Characterisation of Selected Mungbean Genotypes for Tolerance to Waterlogging Stress at Pod Filling Stage

Sobia Ikram *, Surya Bhattarai and Kerry Brian Walsh

Institute for Future Farming Systems, Central Queensland University, Building 361, Bruce Highway, Rockhampton, QLD 4701, Australia; s.bhattarai@cqu.edu.au (S.B.); k.walsh@cqu.edu.au (K.B.W.)
* Correspondence: s.ikram@cqu.edu.au; Tel.: +61-415477229

Abstract: Mungbean is susceptible to waterlogging stress; therefore, breeding tolerant varieties would provide Australian growers with management options for wet summer season planting. Selection for waterlogging tolerance could be improved using vegetative indices that correlate to yield. Five mung bean genotypes were exposed to waterlogging stress at the pod-filling stage and characterised for various morphological and physiological traits governing seed yield. Waterlogging during pod filling decreased stomatal conductance (gs) and photosynthetic rate (A infinitely) to ~27% and 25% compared to control, respectively, resulting in a decline in effective quantum yield of PSII (ΦPSII) and maximum efficiency of PSII of dark-adapted leaves (Fv/Fm) and leaf chlorophyll while increasing excitation pressure (1-qP) significantly. Waterlogging at pod filling reduced leaf count (19%), plant height (23%), leaf dry weight (38%), stem dry weight (33%), pod dry weight (36%), above-ground biomass (34%), root biomass (26%), and 100-seed weight (4%). Seed yield was highly positively correlated with A infinitely (0.86), gs (0.69), chlorophyll content (0.63), and ΦPSII (0.59), with a highly negative correlation with 1-qP (~0.87) at 30 days of treatment imposition. A yield penalty of 32% was recorded under waterlogging stress compared to control plants, while the performance of all genotypes was found to be similar in terms of seed yield. Interestingly, genotype AVTMB#3 produced significantly larger seeds under waterlogging stress relative to other genotypes, including the leading Australian mungbean variety, Jade-AU. Based on a robust and significantly strong correlation with seed yield under waterlogging stress, 1-qP and photosynthetic rates (A infinitely) are recommended as potential indicators for the screening of mungbean genotypes. Thus, the current study presents a framework for screening waterlogging tolerance, which can provide a reasonable basis for the selection of various genotypes in future mungbean breeding programs.

Keywords: waterlogging; chlorophyll a fluorescence; mungbean; photosystems; plant stress

1. Introduction

Waterlogging stress is a global constraint limiting crop yield [1]. It is ranked second to drought based on severe damage to crop production and substantial economic losses [2]. Among all the abiotic stresses, 65% of agricultural crop losses were attributed directly to waterlogging stress, costing the global economy an estimated USD 74 billion each year [3]. Recent data suggests that over 16 percent of the world’s cultivated land is affected by transient waterlogging [4] and over 17 million km² of land is at risk of flooding [5]. According to the current climate change forecast [6], waterlogging events are predicted to increase, and to become a great challenge for sustainable mungbean cultivation.

Despite Australia being one of the driest continents in the world, waterlogging results in economic losses in all sectors of Australian agricultural production, including grains [7], pasturage [8], and cotton [9]. A study by Fernanandez and Shamugasundaram (1988) reported that yield severely diminished under waterlogging stress in the mungbean when annual rainfall exceeded 1000 mm [10]. Mungbean plants exposed to...
waterlogging stress for nine days at the seedling stage experienced a significant decline in photosynthetic rates and seed yield of 83% and 85%, respectively [11]. Inhibition of shoot and root dry mass by 60–65% in mungbean and by 40% in black gram was reported under waterlogging stress for 8–16 days during the seedling stage [12]. Short-term (five days) waterlogging stress in soybean resulted in yield decline of 25% in a waterlogging-sensitive genotype, Kenfeng 16, while a reduction of 16% was reported in Kenfeng 14, a waterlogging-tolerant genotype [13]. A decline in growth has been observed in waterlogging-sensitive genotypes in common beans under three days of waterlogging stress at the seedling stage [14]. The growth decreased in field pea when subjected to waterlogging stress for seven days at vegetative and generative phases in field bean [15]. Previous research on wheat [16], barley [17], and rapeseed [18] has demonstrated that plants subjected to fourteen days of waterlogging during the vegetative stage and reproductive stages showed reduced growth, demonstrating their sensitivity during the early vegetative period.

Waterlogging tolerance is a highly complex phenomenon, and plants have evolved a variety of tolerance mechanisms that include changes in morphological and physiological parameters such as adventitious root production [19,20], parenchyma development, and alteration in leaf (epinasty) and shoot morphology [21], maintaining higher levels of gas exchange [22] and chlorophyll a fluorescence parameters [23]. Moreover, under waterlogging stress, plants adapt various other mechanisms which involve higher acquisition of soluble sugars, enhanced fermentation activity, and reactive oxygen (ROS)-scavenging enzymes [24,25], along with responses involving hormones such as ethylene and abscisic acid.

Waterlogging has been shown to cause a variety of morpho-physiological alterations in mungbean genotypes depending on the growth stage, duration, and soil type [26,27]. Various studies have reported a reduction in stomatal conductance accompanied by CO₂ assimilation under waterlogged situations in the mungbean ([28,29], resulting in reduced growth [30]. The more pronounced early effects found in sensitive genotypes included a drop in photosynthesis and stomatal conductance. In mungbean, several growth parameters including root growth [31], leaf area [11] leaf chlorophyll content, photosynthesis, stomatal conductance, water use efficiency [32], and total biomass [11] are severely impacted by waterlogging and predate the effect on yield, and thus could be used in screening for waterlogging tolerance.

