

Effect of Waterlogging Duration on Germination, Physiological Characteristics, and Yield of Mungbean (*Vigna radiata* L.)

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Abstract

The study was conducted to evaluate the germination, physiological responses, yield-related traits, and seed yield of three mungbean varieties, *viz.* DXVN7, DXVN5, and DX11, under waterlogging conditions in the 2019 Summer. In experiment 1, the seeds of the three mungbean varieties were immersed in distilled water in Petri dishes for 12, 24, 36, 48, and 72h. Afterwards, water was removed and the percentage of germinated seeds was calculated at 84h after sowing. In experiment 2, plants were waterlogged at the seedling stage (25 days after germination) for 3, 6, and 9 days. Waterlogging depth was maintained at 3cm above the soil surface. Physiological traits were determined at the recovery period after termination of waterlogging (45 days after germination). The results showed that waterlogging significantly decreased germination percentages, plant height, root dry weight, leaf relative water content (RWC), SPAD value, Fv/Fm index, leaf photosynthesis, total dry weight, and seed yield of all varieties. Germination percentages at 12 and 24h of waterlogging were not significantly affected, whereas germination was significantly reduced at up to 36h of waterlogging. Seventy-two hours of waterlogging caused failure in germination. Nine days of waterlogging at the seedling stage adversely affected the physiological traits and seed yield of the mungbean varieties with 31% of yield reduction. Meanwhile, plants grew better at 3 days of waterlogging. Among the three varieties, DXVN7 showed the best adaptability under waterlogging conditions, attaining the highest seed germination and yield.

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Keywords

Germination, mungbean, physiological traits, seed yield, waterlogging duration

Introduction

Mungbean (*Vigna radiata* (L.) Wilczek) is a popular and

important pulse crop in Asia because of its rich in digestible protein (approximately 25-28%) by N₂ fixation machinery and a wide range of adaptability.

Climate change is affecting agricultural production in various ways, including leading to changes in average temperatures, rainfall, and climate extremes (i.e., storms, floods, and droughts, etc.). Waterlogging is considered one of the major factors affecting the growth and yield of many crops. Mungbean cannot withstand soil waterlogging, particularly during the early stages of growth (Singh & Singh, 2011). Waterlogging reduces oxygen concentrations around the roots of the plants, so the plants show wilting of their leaves even when enclosed by the excess of water, which affects water and nutrient uptake (Sairam *et al.*, 2008) and inhibits nodule activity and nitrogen fixation (Kumar *et al.*, 2013). Additionally, waterlogging has an adverse effect on the growth and development of the plant, biomass production, pod setting, and seed yield by affecting some physiological processes. The main physiological effect of waterlogging is the inhibition of photosynthesis (Ahmed *et al.*, 2006) that is directly related to the stomatal limitation, carbon assimilation, and disturbance in plant respiratory metabolism (Dat *et al.*, 2004).

Waterlogging in mungbean is not unusual, but despite this fact, there is little information on the responses of germination, physiological characteristics, and yield of mungbean varieties to soil waterlogging. Under the field conditions, waterlogging duration varies with the intensity and duration of rain, and soil types as well (Amin *et al.*, 2017). Furthermore, successful selection and breeding for varieties with waterlogging tolerance can only be attained when the mechanisms of waterlogging tolerance in mungbean are fully understood. Therefore, the aim of this study was to determine the effect of waterlogging duration on germination, physiological characteristics, and yield of some selected mungbean varieties, thereby selecting mungbean varieties with better waterlogging tolerance.

Materials and Methods

Materials and experimental design

Three mungbean varieties, namely DXVN7, DXVN5, and DX11, were used in this study. DX11 was developed by the Legumes Research and Development Center (LRDC), DXVN5 was developed by the Maize Research Institute (MRI), and DXVN7 was developed by LRDC and MRI, Vietnam. Two experiments were conducted at the Faculty of Agronomy, Vietnam National University of Agriculture in the 2019 summer season.

