Tolerance of mungbean (*Vigna radiata* var. *radiata* (L.) R. Wilczek) to waterlogging stress at the germination and seedling stages

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ABSTRACT

Mungbean [*Vigna radiata* var. *radiata* (L.) R. Wilczek] is an important and nutritious grain legume crop. It is widely grown by smallholder farmers in the subtropical regions of Asia in upland and lowland ecosystems. Mungbean production is affected by soil waterlogging caused by unseasonal and increasingly frequent extreme precipitation events coupled with poor agricultural practices. Mungbean is sensitive to transient waterlogging at the germination and seedling stages. However, the novel genetic and phenotypic diversity of wild and domesticated mungbean has not been explored to identify valuable loci and germplasm for waterlogging tolerance.

This thesis focuses on the waterlogging tolerance of 292 mungbean mini core collection genotypes at two critical growth stages (germination and early seedling). The specific research objectives were to: (i) determine the effect of waterlogging duration on germination and survival of mungbean genotypes, (ii) determine a suitable waterlogging duration to screen for waterlogging tolerance (phenotypic screening) of seedlings, (iii) investigate phenotypic variation and identify sources of tolerance as a precursor to understanding the genetic control of waterlogging-tolerance traits, and (iv) understand the genetic basis of waterlogging tolerance in mungbean through genome-wide association studies (GWAS) focusing on associations between single-nucleotide polymorphisms (SNPs) and targeted traits.

The first experimental chapter established an efficient screening methodology to differentiate between susceptible and tolerant germplasm and identifies key traits that best facilitate adaptation or acclimation to waterlogging. Mungbean genotypes were compared under varying durations of waterlogging stress at the germination (0, 1, 2, 3, 4, 5, 6, 7, or 8 days) and seedling (0, 2, 4, 8, or 16 days) stages [15 days after sowing (DAS)] in a pot soil system in temperature-controlled glasshouse conditions. Two mungbean genotypes (green testa) contrasting in seed size and hypocotyl pigmentation—Celera II-AU (small seeded) and Jade-AU (large seeded)—and a benchmark blackgram genotype (Onyx-AU; black testa) were used. Waterlogging reduced soil redox potential, delayed or even prevented germination, decreased seedling establishment, and affected shoot and root development. Adventitious root formation and crown nodulation varied between the waterlogged (WL) mungbean seedlings, and 16 days of waterlogging substantially reduced growth but did not result in plant death. Waterlogging for 8 or 16 days followed by drainage and sampling at 39 DAS reduced shoot and root dry mass by 60–65% in mungbean and 40% in blackgram compared with continuously drained controls, due

at least in part to fewer lateral roots. Leaf chlorophyll content also decreased. Onyx-AU tolerated transient waterlogging at both growth stages better than Jade-AU and Celera II-AU. Celera II-AU had better seedling establishment than Jade-AU after imposing waterlogging at sowing. In contrast, Jade-AU had more plant biomass and greater recovery growth than Celera II-AU after waterlogging and recovery at the seedling stage. Both species had delayed emergence in response to shorter periods (2 days of WL) of transient waterlogging at germination, with longer waterlogging durations preventing germination and emergence. Both mungbean genotypes adapted to waterlogging stress at the seedling stage by forming adventitious roots.

The second experimental chapter characterised phenotypic and genetic diversity for waterlogging tolerance in 292 mungbean mini core collection genotypes under controlled conditions. Several genotypes were identified with waterlogging tolerance at two growth stages, which can be used as parents for pyramiding tolerance alleles and breeding robust cultivars that remain productive in the event of transient waterlogging during the germination and/or seedling stages. The screening methodology created hypoxic soil conditions to identify sources of waterlogging tolerance at both growth stages. At the germination stage, waterlogging tolerance was related to maintaining seed viability under hypoxia and subsequent emergence on the release of hypoxia. At the seedling stage, waterlogging tolerance correlated with rapid adventitious root formation. Frequency distributions of related traits revealed leptokurtic (positive kurtosis) and platykurtic (negative kurtosis) distributions, suggesting polygenic control of waterlogging tolerance with duplicate gene epistasis and dominant-based complementary epistatic gene action. The highest broad-sense heritability estimates occurred for emergence (81%) at the germination stage and shoot (81%) and root (79%) dry mass at the seedling stage. The estimated broad-sense heritability was 56% for adventitious root formation and 56–71% for leaf chlorophyll content at the seedling stage. Such heritability estimates combined with identifying sources of waterlogging tolerance demonstrate the possibility of selecting these traits and accelerating mungbean breeding for climate-resilient cultivars.