Several traits have been used as selection criteria for waterlogging tolerance in many crops, including mungbean. These include plant height, dry weight, photosynthetic rates (Aₜₐₜ), stomatal conductance (gₛ), photochemical efficiency of PSII (ΦPSII), the quantum efficiency of PSII of dark-adapted leaves (Fᵥ/Fm), excitation pressure (v₁qₚ), and leaf chlorophyll content (SPAD units) are considered important parameters that can be used to determine waterlogging tolerance [32,33].

Mungbean is a short-duration and protein-rich grain legume that fits well in different cropping systems globally, and has the potential to dramatically boost nitrogen fixation from the atmosphere, improving soil fertility as well as farmers’ incomes and nutrition for the world’s resource-poor and vegetarian communities [34]. However, mungbean is a waterlogging-susceptible crop.

In Australia, mungbean is generally grown as a rain-fed crop during the summer months in the subtropical environments of NSW and QLD and as an irrigated crop during the winter months in Northern Australia [35]. The summer season crop can experience waterlogging conditions due to the crop growing season coinciding with prolonged rainfall events, particularly in heavy clay and poorly-draining soil types. During the mid-October to January planting window, monsoon rainfalls can cause transient waterlogging [36] at the pod-filling stage.

The mungbean crop has been reported to be susceptible to waterlogging imposed at the seedling stage, while other researchers have demonstrated yield decrease following waterlogging of mungbean across both the vegetative and pod-filling stages [31,32]. Studies on waterlogging stress in mungbean during the pod-filling stage alone are limited.
The identification and development of genotypes tolerant to waterlogging in the pod-filling stage is recommended for the Australian production system, given the likelihood of waterlogging at this stage. The current study was undertaken within a larger study involving the evaluation of several new mungbean genotypes as potential replacements for Jade-AU, the current dominant line used in Australia. These evaluations have been based on growth under optimal condition and in water-limiting conditions. In the present study, four leading candidates from these evaluation trials were characterized for tolerance of waterlogging at pod filling relative to Jade-AU.

The main objectives of this study were:

1. to determine the suitability of various morpho-physiological parameters for screening of waterlogging tolerance of mungbean genotypes at the pod-filling stage; and
2. to identify waterlogging stress-tolerant genotypes based on morpho-physiological traits.

2. Materials and Methods

2.1. Plant Material

Initial field screening of 25 AgriVentis (Sydney, Australia; https://www.agriventis-technologies.com.au/) (accessed on 20 September 2017) mungbean genotypes was carried out in Central and North Queensland, Australia (preliminary trials; in house reports) beginning in 2017 and 2019 under optimum growth conditions. They were developed by crossing high-yield parental genotypes primarily bred to cope with water stress and high temperatures (Supplementary Material, Table S1). Four genotypes (AVTMB#1, AVTMB#2, AVTMB#3 and AVTMB#4) were selected based on their consistently higher seed yield. The seeds of check variety Jade-AU were obtained from the Australian Mungbean Association (https://www.mungbean.org.au) (accessed on 5 September 2020). Plant development stages were used as described by Grain Research and Development Corporation (2017) [37].

2.2. Growth Conditions

A glasshouse trial was undertaken over 84 days from 7 September 2020 using a Complete Randomised Design (CRD). The glasshouse was located at Central Queensland University (23.37° S latitude, 150.52° E longitude), Rockhampton, Australia, with temperatures maintained in the range of 25–35 °C with an average humidity of 55–60% (HOBO Pendant Temp-Light Data Loggers, UA-002-08, Onset, Australia).

Mungbeans were grown in polyvinyl chloride (PVC) pipes (Hollman irrigation pipes, Australia) 15 cm in diameter and 75 cm in height. A height of 75 cm was chosen to avoid root growth restriction. A PVC end cap (Hollman EzyFit caps; Australia) with a diameter of 15 cm and height of 3.5 cm was fitted at the bottom end of each PVC column to contain the vermiculite. Four holes (5 mm) were drilled into each PVC end cap to ensure proper drainage until stress application. Each column was lined with a plastic film to aid extraction of the vermiculite core in order to pull intact roots at harvest. Each column was filled with 2 kg of vermiculite (Ausperl Vermiculite Fine Gr 2, Fernland, Australia). Fertilisers (N/P/K/S) used were urea (46% N), phosphorus (containing 11.6% total phosphorus), and sulphate of potash (41% of potassium and 17% of sulphur) from RICHGRO garden product (Richard Pty LTD, Australia). The fertilizer was applied at the start of the trial at a rate of 150:75:75:75 N:P:K:S kg/ha (calculation based on plants in a hectare for the field-grown crop, assuming 300,000 plants/ha). Fertilizer applications based on gram per pot were N: 0.5, P: 0.25, K: 0.25, and S: 0.25. The additional sulphur was added using elemental sulphur (90%). Mungbean seeds were sterilised by immersion in a 3% H2O2 hydrogen peroxide solution for 5 min, rinsed, and hand sown at a depth of 2 cm at the rate of three seeds in each column. One week after germination, one healthy seedling from each genotype was retained in each column. The combination of PVC columns, vermiculite, and plants will collectively be referred to as pots for the remainder of this document.
Altogether, 60 pots were used to grow five mungbean genotypes with two water treatments in six replications, with 30 pots utilised for each water treatment. The pots were repositioned every week in order to avoid the impact of any microclimatic gradient within the glasshouse while maintaining a plant–plant distance of 50 cm.