Experiment 1: Effect of waterlogging duration on germination of three mungbean varieties

Mungbean seeds were rinsed with distilled water three times. After that, the seeds were sown in Petri dishes (10 seeds per dish) and immersed in distilled water for 12, 24, 36, 48, and 72h (waterlogging duration) followed by a standard germination test along with the control (non-waterlogging) in the dark at 25°C. After that, water was removed and the seeds were germinated in the same condition as the control (in the dark at 25°C and 60% relative humidity). The number of germinated seeds (>2mm radicle) was counted at 84h after sowing. A two factorial experiment was designed in a Completely Randomized Design (CRD) using six waterlogging treatments (factor A) and three mungbean varieties (factor B) with three replications, one Petri dish per replication. Ten seeds were collected from each replicate for the measurement of germination percentage and fresh radicle weight.

Experiment 2: Effect of waterlogging duration on physiological characteristics and yield of three mungbean varieties

Seeds of each variety were sown separately in pots (18 × 16cm) each containing 2kg of dry soil per pot. The potting base contained 0.2 g N + 0.5 g P₂O₅ + 0.3 g K₂O. There were two plants in each pot. Twenty-five days after germination, waterlogging was imitated by applying water and kept at 3cm of water level above the soil surface for 3 days, 6 days, and 9 days. After that, the standing water was removed. The waterlogging treatments included the control (non-

waterlogging) and 3, 6, and 9-day waterlogging treatments. A two factorial experiment was arranged in a Completely Randomized Design (CRD) with four replications, with two pots per replication. Waterlogging treatments were designated factor A, while the three mungbean varieties were designated factor B. A total of 96 pots were used in this experiment. The average of two samples per pot was collected from each replicate for the estimation of growth, physiology, and yield parameters.

Measurement items and analytical methods

In experiment 1: Germination percentage (GP) and fresh radicle weight of mungbean varieties were calculated at 84h after sowing.

$$GP (\%) = (\text{Number of seeds germinated} / \text{Total number of seeds}) \times 100$$

In experiment 2: Growth and physiological traits of mungbean varieties were sampled at the recovery period after termination of waterlogging (45 days after germination). The height of the plant, root dry matter, and SPAD values were measured using a chlorophyll meter (Konica Minolta Chlorophyll meter SPAD 502, Japan). Fv/Fm values were measured using Opti-Sciences Chlorophyll Fluorometer (Hudson, USA-model OS-30p). Leaf relative water content (RWC) was determined by recording the turgid weight of 0.5g fresh leaf samples by keeping it in water for 4h, followed by drying samples at 80°C for 48h or until constant weight achieved (Weatherley, 1950).

$$RWC (\%) = (\text{Fresh weight} - \text{dry weight}) / (\text{Turgid weight} - \text{dry weight}) \times 100$$

Leaf photosynthesis rate ($\mu\text{mol CO}_2/\text{m}^2$ leaf/s) was recorded using LICOR-6400 (USA) at the youngest fully expanded leaf from 11:00 to 13:00h, at 30°C, CO₂ concentration of 370 ppm, light density of 1500 $\mu\text{mol}/\text{m}^2/\text{s}$, and humidity of 60%. Total dry weight accumulation was determined after drying the samples at 80°C for 48h or until constant weight.

Data on pods per plant, seeds per pod, 100-seed weight, and seed yield were recorded at the harvest stage. Seed yield was adjusted at 12% moisture content.

Statistical analysis

Mean data for each trait was used for the analysis of variance (ANOVA) with two factors (waterlogging treatments and varieties) and their interactions using IRRISTART version 5.0. The mean values were compared using the Least Significant Difference (LSD) test.