The final experimental chapter identified several genetic markers significantly associated with waterlogging tolerance using GWAS, increasing our understanding of the possible molecular mechanisms and genetic basis underlying waterlogging tolerance in mungbean. A total of 10,224 high-quality SNPs were used to identify significant loci associated with waterlogging tolerance and possible underlying candidate genes. At the germination stage, zinc finger protein (*ZFP8*) is a candidate gene for emergence after 4 days of waterlogging. At the seedling stage,

FGGY carbohydrate kinase domain-containing protein was associated with adventitious root formation. Significantly, transcription factor HHO5 was a pleiotropic gene related to three traits: shoot, root and total dry mass. Leaf chlorophyll content was associated with 3-oxoacyl-[acyl-carrier-protein] synthase, mitochondrial. Seed weight was associated with three genes: lipopolysaccharide-binding protein/bactericidal permeability-increasing protein (LBP/BPI), trehalose-6-phosphate synthase (TPS), and pentatricopeptide repeat-containing protein (PCMP-E1). These marker-trait associations provide the first insights into the possible molecular genetic control of waterlogging tolerance in mungbean and may provide the basis for adopting marker-assisted selection to increase breeding efficiency for developing waterlogging-tolerant mungbean lines.

Overall, the research presented in this thesis provides a strong foundation for understanding waterlogging tolerance in mungbean, which must be further built upon and validated under field conditions. In particular, it addresses the effect of waterlogging duration on the phenotypic variation of the mini core collection of mungbean genotypes for the first time. In addition, this study offers insight into the significance of waterlogging-tolerance traits and genomic regions for candidate genes at the germination and seedling stages for waterlogging tolerance of mungbean.

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DEDICATION

I dedicate this thesis to my late father, U San Oo, who played a significant role in shaping my personality and was a constant source of support and encouragement throughout my life. To my mother, Daw Tin Ohn, for her unconditional love, giving me the courage to walk all the paths of my life. To my sisters and brothers, for showering me with love and blessings. To my husband, U Than Hla Htay, who has been a constant source of support and encouragement during the challenges of my PhD journey.

AUTHORSHIP DECLARATION: CO-AUTHORED PUBLICATIONS

This thesis contains work that has been published and/or prepared for publication.

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I, Professor William Erskine, certify that the student's statements regarding their contribution to each of the works listed above are correct. As all co-authors' signatures could not be obtained, I hereby authorise inclusion of the co-authored work in the thesis. Coordinating supervisor signature: [redacted] Date: 08/09/2022

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LIST OF ABBREVIATIONS

ACIAR	Australian Centre for International Agricultural Research
$NH_4H_2PO_4$	Dihydrogen ammonium phosphate
ABA	Abscisic acid
ADH	Alcohol dehydrogenase
AMA	Australian Mungbean Association
AMPRIL	Arabidopsis multi-parent RIL
ANOVA	Analysis of variance
ANPs	Anaerobic proteins
APX	Ascorbate peroxidase
AR	Adventitious roots
AVRDC	Asian Vegetable Research & Development Center (The World Vegetable Center)
BLINK	Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway
BPI	Bactericidal permeability-increasing protein
C	Cohort
CAT	Catalase
CCI	Chlorophyll content index
CMLM	Compression mixed linear model
CO_2	Carbon dioxide
DArT	Diversity Arrays Technology
DAS	Days after sowing
df	Degrees of freedom
DGDG	Digalactosyldiacylglycerol
DI	Distilled water
DNA	Deoxyribonucleic acid
EGTA	Ethylene glycol-bis (β-aminoethyl ether
EM	Emergence
FAO	Food and Agriculture Organization
FDR	False Discover Rate
Fe^{2+}	Iron
GAPIT	Genomic association and prediction integrated tool
Gen	Genotype
GIS	Geographic information system
GLM	General linear model
GWAS	Genome-wide association analysis
H	Harvest