2.3. Water Treatments

Two water treatments were applied to plants: a control treatment corresponding to 70–80% water holding capacity under free drainage and a waterlogging stress treatment. The weight of all 60 pots was recorded at the beginning of the trial following saturation and overnight drainage, i.e., water holding capacity. Pots were then weighed twice weekly (on Monday and Thursday) throughout the experiment to evaluate water loss gravimetrically [38]. Water was added to each weighing event to maintain all pots at 70–80% of their water holding capacity until the pod-filling stage at 49 days after sowing (DAS). The pod-filling stage (R4 stage) was achieved when one pod on any of the top three nodes of the plant developed a constriction belt between seeds [37]. Waterlogging stress treatment was imposed in 30 pots at the pod-filling stage (R4; 49 DAS) by maintaining a 5 cm standing water layer over the vermiculite surface. The control treatment corresponding to 70–80% water holding capacity was continued in the remaining 30 control pots throughout the trial period. Substrate oxygen concentration was assessed using a Fibox-3 oxygen meter (PreSens GmbH, Regensburg, Germany) [39] by placing oxygen sensors in pots (four pots from each treatment per genotype) at 15 cm depth in the pot.

2.4. Gas Exchange and Chlorophyll a Fluorescence

Light-adapted chlorophyll a fluorescence and gas exchange measurements were simultaneously recorded at 1, 9, 23, and 30 days after waterlogging stress (WL) imposition using an open gas exchange system with an integrated Fluorometer (Li-6800 Multiphase Flash™ Fluorometer, Portable Photosynthesis System, Li-Cor, Lincoln, NE, USA). The gas exchange parameters included photosynthetic rates \( (\text{A}_{\text{act}}; \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}) \), stomatal conductance \( (g_{\text{s}}; \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}) \), and intrinsic water use efficiency \( (i\text{WUE}; \mu \text{mol} \cdot \text{mol}^{-1}) \), and light-adapted chlorophyll a fluorescence included the effective quantum yield of PSII \( (\Phi_{\text{PSII}}) \) and the excitation pressure \( (1-qP) \).

For gas exchange and light-adapted chlorophyll a fluorescence measurements, the topmost fully expanded middle leaflet of a trifoliate leaf was used. The leaflet was carefully placed into the fluorescence chamber and allowed to acclimate to the chamber conditions for 10–15 min until the photosynthesis rate was steady \( (\text{d}F/\text{d}t < 5) \). Measurements were made each day from 8:00 am to 12:30 pm at a block temperature of 25 °C, a flow rate of 700 \( \mu \text{mol} \cdot \text{s}^{-1} \), a reference carbon dioxide concentration of 400 \( \mu \text{mol} \cdot \text{mol}^{-1} \), and a light-saturating intensity of 1500 \( \mu \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \). The time taken for each plant was ~10–15 min, and after recording all parameters, the leaflets were carefully removed from the chamber.

Dark-adapted chlorophyll a fluorescence \( (\text{Fv}/\text{Fm}) \) was determined in situ using a chlorophyll fluorometer (OS-30p; OptiSciences, Hudson NH, USA). The Fv/Fm ratio represents an estimation of the maximum quantum efficiency of PSII reaction centers of dark-adapted leaves [40]. For dark-adapted chlorophyll a fluorescence \( (\text{Fv}/\text{Fm}) \) measurement, the recent most fully expanded leaves (trifoliate) of four plants \( (n = 4) \) were covered with aluminium foil overnight and the maximum quantum yield in the dark \( \text{Fv}/\text{Fm} = (\text{Fm} - \text{Fo}) / \text{Fm} \) [40] was determined between 6:00 am and 7:00 am next morning. The aluminium foil was carefully removed to ensure that no light reached the leaf before the dark-adapted values were recorded.

2.5. Relative Chlorophyll Measurement

After gas exchange and chlorophyll a fluorescence measurements, leaf chlorophyll content was assessed using a handheld SPAD-502 m (Minolta Co. Osaka, Japan) on four
replicates sets used for gas exchange and chlorophyll a fluorescence measurements. This occurred on all measurement dates, i.e., at 1, 9, 23, and 30 days after WL stress imposition.

2.6. Morphology and Crop Biomass

Leaf count (#/plant) and plant height (cm) were recorded after harvest by the straight ruler method. At harvest, 84 days after sowing (DAS), plants were cut from the stem base and separated into leaves, stems, and pods. Except for pods, plant parts were put into paper bags and dried in the oven at 65 °C for 72 h to determine the dry weights (DW). Pods were sun-dried for use in further trials.

To determine root biomass (g DW/plant), the plastic liner containing vermiculite and roots was gently extracted from the pots. Roots were gently separated from the vermiculite and placed on a sieve with a mesh width of 2 mm. Extracted roots were then gently and thoroughly washed with tap water. The following parameters were recorded on a dry weight basis: leaf (g DW/plant), stem (g DW/plant), pods with seeds (g DW/plant), and root biomass (g DW/plant) (Table 1). Above-ground biomass (g DW/plant) was determined by combining the dry weights of all above-ground plant parts (stem, leaves, and pods with seeds inside). Root to shoot ratio was calculated by dividing the root biomass by the above-ground biomass. Seeds were separated from pods and weighed to record the seed yield (g/plant).
Table 1. Summary statistics for the effect of genotypes and treatment and their interaction with leaf count (LC; #/plant), plant height (PH; cm), leaf dry weight (LDW; g DW/plant), stem dry weight (SDW; g DW/plant), pods dry weight (PDW; g DW/plant), above-ground biomass dry weight (AGB; g DW/plant), root biomass (RB; g DW/plant), root: shoot ratio (RS), yield dry weight (YD; g/plant), 100-seed weight (100-SW; g), and harvest index (HI) of five mungbean genotypes (Four AgriVentis lines AVTMB#1–4 and Australian commercial variety Jade-AU). The presented values are the mean of four replications (n = 4; standard error). Different letters indicate significant differences according to the Tukey test (Two-way ANOVA). Values followed by the same letters are not significantly different (LSD < 0.05).