Results and Discussion

Effect of waterlogging duration on germination of mungbean

Germination percentage and fresh radicle weight of the three mungbean varieties at 84h after sowing are shown in **Table 1** and **Figure 1**. Waterlogging duration decreased the percentage of germination compared with the control in all mungbean varieties. Twelve and twenty-four hours of waterlogging showed a significantly higher germination percentage than the others. But longer than 24h of waterlogging significantly reduced seed germination compared with the control, below 77% of germination percentage. Notably, waterlogging for 72h caused serious damages to the germination of seeds in all varieties, with the germination percentage of only 10% and 3.3%. Waterlogging treatments also led to the decline of fresh radicle weight. At 24h and more of waterlogging duration, there was significant reductions compared with the control and 12h of waterlogging. The highest reduction of fresh radicle weight was at 48h and 72h of waterlogging in the three mungbean varieties. In all varieties, the germination percentage and fresh radicle weight of DXVN7 were higher than those of DXVN5 and DX11. Germination percentage of DX11 variety at 72h of waterlogging was the lowest.

Soaking is a method to increase the percentage of germination in many crops (Chippendale, 2008). However, over-soaking (more than 48h) causes a drastic reduction in germination (**Figure 1**). The germination decreased significantly as the waterlogging duration increased. Maybe, some useful biochemical and hormones in seeds filtered out, thereby decreasing the vigor of seeds. Our results also agreed with the research of Ullah (2006) in

Table 1. Effect of waterlogging duration on germination and fresh radicle weight of mungbean

| Varieties | Waterlogging (H) | Germination percentage (%) | Fresh radicle weight (gram) |
|---------------------------|------------------|----------------------------|-----------------------------|
| DX11 | 0 | 100.0 ^a | 0.196 ^a |
| | 12 | 90.0 ^{ab} | 0.174 ^b |
| | 24 | 86.7 ^{ab} | 0.061 ^d |
| | 36 | 50.0 ^c | 0.025 ^{fg} |
| | 48 | 36.7 ^c | 0.018 ^g |
| | 72 | 3.3 ^d | 0.009 ^g |
| DXVN5 | 0 | 100.0 ^a | 0.197 ^a |
| | 12 | 93.3 ^a | 0.185 ^{ab} |
| | 24 | 86.7 ^{ab} | 0.087 ^c |
| | 36 | 70.0 ^b | 0.039 ^{ef} |
| | 48 | 40.0 ^c | 0.023 ^f |
| | 72 | 10.0 ^d | 0.015 ^g |
| DXVN7 | 0 | 100.0 ^a | 0.206 ^a |
| | 12 | 96.7 ^a | 0.189 ^{ab} |
| | 24 | 90.0 ^{ab} | 0.102 ^c |
| | 36 | 76.7 ^b | 0.051 ^{de} |
| | 48 | 43.3 ^c | 0.036 ^{ef} |
| | 72 | 10.0 ^d | 0.015 ^g |
| CV% | | 1.8 | 2.5 |
| LSD _{0.05} (V) | | 12.5 | 0.014 |
| LSD _{0.05} (W) | | 17.3 | 0.027 |
| LSD _{0.05} (VxW) | | 19.1 | 0.021 |

Note: Values followed by the same letter in each treatment column are not significantly different at the 5% level. (V): Variety; (W): Waterlogging treatment. The data were recorded at 84h after sowing.

which waterlogging over 24h was found to significantly decrease the germination of mungbean (*Vigna radiate*) vc. Kanti and waterlogging up to 72h caused failure in germination.