K	Most likely number of subpopulations
LBP	Lipopolysaccharide-binding protein
LD	Linkage disequilibrium
LiDaR	Light Detection and Ranging
LSD	Least significant differences
MAF	Minor allelic frequency
MAGIC	Multiparent advanced-generation intercross
MAS	Marker-assisted selection
MGDG	Monogalactosyldiacylglycerol
MLM	Mixed linear model
Mn^{2+}	Manganese
N	Nitrogen
P	Phosphorus
PCA	Principal component analysis
PCMP-E1	Pentatricopeptide repeat-containing protein
QQ plots	Quantile-Quantile plot
QTL	Quantitative trait locus
r^2	Squared correlation coefficient
RDM	Root dry mass
REML	Restricted maximum likelihood
RGR	Relative growth rate
RIL	Recombinant line
ROL	Radial oxygen loss
SDM	Shoot dry mass
SE	Standard error
SeedM	100-seed weight
SNP	Single-nucleotide polymorphism
SO_4^{2-}	Sulphate
SOD	Superoxide dismutase
SPAD	Soil plant analysis development
TDM	Total dry mass
TPS	Trehalose-6-phosphate synthase
Treat	Treatment
UDP	Alpha-trehalose-phosphate synthase
WL	Waterlogging
ZFP8	Zinc finger protein

CHAPTER 1. GENERAL INTRODUCTION

1.1 BACKGROUND ON MUNGBEAN

Mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) is a popular, short-season legume that can tolerate dryland farming systems, fix atmospheric nitrogen and decrease soil nitrogen depletion. It is a highly nutritious crop with seeds rich in iron and protein, making it an important supplement to cereal-based diets in South Asia (Nair et al., 2013). The crop belongs to the family Fabaceae or Leguminosae, subgenus Ceratotropics in the genus *Vigna* Savi (Baudoin and Marechal, 1988). Being a nitrogen-fixing legume, it represents an important renewable nitrogen source for agricultural soils (Peoples et al., 2009). Mungbean is predominantly grown in Asia, with some cultivated in Africa, Oceania, Canada and the United States of America. South Asia has the world's largest cultivated area (56%), followed by Southeast Asia (25%), East Asia (11%), and East Africa (7%) (Figure 1.1; Nair and Schreinemachers, 2020).

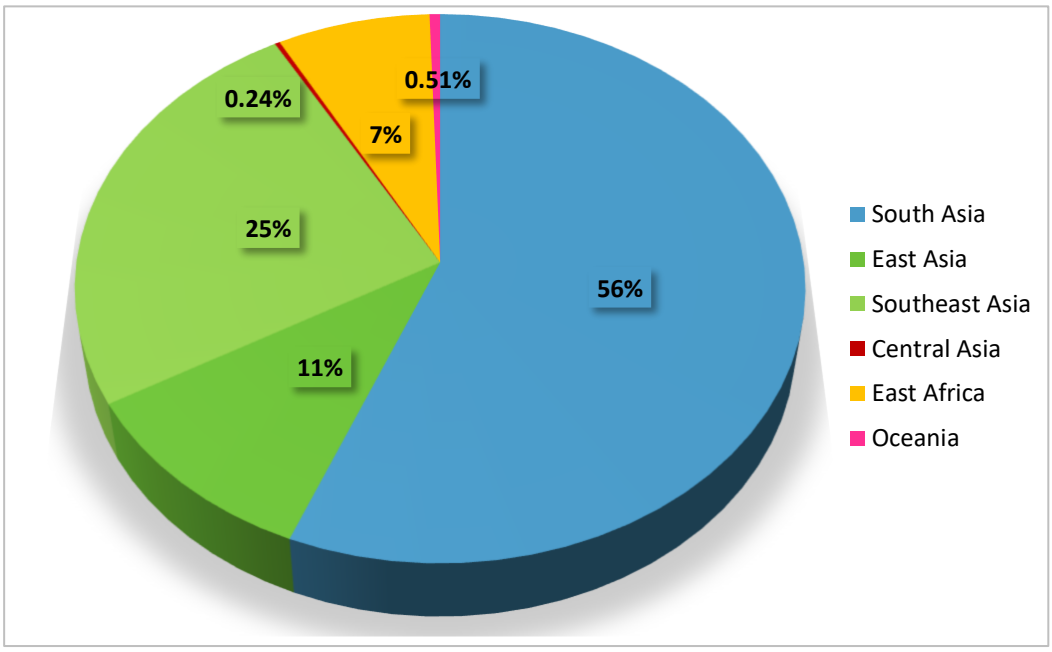


FIGURE 1.1 PERCENTAGE OF AREA SOWN TO MUNGBEAN FOR SELECTED REGIONS. DATA TAKEN FROM THE MUNGBEAN GENOME BOOK, 2020. SOURCE NATIONAL STATISTICAL AGENCIES FOR ALL COUNTRIES EXCEPT CHINA (USDA, 2009); THAILAND (USDA, 2014); KOREA (LEE, 2003); UZBEKISTAN (RANI ET AL., 2018) AND AUSTRALIA (AMA, 2014).