| Treatments | LC (#/Plant) | PH (cm) | LDW (g DW/Plant) | SDW (g DW/Plant) | PDW (g DW/Plant) | AGB (g DW/Plant) | RB (g DW/Plant) | RS | YD (g/Plant) | 100-SW (g) | HI |
|------------|--------------|---------|------------------|------------------|------------------|------------------|----------------|----|--------------|------------|----|
| Control    | 7.25 ± 0.91 bc | 30.75 ± 0.26 cde | 2.42 ± 0.10 cd | 1.22 ± 0.06 bcd | 4.05 ± 0.16 bcd | 8.09 ± 0.16 cdef | 0.50 ± 0.02 bcde | 0.06 ± 0.001 a | 2.92 ± 0.09 cde | 5.29 ± 0.19 bc | 0.36 ± 0.01 a |
| AVTMB#1    | 6.5 ± 0.53 abc | 33.88 ± 0.71 cde | 2.11 ± 0.22 bcd | 1.39 ± 0.12 d | 4.66 ± 0.27 cde | 8.57 ± 0.46 def | 0.60 ± 0.01 de | 0.07 ± 0.01 ab | 3.42 ± 0.23 e | 5.28 ± 0.01 bc | 0.40 ± 0.01 a |
| AVTMB#2    | 7 ± 1.15 abcd | 27.75 ± 1.74 bcde | 2.26 ± 0.23 bcd | 1.20 ± 0.12 d | 3.78 ± 0.45 bcd | 7.72 ± 0.84 cde | 0.50 ± 0.02 bcde | 0.07 ± 0.01 ab | 2.82 ± 0.35 cde | 5.58 ± 0.04 cd | 0.36 ± 0.01 a |
| AVTMB#3    | 7.25 ± 0.77 bc | 33.43 ± 2.97 de | 2.74 ± 0.11 d | 1.38 ± 0.05 cd | 4.98 ± 0.26 de | 9.75 ± 0.39 ef | 0.66 ± 0.11 e | 0.07 ± 0.01 ab | 3.62 ± 0.28 e | 5.76 ± 0.21 d | 0.37 ± 0.01 a |
| Jade-AU    | 8.25 ± 0.70 c | 33.50 ± 1.92 de | 2.65 ± 0.25 d | 1.60 ± 0.10 d | 5.60 ± 0.92 e | 10.42 ± 1.27 f | 0.58 ± 0.05 cde | 0.06 ± 0.001 a | 3.25 ± 0.09 de | 5.25 ± 0.03 abc | 0.33 ± 0.03 a |

Waterlogging

| Genotypes | p-values (LSD) | p-values (LSD) | p-values (LSD) | p-values (LSD) | p-values (LSD) | p-values (LSD) | p-values (LSD) | p-values (LSD) | p-values (LSD) | p-values (LSD) | p-values (LSD) |
|------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| AVTMB#1    | 5.75 ± 0.51 abc | 22.25 ± 0.63 ab | 1.19 ± 0.14 a | 0.79 ± 0.15 ab | 2.61 ± 0.43 ab | 5.03 ± 0.68 ab | 0.31 ± 0.01 a | 0.07 ± 0.01 ab | 1.83 ± 0.32 ab | 5.19 ± 0.01 ab | 0.36 ± 0.02 ab |
| AVTMB#2    | 4.5 ± 0.24 ab  | 19.25 ± 2.04 a | 1.01 ± 0.15 a | 0.66 ± 0.06 a | 2.16 ± 0.25 a | 4.20 ± 0.24 a | 0.37 ± 0.01 ab | 0.09 ± 0.01 b | 1.54 ± 0.17 ab | 4.91 ± 0.05 a | 0.36 ± 0.02 a |
| AVTMB#3    | 5 ± 0.33 ab    | 17.23 ± 2.14 abc | 0.93 ± 0.07 abc | 2.95 ± 0.34 ab | 9.45 ± 0.57 abc | 0.43 ± 0.04 abc | 0.07 ± 0.01 abc | 2.21 ± 0.26 abc | 5.43 ± 0.10 bcd | 0.37 ± 0.02 a |
| Jade-AU    | 7.50 ± 0.97 bc | 28.25 ± 2.57 bc | 2.06 ± 0.44 bc | 1.38 ± 0.29 cd | 3.48 ± 0.54 bc | 7.62 ± 1.31 bc | 0.48 ± 0.04 bc | 0.07 ± 0.01 ab | 2.81 ± 0.33 cd | 5.17 ± 0.03 ab | 0.39 ± 0.05 a |

Treatment (T)

| p-values | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| LSD       | 0.07   | 0.09   | 0.15   | 0.20   | 0.24   | 0.30   | 0.35   | 0.36   | 0.38   | 0.40   | 0.43   |

G x T

| p-values | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| LSD       | 0.16   | 0.17   | 0.34   | 0.45   | 0.56   | 0.68   | 0.78   | 0.80   | 0.85   | 0.91   | 0.94   |

Mean

| 6.60 | 28.12 | 1.97 | 11.3 | 3.76 | 7.38 | 0.49 | 0.07 | 2.68 | 5.31 | 0.36 |
2.7. Statistical Analysis

A two-way analysis of variance (ANOVA) was performed for gas exchange and chlorophyll a fluorescence parameters measured at four-time intervals for each measurement day using the statistical software GenStat version 20th edition (VSN International UK) after testing the data for normality, outliers, and homogeneity of error variances [41]. The Shapiro–Wilk test was carried out to test normality [42]. To test the homogeneity of variance of the residues, Bartlett’s test of homogeneity was conducted [42]. Main and interaction effects (genotype × treatment) were analysed. The F ratios were used to note treatment significance (p ≤ 0.05), and mean separation was carried out using Tukey’s HSD [43]. Correlation of coefficient was determined by using the “corrplot” R package [44,45]. Graphs were prepared using the R package “ggplot2” [46].