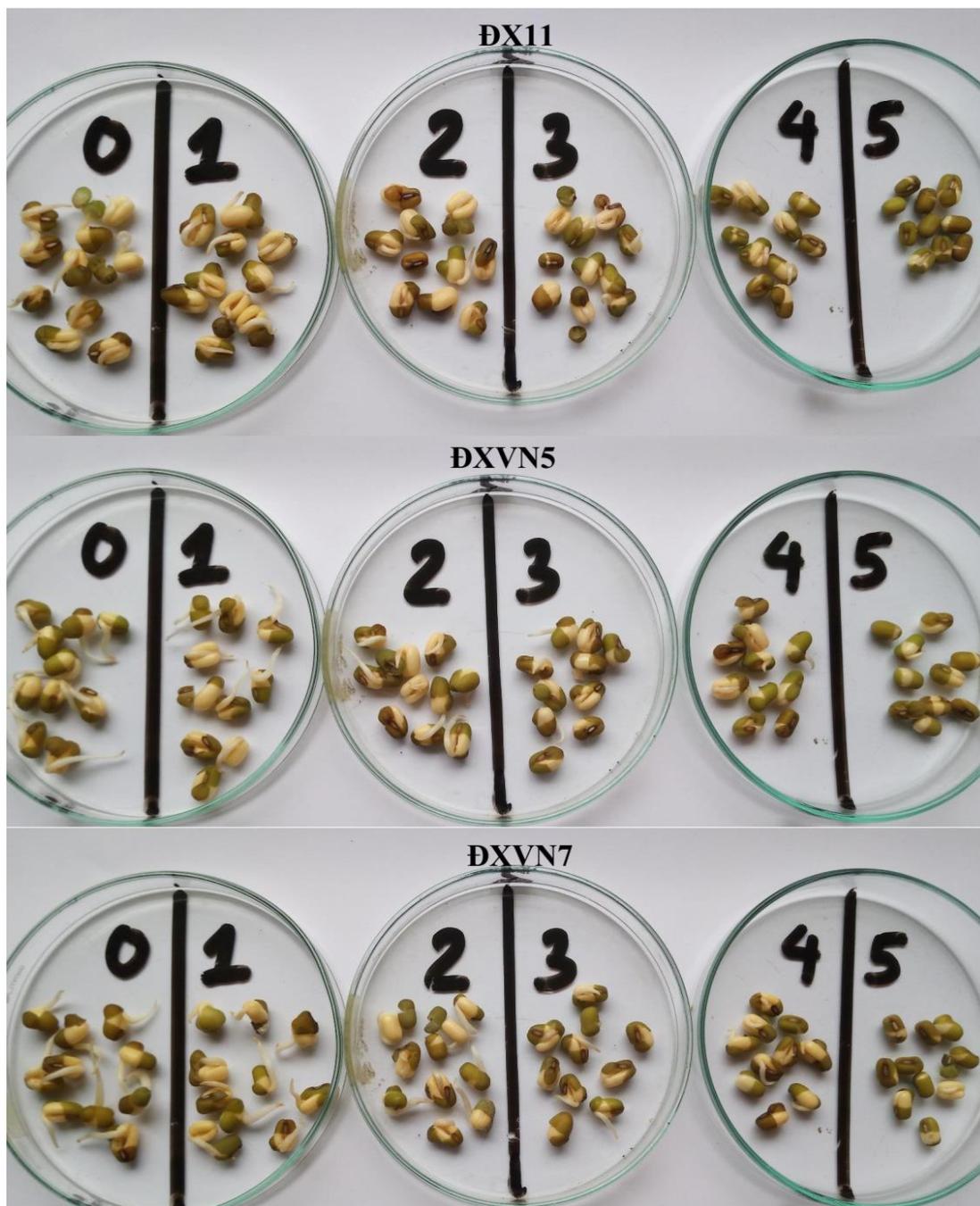
Effect of waterlogging duration on plant height and root dry weight of mungbean

The results as can be seen in **Table 2** show that the waterlogged treatments affected the development of all varieties. The longer the waterlogging duration at the seedling stage, the slower the growth of the height of plant and root dry weight. These two parameters showed the highest reduction at 9 days of waterlogging in the three varieties of mungbean, followed by 6 days with significant differences compared with the control. Plant height and root dry weight at 3

days of waterlogging were slightly reduced but not significantly in comparison with non-waterlogging treatment. DXVN7 variety had the better growth of plant height and root dry weight. The lowest plant height was recorded in DXVN5, and the lowest root dry weight was found in DX11 under waterlogging conditions (**Figure 2**).

