri Sci 28:135-142

Rocío AP, Miralles DJ, Colmer TD, Polischuk EL, Striker GG (2018) Waterlogging of winter crops at early and late stages: impacts on leaf physiology, growth and yield. Front Plant Sci 9:1863.

Satter MA, Rahman MA, Rashid HU, Ali MS, Alom MS (2005) Krishi Projukti Hatboi (Handbook on Agrotechnology), Bangladesh Agricultural Research Institute 25:144-151.

Sun J, Yang L, Zhao F, Wu W (2020) Domestic dynamics of crop production in response to international food trade: evidence from soybean imports in China. J Land Use Sci 15(1):91-98.

Sun M, Zhao K, Wang J, Mu W, Zhan Y, Li W, Teng W, Zhao X, Han Y (2022) Identification of quantitative trait loci underlying lodging of soybean across multiple environments. Crop Pasture Sci 73(6):652-662.

Twum LA, Ocloo FC, Duah-Bisiw D, Odai BT (2021) Determining the effect of heat treatment on iron fortified soybean gari blend and its potential bioavailability. Sci Afr 12:e00763.

USDA (2022) United States Department of Agriculture. https://ipad.fas.usda.gov/countrysummary/default.aspx?id=BG&crop=Soybean

Wu C, Mozzoni LA, Moseley D, Hummer W, Ye H, Chen P, Shannon G, Nguyen H (2020) Genome-wide association mapping of flooding tolerance in soybean. Mole Breed 40:1-14.

Wu C, Zeng A, Chen P, Hummer W, Mokua J, Shannon JG, Nguyen HT (2017) Evaluation and development of flood-tolerant soybean cultivars. Plant Breed 136(6):913-923.

Wuebker EF, Mullen RE, Koehler K (2001) Flooding and temperature effects on soybean germination. Crop Sci 41(6):1857-1861.

Yaklich RW, Abdul-Baki AA (1975) Variability in Metabolism of Individual Axes of Soybean Seeds and Its Relationship to Vigor 1. Crop Sci 15(3):424-426.

Zeng H, Zhang Y, Zhang X, Pi E, Zhu Y (2017) Analysis of EF-hand proteins in soybean genome suggests their potential roles in environmental and nutritional stress signaling. Front Plant Sci 8:877.