3. Results

3.1. Soil Oxygen

A significant decline (71%) in oxygen concentration (ppm) in waterlogging treatment compared to control plants was recorded after the imposition of waterlogging at the pod-filling stage and continued until the end of the crop growth stage R7 [37] (Figure 1).

![Figure 1. Time course of substrate oxygen levels after imposition of waterlogging (49 DAS); 0 DAS was the day immediately before waterlogging stress treatment imposition. Each data point represents the mean ± standard error (SE) of n = 4. Red line for waterlogging and green without (control).](image)

3.2. Gas Exchange Parameters

There were significant differences between mungbean genotypes, treatments, and their interaction for the gas exchange parameters (Asat and gs) throughout the measurement period. The considerable variations in mungbean genotypes and genotypes × treatments under waterlogging and control indicate that there is sufficient genetic variability among the genotypes studied. Henceforth, days after the imposition of waterlogging stress (WL) is abbreviated as DAIS.

Significant interaction effects between genotypes × treatment were observed for light-saturated photosynthetic rate (Asat; µmol m⁻² s⁻¹) on all the measurement days (1, 9, 23, and 30 DAIS). On 1 DAIS, Asat was significantly (p = 0.007) affected by the interaction of genotype and treatment, with WL causing a reduction of ~20% (averaged across all the genotypes relative to control plants) and AVTMB#1 reporting the highest mean value for Asat especially under control treatment (Figure 2A and Supplementary Table S2). Under WL, Asat was reduced by 29% overall across all the genotypes at 9 DAIS compared to the control treatment, while AVTMB#2 exhibited the highest mean value for Asat under WL.
compared to the other genotypes (see Figure 2B and Supplementary Table S2). There was a significant \( (p = 0.012) \) interaction effect for \( A_{sat} \) on 23 DAIS, with WL significantly reducing \( A_{sat} \) in AVTMB#1 relative to other genotypes (see Figure 2C and Supplementary Table S2). A significant \( (p < 0.001) \) genotype \( \times \) treatment interaction effect occurred at 30 DAS for \( A_{sat} \) in which genotypes AVTMB#1 and AVTMB#2 maintained relatively higher mean \( A_{sat} \) (12.50 and 11.63 \( \mu \)mol m\(^{-2} \) s\(^{-1} \), respectively) under control treatment, while the rest of the genotypes experienced a significant reduction under both control and WL treatments (see Figure 2D and Supplementary Table S2).

**Figure 2.** Light-saturated photosynthetic rate \( (A_{sat}; \mu \text{mol m}^{-2} \text{s}^{-1}) \) on days 1 (A), 9 (B), 23 (C), and 30 (D) and Stomatal conductance \( (g_s; \mu \text{mol m}^{-2} \text{s}^{-1}) \) on days 1 (E), 9 (F), 23 (G) and 30 (H) after imposition of waterlogging stress on five mungbean genotypes (AVTMB#1, AVTMB#2, AVTMB#3, AVTMB#4, and Jade-AU). Vertical bars show ± standard error for means of four replications. Capital letters represent interaction effects among genotypes in the control treatment \( (p > 0.05) \) and small letters illustrate interaction effects among genotypes under waterlogging stress. There is a substantial difference between mean values that do not share a common letter \( (p > 0.05) \). Red bars for waterlogging and green without (control).
Stomatal conductance (gs) was significantly (p < 0.001) reduced (~26%) by WL treatment relative to control plants after 1 DAIS. The mean gs of genotypes AVTMB#4 and Jade-AU were significantly reduced in WL (significant genotype effect; p < 0.001), compared to other genotypes (see Figure 2E and Supplementary Table S2). Significant interaction effects between genotype and treatment were observed at 9 (p < 0.001), 23 (p < 0.001), and 30 (p < 0.013) DAIS, resulting in an overall reduction of 22%, 20%, and 38%, respectively, relative to control plants. At 9 DAIS, AVTMB#1 exhibited significantly higher mean gs under control treatment, while a contrasting pattern in performance under waterlogging stress was reported (see Figure 2F and Supplementary Table S2). Nevertheless, at 23 DAIS, AVTMB#2 and AVTMB#3 had significantly greater mean gs under WL (see Figure 2G and Supplementary Table S2), while AVTMB#1 experienced significant reduction under both control and waterlogging treatment. Under waterlogging stress at 30 DAIS, Jade-AU exhibited considerably greater mean gs (see Figure 2H and Supplementary Table S2).

Increased Asat and decreased gs were associated with an increase in iWUE, which tended to increase more dramatically in the presence of waterlogging stress. Throughout the research period, a significant variation in iWUE was seen when waterlogging stress was applied. Significant interaction effects between genotype and treatment for iWUE were seen at 9 (p = 0.003), 23 (p = 0.036), and 30 (p = 0.001) DAIS (see Figure 3A–D and Supplementary Table S2). At 9 DAIS, genotype AVTMB#2 showed significantly higher mean iWUE under WL, although surprisingly at 23 DAIS, genotype AVTMB#1 demonstrated significantly higher mean iWUE under control treatment. Jade-AU had significantly higher iWUE at 30 DAIS, which indicates that Jade-AU can moderate the stress impact by shutting stomata while keeping Asat high.
3.3. Chlorophyll a Fluorescence

The quantum yield of photochemical energy conversion in PSII (ΦPSII) was significantly (p < 0.001) affected by WL at 1 DAIS and experienced a drop of 15%, relative to control plants. Significant (p < 0.001) genotypic variation was reported at 1 DAIS, where Jade-AU sustained a significantly higher mean ΦPSII under WL compared to other genotypes (see Figure 3E–H and Supplementary Table S2). However, no significant interaction effects were reported for ΦPSII at 1 DAIS.