Effect of waterlogging duration on leaf relative water content (RWC), SPAD value, and Fv/Fm index of mungbean

Leaf relative water content (RWC), SPAD, and Fv/Fm index of all mungbean varieties under waterlogging conditions are demonstrated in **Table 3**. All parameters of RWC, SPAD, and Fv/Fm decreased with an increased waterlogging duration. The values of RWC at waterlogging treatments of the three mungbean varieties were



Notes: (0): Control treatment; (1), (2), (3), (4), and (5): 12, 24, 36, 48, and 72h of waterlogging, respectively.

Figure 1. Comparison of germination percentages between the control and waterlogging treatments of three mungbean varieties at 84h after sowing

below 85%. The highest reduction in RWC was observed at 9 days of waterlogging, followed by 6 days. Waterlogging also caused a significant decrease in SPAD values and F_v/F_m indexes of mungbean varieties with an increase in the waterlogging duration. The lowest values of both parameters were obtained at 9 days of waterlogging. SPAD values and F_v/F_m indexes of

the three mungbean varieties at 3 days of waterlogging were lower than the control but did not differ significantly. Among the three mungbean varieties, the highest values of RWC, SPAD, and F_v/F_m index were observed in DXVN7 mungbean variety, followed by DXVN5 mungbean variety. The lowest was found in DX11 mungbean variety.

Effect of waterlogging duration on photosynthesis and total dry weight of mungbean

Waterlogging also inhibited the photosynthesis and total dry weight of all mungbean varieties. Both parameters declined when the duration of waterlogging increased. The values at 6 and 9 days of waterlogging significantly decreased compared with the

control. At 3 days of waterlogging, the photosynthesis and total dry weight also decreased but did not differ significantly compared with the control. The lowest in both parameters were observed at 9 days of waterlogging, followed by 6 days. Among the three varieties, photosynthesis and total dry weight of DXVN7 were higher than the other varieties. The lowest figures were observed in DX11 variety (**Table 4**).

Table 2. Effect of waterlogging duration on plant height and root dry weight of mungbean at the recovery period

| Varieties | Waterlogging (days) | Plant height (cm) | Root dry weight (gram) | Adventitious root formation |
|---------------------------|---------------------|--------------------|------------------------|-----------------------------|
| DX11 | 0 | 41.8 ^{ab} | 0.26 ^{cd} | - |
| | 3 | 39.6 ^{bc} | 0.24 ^{ef} | + |
| | 6 | 35.2 ^e | 0.23 ^f | + |
| | 9 | 33.2 ^{ef} | 0.21 ^g | ++ |
| DXVN5 | 0 | 40.5 ^b | 0.27 ^{bc} | - |
| | 3 | 38.2 ^{cd} | 0.26 ^{cd} | + |
| | 6 | 33.6 ^e | 0.24 ^{ef} | ++ |
| | 9 | 30.5 ^f | 0.23 ^f | ++ |
| DXVN7 | 0 | 43.9 ^a | 0.29 ^a | - |
| | 3 | 42.1 ^{ab} | 0.28 ^{ab} | + |
| | 6 | 38.4 ^{cd} | 0.26 ^{cd} | ++ |
| | 9 | 35.7 ^{de} | 0.25 ^{de} | +++ |
| CV% | | 3.9 | 1.4 | |
| LSD _{0.05} (V) | | 4.6 | 0.021 | |
| LSD _{0.05} (W) | | 3.1 | 0.018 | |
| LSD _{0.05} (VxW) | | 2.8 | 0.015 | |

Note: Values followed by the same letter in each treatment column are not significantly different at the 5% level. (V): Variety; (W): Waterlogging treatment. (-): Non; (+): Little; (++): Many; (+++): So many.



Figure 2. Comparative adventitious root formation in DXVN7 variety after 3 (A), 6 (B), and 9 (C) days of waterlogging

Waterlogging caused some physiological disturbances, including reductions in RWC, SPAD, F_v/F_m index, photosynthesis, and total dry weight of mungbean. Wilting in crops under waterlogging has been ascribed to higher resistance to the mass flow of water through the roots (Jackson & Drew, 1984). Waterlogging-tolerant plant varieties develop adaptive mechanisms to maintain better water relationships using stomatal conductance (Malik *et al.*, 2001). In our research, waterlogging treatments decreased RWC of all mungbean varieties. The same results have also been shown in pineapples at the flowering stage (Min & Bartholomew, 2005) and in mungbean genotypes (Kumar *et al.*, 2013) after 6 days of waterlogging.