1.2 MUNGBEAN CROPPING PATTERN

In Asia, mungbean is widely grown in upland and lowland ecosystems. In upland ecosystems of Southeast and South Asia, mungbean is grown as an intercrop with other legumes, such as pigeonpea (*Cajanus cajan* L.), oilseeds [sesame (*Sesamum indicum* L.) or groundnut (*Arachis hypogaea* L.)] or cereal crops [sorghum (*Sorghum bicolor* L.) and maize (*Zea mays* L.)] in pre-monsoon and monsoon seasons (Islam et al., 1993; Herridge et al., 2019). In lowland ecosystems, mungbean is adopted as a relay crop, broadcast onto the standing rice crop 7–10 days before harvest or dibbled manually after harvest (Gupta et al., 2016). In Australia, mungbean is grown as a summer crop incorporated into the dryland cropping pattern, helping to fix up to 100 kg ha^{-1} N. Australia produces the highest mungbean grain quality, annually exporting approximately 66,000 tonnes to North America, Europe and the Middle East for net income of $6.7 million (www.pulseaus.com.au).

In the last two decades, the global mungbean area has increased from 4.6 to 7.3 million ha, with production and productivity increasing from 2.3 to 5.3 million tonnes and 500 to 721 kg ha^{-1} (Hartman et al., 1993; Nair and Schreinemachers, 2020). However, it is yet to reach its genetic yield potential (Douglas et al., 2020), with abiotic stresses due to extreme weather events challenging crop productivity (Charles, 2011). Among abiotic stresses, increased waterlogging has devastated crop production in some parts of the world due to a high frequency of unseasonal rainfall caused by adverse effects of global climate change (IPCC, 2021). For example, in Yezin, Myanmar, mungbean crops received about 100 mm of rain within a day in May 2018, causing transient flooding (Figures 1.2 and 1.3), threatening plant survival, and substantially reducing yield. Similarly, the 69-year average annual weather data from Barisal, Bangladesh, points to the cumulative frequency of heavy rainfall as challenging farmers to find an optimum sowing time for mungbean after a T-Aman rice crop (unpublished ACIAR annual finding, June 2018).

1.3 WATERLOGGING STRESS ON MUNGBEAN

Waterlogging can occur at any stage of plant development in rainfed and irrigated cropping systems due to irregular rainfall patterns (Setter and Waters, 2003). In addition, excess soil water immediately before or after rice harvest exposes seeds of the succeeding crop to waterlogging stress, resulting in reduced germination and/or poor crop establishment, as documented in field and glasshouse experiments for different legumes (Zaman et al., 2018). This type of waterlogging stress has been observed in some grain legumes grown as relay crops

after rice, including field pea (*Pisum sativum* L.), lentil (*Lens culinaris* L.), grass pea (*Lathyrus sativus* L.), and soybean (*Glycine max* L.), in countries in South, Southeast and East Asia, including Bangladesh, India, Nepal, Pakistan and Japan (Araki, 2006; Malik et al., 2015; Zaman et al., 2018). However, while farmers face production losses due to soil waterlogging, there is limited recorded data for yield losses in mungbean.

Understanding the genetic basis of waterlogging tolerance and developing climate-resilient varieties is essential for overcoming crop yield limitations due to waterlogging stress. Mungbean is considered highly sensitive to soil waterlogging, mainly during early stages of growth (Douglas et al., 2020). This PhD investigates waterlogging tolerance in mungbean using a set of mini core collection genotypes developed by the World Vegetable Center (Schafleitner et al., 2015). The three experimental research chapters focus on two critical growth stages in mungbean: germination and early seedling.