The WL treatment declined ΦPSII by 9%, indicating a significant (p = 0.020) treatment effect only at 9 DAIS. The WL stress did not influence ΦPSII at 23 DAIS, representing no individual or interactive significant effects (see Figure 3E–H and Supplementary Table S2). However, a significant interaction effect between genotype and treatment was seen at 30 DAIS, with Jade-AU and AVTMB#4 exhibiting significantly greater mean ΦPSII under waterlogging stress (see Figure 3E–H and Supplementary Table S2).

Fv/Fm values ranged from 0.80–0.85 in control plants while being as low as 0.63–0.78 in plants subjected to waterlogging stress (see Figure 4A–D and Supplementary Table S2). Significant (p < 0.001) genotype × treatment interaction effects occurred at 1 DAIS due to significant reduction caused by WL treatment, particularly in Jade-AU (see Figure 3E–H and Supplementary Table S2). There was a 4% and 9% drop at 23 and 30 DAIS, respectively, while no significant genotypic or interaction effects were identified for 23 and 30 DAIS (see Figure 4A–D and Supplementary Table S2).
Figure 4. The maximum quantum yield of PSII in dark-adapted leaves (Fv/Fm) on days 1 (A), 9 (B), 23 (C), and 30 (D) and excitation pressure (1-qP) on days 1 (E), 9 (F), 23 (G), and 30 (H) after imposition of waterlogging stress; control treatment (green bars) and waterlogging stress (red bars) in five mungbean genotypes (AVTMB#1, AVTMB#2, AVTMB#3, AVTMB#4, and Jade-AU). Vertical bars show mean values (n = 4) and error bars represent standard errors. Values followed by the same letters (Tukey; Two-way ANOVA) are not significantly different (LSD < 0.05).

Throughout the measurement period, excitation pressure (1-qP) was significantly (p < 0.001) influenced by the interaction effect of genotypes and treatment (see Figure 4E–H and Supplementary Table S2). On 1 DAIS, WL resulted in a significant rise (~8%, averaged across all the genotypes relative to control plants) in 1-qP; genotype AVTMB#2 had a significantly higher mean 1-qP, whereas Jade-AU and AVTMB#4 had a significantly lower
mean 1-qP under WL. At 30 DAIS the total drop was 6%, with Jade-AU exhibiting the least rise in excitation pressure (see Figure 4H and Supplementary Table S2).

3.4. Relative Chlorophyll Measurement

Leaf chlorophyll content (SPAD units) dropped considerably during waterlogging stress and showed genotypic variations in comparison with the control treatment (see Figure 5A–D and Supplementary Table S2). Significant interaction effects between genotype and treatment for SPAD units were observed at 1 ($p = 0.001$), 23 ($p = 0.003$), and 30 ($p = 0.023$) DAIS. At 1 DAIS, leaf chlorophyll content began to fall (~6% averaged across all genotypes relative to control plants), with AVTMB#1 showing a significantly higher decline in mean SPAD units under waterlogging stress relative to the other genotypes (see Figure 5A–D and Supplementary Table S2). On 30 DAIS, AVTMB#1 showed the greatest drop in mean SPAD units, while AVTMB#4 demonstrated the least.

![Figure 5. Leaf chlorophyll content (SPAD units) on days 1 (A), 9 (B), 23 (C), and 30 (D) after imposition of waterlogging stress; control treatment (green bars) and waterlogging stress (red bars) in five mungbean genotypes (AVTMB#1, AVTMB#2, AVTMB#3, AVTMB#4, and Jade-AU). Vertical bars show mean values (n = 4) and error bars represent standard errors. Values followed by same letters (Tukey; Two-way ANOVA) are not significantly different (LSD < 0.05).](image)

3.5. Growth Attributes and Yield Attributes

Five mungbean genotypes were compared under two water treatments (control and waterlogging stress) to determine how waterlogging stress affects plant growth, dry weight (DW), and yield parameters. Significant genotypes and treatment effects were observed for leaf and stem DW, above-ground biomass, root biomass, and 100-seed weight. However, no significant interaction effects between genotype × treatment were observed for plant growth, dry weight, or yield parameters (Table 1).

Leaf count (LC; #/plant) was significantly ($p = 0.020$) reduced (~19%) under WL stress compared to control plants (Table 2). Similarly, WL significantly ($p < 0.001$) reduced plant height (PH; cm) by 23% (averaged across all the genotypes relative to control plants); see Table 1.
Waterlogging stress significantly ($p < 0.001$) reduced the above-ground biomass (g/plant) (AGB) by 34%. A significant genotype effect ($p = 0.025$) was observed for AGB in this study, with the lowest mean AGB exhibited by genotype AVTMB#2, while AVTMB#3 showed the highest mean AGB in WL the treatment as compared to the other genotypes (Table 1).

Root biomass (RB) was significantly ($p < 0.001$) affected by WL, and experienced a significant reduction of 26%, compared to the control treatment. The genotypic variations ($p = 0.043$) were observed in RB, with AVTMB#2 impacted the most and AVTMB#3 maintaining significantly higher mean RB relative to the other genotypes (Table 1). The genotypic responses in AGB and RB were found to be similar in the present study.