According to our experiment, yellow leaves of mungbean plants (a reduction in the chlorophyll content through SPAD index) (**Figure 3**) might be due to a decrease in leaf nitrogen, nodulation, N fixation, and production of toxic substances such as nitrites and sulfides which move from the soil through roots to the leaf if carried upward in large quantities (Ezin *et al.*, 2010). In addition, chlorophyll has an important role in light absorption during the photosynthetic process. The decrease in the chlorophyll content could lower the efficiency of

plants to convert light energy into chemical energy, thereby suppressing the photosynthetic, reducing the total amount of organic synthesis, and ultimately leading to a reduction in the biomass of plants (Zhang *et al.*, 2015). Moreover, Ahmed *et al.* (2006) showed that a reduction in photosynthesis is regulated by internal damages to photosystem II (PSII) and ultimately limits light interception. Reductions in the chlorophyll content (SPAD index), photosynthesis, and total dry matter of plants under waterlogging stress were also reported by Ahmed *et al.* (2006) and Kumar *et al.* (2013) in mungbean; by Wang *et al.* (2017) in cotton; and by Vu Tien Binh & Nguyen Viet Long (2015) in soybean.

Effect of waterlogging duration on yield components and seed yield mungbean

Yield components and seed yield of mungbean varieties were affected by the waterlogging conditions (**Table 5**). There were significant reductions in the number of pods per plant, 100-seed weight, and seed yield between the waterlogged and control treatments in all mungbean varieties. The highest decreases in yield components and seed yield were at 9 days of waterlogging that reduced the yield of DX11,

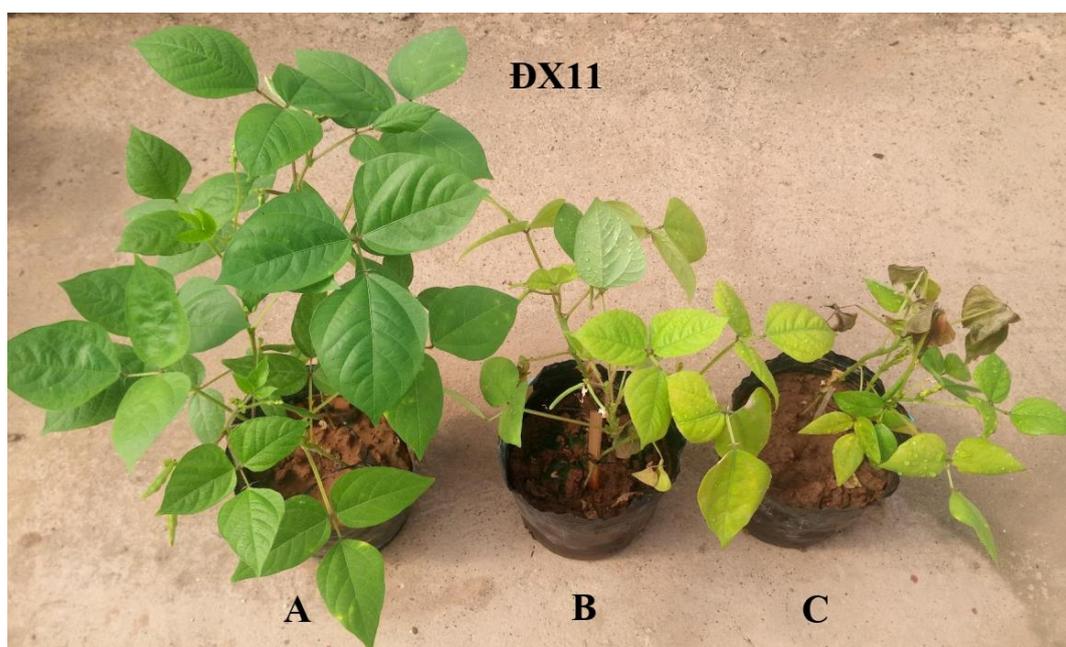


Figure 3. DX11 mungbean variety at the recovery period in the control treatment (A); 6 days of waterlogging treatment (B); and 9 days of waterlogging treatment (C)