1.4 THESIS OUTLINE AND STRUCTURE

This thesis contains six chapters presented as a series of scientific articles, including one published manuscript (Chapter 3) and two manuscripts in preparation for submission to peer-reviewed journals. Chapter 1 includes a general introduction to mungbean and the issue of waterlogging stress impacting some crops Chapter 2 reviews the literature relevant to this thesis and outlines the motivation and gaps in knowledge that give rise to the research questions and objectives of this thesis. Chapter 3 is the first experimental chapter, examining the effect of waterlogging duration on germination and survival to identify appropriate conditions to screen for waterlogging tolerance (i.e. phenotypic screening) in the mungbean mini core collection. This chapter has been published in the peer-reviewed journal 'Frontiers in Plant Science' and reformatted for consistency within the thesis. Chapter 4 discusses phenotypic variation in the mungbean mini core collection under waterlogging stress and identifies sources of tolerance as a precursor to understanding its genetic control. Chapter 5 comprises a genome-wide association study to predict specific loci underlying the phenotypic traits of interest and understand the molecular mechanisms and genetic basis underlying waterlogging tolerance in mungbean. Chapter 6 summarises the findings and their collective implications for breeding waterlogging-tolerant mungbean crops.

FIGURE 1.2 WATERLOGGING IN A MUNGBEAN CROP

FIGURE 1.3 RECOVERY OF A MUNGBEAN CROP AFTER WATERLOGGING

CHAPTER 2. LITERATURE REVIEW

2.1. BACKGROUND

Mungbean (*Vigna radiata* var *radiata* (L.) R. Wilczek) is a short-season subtropical grain legume that is an important crop for smallholder farmers in Asia. As a nitrogen-fixing legume, it represents an important renewable source of nitrogen for agricultural soils (Peoples et al., 2009). Mungbean performs well under heat and drought stress (Nair and Schreinemachers, 2020) but is sensitive to waterlogging (Douglas et al., 2020). Mungbean is grown in both upland and lowland cropping systems. In the uplands, waterlogging has become major abiotic stress due to climate change. In the lowlands, excess soil moisture in relay cropping systems results in the crop experiencing transient waterlogging during early growth.

This literature review (1) outlines the effects of waterlogging on soil redox potential, nutrient uptake and plant acclimation during the recovery stage; (2) describes how waterlogging affects crop growth rate, agronomic management approaches, and adaptation traits at the germination and seedling stages; (3) explains genetic variation for waterlogging tolerance and associated loci uncovered thus far through genome-wide association analysis (GWAS); (4) discusses waterlogging tolerance mechanisms, genomic resources, and strategies for crop improvement. Overall, the literature review highlights the need to improve our understanding of the genetic basis of waterlogging tolerance in mungbean.

2.2 CHANGES IN SOIL REDOX POTENTIAL AND NUTRIENT UPTAKE LIMITATIONS

Waterlogging stress results from excess water in the topsoil profile creating an oxygen deficiency and anaerobic conditions in the plant root zone. Oxygen depletion creates a major crisis for root tissues in the rhizosphere through increased carbon dioxide (CO_2) and toxic ion accumulation (e.g. Fe^{2+}) and plant water and nutrient uptake deficiencies (Colmer and Voesenek, 2009). In waterlogged soil, soil microorganisms alternatively acquire oxygen from NO_3^- as an electron acceptor for respiration, reducing NO_3^- to NH_4^+ as a mineral nitrogen source. Nonetheless, radial oxygen loss (ROL) in the root zone oxidises NH_4^+ to NO_3^-, changing the available nitrogen form for root uptake. Consequently, manganese and iron oxidise to Mn^{2+} and Fe^{2+}, and sulphate (SO_4^{2-}) ions reduce to hydrogen sulphide (H_2S). Elevated amounts of these three ions lead to toxic levels in the soil solution (Sharma and Swarup, 1989; Smethurst et al., 2005; Khabaz-Saberi et al., 2010). As a result, the soil redox potential (i.e. soil anaerobic condition) decreases significantly in waterlogged soil (Pezeshki

and DeLaune, 2012; Pezeshki, 2001; Boivin et al., 2002; Lu et al., 2004). Hence, the soil redox potential reflects the changes occurring during waterlogging (Pezeshki and DeLaune, 2012).

Soil waterlogging causes oxygen deprivation and inhibits mitochondrial respiration due to the lack of the final electron acceptor, leading to an energy crisis that influences growth and biosynthetic processes (Drew, 1997; Bailey-Serres and Voesenek, 2008; Bailey-Serres et al., 2012). Under these circumstances in *Arabidopsis thaliana*, the expression of stress tolerance genes and metabolism-related proteins such as enzymes involved in glycolysis and fermentation is essential for producing the required energy to build up complex molecules and the anatomical response of the plant for water and nutrient uptake (Mustroph et al., 2009).