Pod DW significantly ($p < 0.001$) decreased by 36% under WL treatment compared to control plants (Table 1). The 100-seed weight (g) was significantly ($p = 0.004$) affected by WL, and a decline of 4% was reported when plants were subjected to WL at the pod-filling stage compared to the control treatment. In addition, there was a significant ($p = 0.004$) genotypic difference for 100-seed weight, with AVTMB#3 demonstrating a considerably greater mean 100-seed weight under WL stress than other genotypes, including Jade-AU (Table 1). The root to shoot ratio and harvest index remained unaffected by waterlogging stress in the present trial (Table 1).

Seed yield (YD; g/plant) ranged from 1.5–2.8 (g/plant) in both treatments, and yield was significantly ($p<0.001$) reduced by ~32% when exposed to WL stress compared to the control treatment. The lowest seed yield reduction was observed in Jade-AU and AVTMB#3 under waterlogging treatment (Tables 1 and 2). However, genotypic variation for YD was not statistically significant. Additionally, the interaction between genotype and treatment did not affect seed production (Table 1), suggesting that all genotypes responded similarly regardless of treatments, which is indicative of no significant yield penalty under waterlogging conditions when new AgriVentis genotypes are planted instead of Jade-AU (check variety).

Table 2. Percentage decline (%) in seed yield (g/plant) under waterlogging treatment at pod filling stage compared to control treatment in five mungbean genotypes (four AgriVentis genotypes AVTMB#1–4 and Australian commercial variety Jade-AU).

| Genotypes | Control Treatment Seed Yield (g/Plant) | Waterlogging Stress Seed Yield (g/Plant) | % Decline |
|-----------|---------------------------------------|----------------------------------------|-----------|
| AVTMB#1   | 2.92                                  | 1.83                                   | 37.5      |
| AVTMB#2   | 3.42                                  | 1.54                                   | 55.0      |
| AVTMB#3   | 2.82                                  | 2.21                                   | 21.8      |
| AVTMB#4   | 3.62                                  | 2.41                                   | 33.6      |
| Jade-AU   | 3.25                                  | 2.81                                   | 13.6      |

3.6. Correlation Analysis

Correlations among the gas exchange, chlorophyll a fluorescence, and yield-related parameters were observed (Figure 6). Gas exchange and chlorophyll a fluorescence parameters were recorded 30 days after stress imposition, while yield-related parameters were recorded at harvest. Of the gas exchange parameters, $A_{\text{net}}$ was highly positively correlated ($r = 0.86$) and $g_c$ was moderately positively correlated ($r = 0.69$) with seed yield (YD). For the chlorophyll a fluorescence parameter, seed yield (YD) was moderately positively correlated with $\Phi_{PSII}$ ($r = 0.59$) and $Fv/Fm$ ($r = 0.42$), while a strong negative correlation ($-0.87$) was observed for YD and $1-qP$. Under waterlogging stress, plants experienced a significant increment in $1-qP$; therefore, a negative correlation of $1-qP$ with seed yield exists. A negative correlation indicates a robust associative response to stress in plants. All yield-related characteristics were extremely significantly positive predictors of seed yield (Figure 6).
4. Discussion

Waterlogging stress can induce dynamic changes in morphological and physiological parameters in the mungbean with the resultant effects on yield reduction [47]. Furthermore, new climate change and crop yield models predict a drop in mungbean production. Thus, this issue needs to be addressed with potential solutions and waterlogging tolerant genotypes present a potential solution to waterlogging stressed environments. Insights regarding the performance of mungbean genotypes under waterlogging stress and their subsequent tolerance mechanisms can be integrated into future breeding programs [48]. In summary, developing tolerant mungbean genotypes to waterlogging stress is an essential approach to address this issue. Though long-established yield declines are reported in various crops, information addressing waterlogging tolerance strategies in mungbean is still limited.

4.1. Gas Exchange

Other than rice, most crops are not adapted to waterlogging stress [23,39]; therefore photosynthetic rates are severely impacted by waterlogging stress, whether transient or prolonged [49]. In the present study, we observed a significant reduction in photosynthetic rates (Asat) and stomatal conductance (gs) under waterlogging stress imposed at the pod-filling stage, to ~27% and 25% compared to control, respectively. Overall, the genotypes AVTMB#3 and Jade-AU experienced the least reduction in photosynthetic rates and stomatal conductance in waterlogging stress. At the leaf level, iWUE is defined as the ratio between Asat and gs, and is calculated as Asat/gs. Achieving higher iWUE (high Asat at a
lower g₀) is a desired trait and can be used as an indicator of stress tolerance. Closing stomata is the first response of plants after sensing stress, and therefore maintenance of high Aᵣ at low g₀ is a desirable trait for future breeding programs targeting the identification of stress-tolerant genotypes. In order to achieve higher iWUE, increased rubisco activity and electron transport ability are required. Thus far, many studies have attempted to explore this interrelationship; however, most studies have been carried out in response to elevated CO₂ and drought stress [50,51]. In the present investigation, Jade-AU exhibited higher iWUE on the last measurement day, indicating the desired potential for maintaining comparatively high Aᵣ at low g₀.