Table 3. Effect of waterlogging duration on RWC, SPAD, and F_v/F_m index of mungbean at the recovery period

| Varieties | Waterlogging (days) | RWC (%) | SPAD index | F_v/F_m index |
|---------------------------|---------------------|---------|--------------------|--------------------|
| DX11 | 0 | 88.57 | 42.9 ^{ab} | 0.79 ^a |
| | 3 | 84.64 | 40.6 ^b | 0.75 ^{ab} |
| | 6 | 79.35 | 31.1 ^{de} | 0.62 ^{cd} |
| | 9 | 75.14 | 28.5 ^e | 0.57 ^f |
| DXVN5 | 0 | 90.07 | 45.2 ^a | 0.80 ^a |
| | 3 | 84.49 | 42.8 ^{ab} | 0.75 ^{ab} |
| | 6 | 80.26 | 34.9 ^c | 0.65 ^c |
| | 9 | 78.53 | 31.3 ^{de} | 0.61 ^{cd} |
| DXVN7 | 0 | 90.17 | 44.3 ^a | 0.82 ^a |
| | 3 | 85.32 | 42.6 ^{ab} | 0.76 ^a |
| | 6 | 81.83 | 35.2 ^c | 0.68 ^{bc} |
| | 9 | 80.52 | 33.6 ^{cd} | 0.64 ^{cd} |
| CV% | | | 3.1 | 2.7 |
| LSD _{0.05} (V) | | | 2.4 | 0.04 |
| LSD _{0.05} (W) | | | 2.5 | 0.07 |
| LSD _{0.05} (VxW) | | | 2.8 | 0.07 |

Note: Values followed by the same letter in each treatment column are not significantly different at the 5% level. (V): Variety; (W): Waterlogging treatment.

Table 4. Effect of waterlogging duration on photosynthesis and total dry weight of mungbean at recovery period

| Varieties | Waterlogging (days) | Photosynthesis ($\mu\text{mol CO}_2/\text{m}^2$ leaf/s) | Total dry weight (gram) |
|---------------------------|---------------------|----------------------------------------------------------|-------------------------|
| DX11 | 0 | 29.33 ^{ab} | 3.97 ^{bc} |
| | 3 | 27.51 ^c | 3.76 ^{cd} |
| | 6 | 22.08 ^{de} | 3.04 ^e |
| | 9 | 21.40 ^{de} | 2.93 ^e |
| DXVN5 | 0 | 30.26 ^a | 4.22 ^{ab} |
| | 3 | 29.11 ^a | 4.01 ^{bc} |
| | 6 | 22.96 ^d | 3.36 ^{de} |
| | 9 | 20.54 ^e | 3.07 ^e |
| DXVN7 | 0 | 29.52 ^{ab} | 4.59 ^a |
| | 3 | 28.47 ^{bc} | 4.26 ^{ab} |
| | 6 | 23.14 ^d | 3.61 ^{cd} |
| | 9 | 22.62 ^d | 3.20 ^e |
| CV% | | 3.4 | 3.8 |
| LSD _{0.05} (V) | | 2.1 | 0.23 |
| LSD _{0.05} (W) | | 2.3 | 0.47 |
| LSD _{0.05} (VxW) | | 1.7 | 0.42 |

Note: Values followed by the same letter in each treatment column are not significantly different at the 5% level. (V): Variety; (W): Waterlogging treatment.