During waterlogging, endogenous nutrient levels decrease significantly in different plant parts. Hypoxia and subsequent anoxia in the root zone lead to losses in root membrane permeability. As a result, root membranes fail to uptake and mobilise essential nutrients, including nitrogen, phosphorous, potassium, magnesium, calcium, copper and zinc, into the shoots of canola (*Brassica napus* L.), wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) under transient waterlogging stress (Atwell and Steer, 1990; Stieger and Feller, 1994; Boem et al., 1996).

2.3 ACCLIMATION AT THE RECOVERY STAGE

Acclimation to waterlogging stress involves numerous processes, including energy generation through the glycolysis fermentation pathway, the ability to deal with carbon depletion, and buffering processes for toxic ions in the rhizosphere (Visser and Voesenek, 2004). During the recovery phase, plants suffer from dehydration due to the receding water levels. The sudden rise in oxygen enhances reactive oxygen species (ROS) production (Vashisht et al., 2016), damaging cells and tissues, particularly when oxygen first becomes available in the recovery phase (Bailey-Serres and Chang, 2005; Pucciariello et al., 2012). ROS includes peroxides, superoxides, hydroxyl radicals, singlet oxygen, and alpha-oxygen, all of which can age seeds and lead to a rapid loss of seed viability through cell membrane phospholipid degradation and the structural and functional deterioration of proteins and genetic material (Baxter et al., 2014; Kurek et al. 2019).

ROS can also act as signalling molecules. For example, the expression of the NADPH oxidase-related gene, *Atrboh* D, in *Arabidopsis* regulates hydrogen peroxide production, increasing *ADH1* gene expression to improve ethanol fermentation and plant survival under waterlogging (Sun et al., 2018). Plants also rely on antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and polyphenol oxidase to maintain the

dynamic balance of ROS, reducing the extent of oxidative damage (Bin et al., 2010; Hasanuzzaman et al., 2020; Bansal and Srivastava, 2012). In mungbean, SOD and APX in the roots of waterlogged plants are involved in stress tolerance (Sairam et al., 2009). These findings provide new insights into plant acclimation to waterlogging stress.

2.4 EFFECT OF WATERLOGGING ON PLANT BIOLOGY

Waterlogging stress disturbs the biochemical, anatomical and physiological systems of root, stem and shoot tissues, decreasing plant growth and productivity. Waterlogging changes the soil conditions from normoxic to hypoxic/anoxic (Loreti et al., 2016). Under hypoxia/anoxia, the insufficient oxygen supply compromises most cellular functions, leading to plant death. Under anoxic conditions, fluctuations in cytoplasmic pH influence cell integrity and function, energy consumption and anaerobic enzyme activities in most organisms, including plants (Roberts, 1984; Raven, 1986; Kennedy et al., 1992; Ratcliffe, 1997; Gout et al., 2001). Even transient waterlogging has long-term adverse effects on plant growth following soil drainage in sensitive crops. Taking wheat as an example, short-duration transient waterlogging can kill root apices, inhibiting seminal root growth and thus reducing root dry weight by two- to three-fold after only 3 or 7 days of stress (Malik et al., 2002).

In mungbean, waterlogging restricts root nodule activity and N fixation (Singh and Singh, 2011). The consequential yield reductions vary depending on the depth of soil waterlogging; for instance, crop losses were 12–17% when the water table was 5 cm below the surface but 40–100% when the soil water table reached the surface layer for 6 days (Garrity and Pernito, 1996). Singh and Singh (2011) reported that waterlogging for 48 h reduced plant height by 36–76%, leaf area by 12–46% and dry matter production by 25–57%. Waterlogging for 9 days at 30 days after sowing reduced the photosynthetic rate by 83%, decreasing productivity in the susceptible genotype (MH-1K-24) by 85% (Kumar et al., 2013). Eight days of waterlogging stress at the vegetative or reproductive stage decreased photosynthesis by 75% and 81%, transpiration by 58% and 62%, and stomatal conductance by 75% and 78%, respectively (Ahmed et al., 2002). In addition, fungal disease attacks and nitrogen limitations through leaching processes can reduce crop yields under waterlogging (Hunt and Gilkes, 1992). Reduced plant growth rates under waterlogging depend on genotypic adaptation; in a field experiment using 40 genotypes, total dry matter decreased by 43–84%, leaf area decreased by 6–80%, and plant height decreased by 3.85–76% (Amin et al., 2015). These findings reflect the adverse effects of soil waterlogging on plant growth and biomass based on only a few

mungbean genotypes. It is unknown whether genetic variation for waterlogging tolerance in mungbean exists and, if so, the genetic basis of its control.