4.2. Chlorophyll a fluorescence

Chlorophyll a fluorescence is a non-invasive measurement of photosystem II (PSII) activity; thanks to the sensitivity of PSII activity to environmental stressors, it has become a critical approach for understanding the photosynthetic process as well as a wide indication of how plants adapt to environmental change [52,53]. One of the widely studied chlorophyll a fluorescence parameters for early stress detection is ΦPSII, which indicates the quantum efficiency of PSII electron transport in light, referred to as the operating efficiency of PSII [52]. It quantifies the amount of light absorbed by chlorophyll associated with photosystem II (PSII) and the rate of linear electron transport [40]. When plants are stressed, their first reaction is to close their stomata; this reduces the efficacy of light, which in turn reduces ATP and NADPH consumption. Hence, this reduction in electron transfer leads to a decrease in PSII [52,54]. The drop in ΦPSII is because PSII’s working efficiency diminishes, which results in fewer photons being employed in photochemistry under stressful conditions [40]. Several previous studies have indicated a decline in ΦPSII under waterlogging stress [2,23,55]. ΦPSII was reduced in all genotypes studied in this research, with the lowest reduction seen in Jade-AU at 1 and 30 days after stress imposition, suggesting that Jade-AU can avoid photodamage better than the other genotypes. Zhu et al. [56] credited this tolerance to the xanthophyll cycle’s capacity to protect photosynthetic machinery from photoinhibition damage on exposure to waterlogging stress. Moreover, the quickest response under waterlogging stress was interestingly observed in ΦPSII (14%, after one day of stress initiation); therefore, it can be used as an early screening parameter for waterlogging stress.

Fv/Fm was formerly believed to be a proxy for the effects of environmental stress on photosynthesis, and a drop in Fv/Fm is a useful predictor of photosynthetic impairment due to waterlogging stress. Throughout our investigation we found a decrease in Fv/Fm, suggesting PSII damage in dark-adapted leaves with the overall least reduction in AVTMB#3 and AVTMB#4 DAIS. These results are in agreement with an earlier study that reported a decline in Fv/Fm in mungbean subjected to waterlogging [32].

Stress causes stomata to close, causing an imbalance in photosynthesis that results in excess excitation energy. Inadequate dissipation of excess excitation energy can exacerbate photooxidative stress, which is generated by an abundance of electrons in photosynthetic light processes and eventually increases the excitation pressure (1-qP). The present study documented the increment in excitation pressure. The lower 1-qP reflects higher stress tolerance. In the present study, a significant increment in 1-qP occurred; overall, Jade-AU maintained a lower mean 1-qP.

SPAD units were reduced under waterlogging; overall, higher SPAD units under waterlogging at 30 DAIS were found in AVTMB#4. Many studies have reported a decline in SPAD values under waterlogging stress [12,57,58]. Reduction in SPAD units in mungbean has been found to occur due to degradation of chlorophyll caused by the accumulation of superoxide radicals under waterlogging stress [59].
4.3. Waterlogging Effect on Growth Attributes and Yield Attributes

Waterlogging stress at the pod-filling stage severely impaired plant development, gas exchange, and chlorophyll a fluorescence parameters. The reduction in these parameters was mirrored by reduced biomass (both above-ground and root biomass) and seed yield [60,61]. Other investigators have reported that prolonged waterlogging severely reduced dry weights [23,62]. Therefore, previous research corroborated the use of shoot development indices to assess the effects of waterlogging stress in the selection of waterlogging-tolerant genotypes [63].

The primary issue that appears under waterlogging stress is a reduction in oxygen content, which has a detrimental effect on the root system and, as a result, on above-ground development [12,64]. Waterlogging stress severely reduced the root biomass in almost all of the genotypes under waterlogging stress compared to control, however, lower-yielding genotypes experienced a more severe decline compared to higher-yielding ones in this study, which has been reported in previous studies as well. Under waterlogging stress, the growth of shallow roots restricts the absorption of nutrients, resulting in a decrease in above-ground biomass and pods that may not fill adequately or abort, leading to a decrease in yield [64]. A reduction in above-ground and root biomass was observed in the present study, with the least decline observed in genotype AVTMB#3 under waterlogging stress compared to other genotypes. Seed yield decline reported in this study was strongly correlated with decreased root biomass. The drop in seed yield seen in this study was substantially linked with a reduction in root biomass. Primarily, the number of pods per plant and pod setting decreased as a result of waterlogging stress. In the present study, pod abortion may be assigned as the primary reason for decreased yield. A similar reduction under waterlogging stress has been reported for mungbean [11,32], soybean [65] and snap bean [66].

In summary, plants can respond in a number of ways to waterlogging stress during the pod-filling stage to maintain their growth and development by utilizing their morphological characteristics and maintaining higher above-ground biomass. In the present study, AVTMB#3 maintained higher dry weights of leaf, stem, above-ground biomass, root biomass, and 100-seed weight.

5. Conclusions

Mungbean reproductive development was interrupted by waterlogging stress, leading to loss of pod dry weight and seed yield. A correlation study demonstrated that the seed yield was correlated with 1-qP, Asat, gS, and ΦPSII under waterlogging stress and differed between the genotypes. Therefore, evaluation of mungbean genotypes for waterlogging tolerance using rapid screening methods such as 1-qP, Asat, gS, and ΦPSII are suggested for mungbean breeding programs.

The assessments of genotypes for morpho-physiological traits suggest that there were significant differences between genotypes in terms of tolerances to waterlogging during the pod-filling stage. A significant difference between the genotype was observed for seed quality as well, as AVTMB#3 produced significantly larger seeds under waterlogging stress relative to other genotypes, including control (Jade-AU).

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy20121663/s1, Table S1: AgriVentis mungbean genotypes were selected for the screening of waterlogging tolerance at the pod filling stage with their line ID, progeny details, and country of origin; Table S2: Degrees of freedom (df), F-values, and probability (represented in parenthesis) of two-way ANOVA for four measurement periods (1, 9, 23, and 30 days after stress imposition) in five mungbean genotypes.

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