Table 5. Effect of waterlogging duration on yield components and seed yield of mungbean

| Varieties | Waterlogging (days) | Pods per plant | Seeds per pod | 100-seed weight (g) | Seed yield (g plant ⁻¹) | Yield reduction (%) |
|---------------------------|---------------------|--------------------|------------------|---------------------|-------------------------------------|---------------------|
| DX11 | 0 | 14.3 ^{ab} | 7.1 ^a | 5.05 | 5.12 ^{ab} | |
| | 3 | 11.6 ^{cd} | 6.9 ^a | 4.83 | 4.67 ^b | 8.9 |
| | 6 | 7.1 ^{ef} | 6.7 ^a | 4.53 | 3.34 ^{cd} | 33.9 |
| | 9 | 6.6 ^f | 6.7 ^a | 4.32 | 3.08 ^d | 39.1 |
| DXVN5 | 0 | 14.8 ^a | 7.1 ^a | 5.11 | 5.27 ^a | |
| | 3 | 12.3 ^{bc} | 6.9 ^a | 5.03 | 4.86 ^{ab} | 7.8 |
| | 6 | 8.4 ^{ef} | 6.8 ^a | 4.65 | 3.73 ^c | 29.2 |
| | 9 | 8.2 ^{ef} | 6.8 ^a | 4.57 | 3.41 ^{cd} | 35.3 |
| DXVN7 | 0 | 15.1 ^a | 7.2 ^a | 5.19 | 5.33 ^a | |
| | 3 | 13.8 ^{ab} | 7.1 ^a | 5.06 | 5.02 ^{ab} | 5.6 |
| | 6 | 9.3 ^{de} | 6.9 ^a | 4.74 | 3.81 ^c | 28.5 |
| | 9 | 8.5 ^{ef} | 6.8 ^a | 4.68 | 3.65 ^c | 31.4 |
| CV% | | 2.3 | 2.6 | | 3.5 | |
| LSD _{0.05 (V)} | | 1.9 | 0.8 | | 0.22 | |
| LSD _{0.05 (W)} | | 2.7 | 1.2 | | 0.45 | |
| LSD _{0.05 (VxW)} | | 2.4 | 1.3 | | 0.48 | |

Note: Values followed by the same letter in each treatment column are not significantly different at the 5% level. (V): Variety; (W): Waterlogging treatment.

DXVN5, and DXVN7 by 39.1%, 35.3%, and 31.4%, respectively; followed by 6 days of waterlogging. Seed yield also declined at 3 days of waterlogging but did not differ significantly compared to the control. Among three mungbean varieties, the highest values of yield components and seed yield were observed in DXVN7 variety under waterlogging conditions, followed by DXVN5 variety. The lowest values of yield components and seed yield were recorded in DX11 under waterlogging conditions.

Plant yield was also affected under waterlogging stress. In our experiment, waterlogging at the seedling stage decreased seed yield primarily by reducing the number of pods per plant and 100-seed weight. On the other hand, the reductions in root growth, leaf water potential, and leaf photosynthesis under waterlogging in mungbean might have resulted in decreasing yield components and seed yield. It was reported in mungbean with a waterlogging duration of more than 6 days (Kumar *et al.*, 2013;

Ahmed *et al.*, 2002) and at 4 days of waterlogging (Amin *et al.*, 2017; Amin *et al.*, 2016).

Conclusions

Waterlogging duration affected the germination of mungbean varieties. The germination percentages and fresh radicle weights of waterlogging treatments were lower than those in the control treatment. Seed germination at 12 and 24h of waterlogging did not decreased significantly; whilst germination percentages and fresh radicle weight decreased the most significantly at 48 and 72h of waterlogging.

Waterlogging treatments also inhibited the growth and development of three mungbean varieties at the seedling stage. A longer waterlogging duration caused a higher decrease in the height of plant, root dry weight, RWC, SPAD, Fv/Fm index, leaf photosynthesis rate,

total dry weight, and seed yield of all mungbean varieties. At 9 days of waterlogging, the highest reduction in physiological traits and seed yield was observed, followed by 6 days of waterlogging. Among the three mungbean varieties, DXVN7 had the highest figures in the above parameters, whereas the lowest figures were seen in the DX11 variety.

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