2.5 AGRONOMIC APPROACHES TO MANAGING WATERLOGGING

Waterlogging affects 16% of the world's arable land area and accounts for 65% of agricultural crop losses, costing an estimated USD 74 billion annually (Conforti et al., 2018; Ploschuk et al., 2018). The adverse economic effects of soil waterlogging can be alleviated by adopting agronomic management practices that prevent or minimise crop production losses, such as sowing time adjustments, use of tolerant cultivars and cover crops, nutrient management practices (e.g. enhanced fertilise efficiency, rescue N application), and adaptive water management practices (e.g. improving the drainage system, raised bed planting) (Kaur et al., 2020). For example, raised bed planting systems for irrigated non-rice crops started in the 1970s on heavy clay soils in Australia (Maynard, 1991) and have been used widely for mungbean (Ram et al., 2018). This planting system improves irrigation management, nutrient availability to crop roots, crop establishment and weed management and decreases soil compaction (Aggarwal et al., 2000).

In addition, precision agricultural technologies based on yield maps, soil productivity maps, high-resolution digital elevation models from LiDaR (Light Detection and Ranging), flow accumulation, and soil electrical conductivity maps enable site-specific management to increase crop productivity (King et al., 2005). Many models are available to determine how management practices can alleviate the adverse effects of waterlogging and simulate crop growth and yield, such as the Agricultural Production Systems Simulator (APSIM; Asseng et al., 1997). In wheat, Bassu et al. (2009) used the APSIM-wheat model to predict the waterlogging effect for multiple planting dates. Similarly, the APSIM mungbean model (Brown et al., 2014) can be used to understand the impacts of soil waterlogging on plant growth and crop production which will help improve crop management practices to mitigate the adverse effects of waterlogging on mungbean. In addition, geographic information system (GIS) and remote sensing technologies can be used to identify precise areas in fields that are vulnerable to soil waterlogging and would benefit from crop or nutrient management practices to reduce waterlogging stress (Kaur et al., 2020).

2.6 ADAPTATION TRAITS

Plants can change their morphological and anatomical features, including hypertrophied lenticels in woody species and adventitious root formation and aerenchyma development in

short-duration crops, as adaptive mechanisms in response to soil waterlogging. Oxygen deprivation can be tolerated by some tissues when cells adjust metabolism, but even then a decrease in root hydraulic conductivity can result in shoot water deficits reducing the rate of photosynthesis as reported for pea (Zang and Zang, 1994) and barley (Yordanova et al., 2005). However, traits associated with waterlogging tolerance can vary according to the specific plant growth stage.

2.6.1 Germination stage

At germination, the waterlogging tolerance mechanism is related directly to the capacity of catalyst enzymes to degrade starchy endosperm into simple sugars to provide substrate for anaerobic metabolism (e.g. ethanol production) to release energy for growth and development. Faster germination and rapid coleoptile development through carbohydrate catabolism and cellular extensibility are essential traits for developing embryo and seed longevity, as reported in rice (Ismail et al., 2009; Angaji et al., 2010). Seed viability is important for germination because older seeds generally increase lipid peroxidation and reduce SOD and other enzyme activities resulting in poor germination (Cakmak et al., 2010). In pea (*Pisum sativum* L.), seed testa integrity under waterlogging is a key tolerance trait at the germination stage (Zaman et al., 2019). Anaerobic enzymes such as α-amylases and others also function to increase stress tolerance.

2.6.2 Seedling stage

Root system architecture plays a significant role in acquiring nutrients and water from the rhizosphere for seedling survival under waterlogging stress. Adventitious root formation, root extension rate, and increased gas-filled porosity of roots are major physiological traits for waterlogging tolerance in faba bean and chickpea (Munir et al., 2017). In maize, associated physiological traits for constitutive aerenchyma, an inducible tight ROL barrier and adventitious root formation play significant roles in waterlogging tolerance (Yamauchi et al., 2018). When seedlings elongate, aerenchyma tissues develop in the internal organs to support oxygen to hypoxic roots, known as the snorkel effect (Alpi and Beevers, 1983; Kawai and Uchimiya, 2000).

Tolerance mechanisms during the early vegetative stage evolve through increased available soluble sugars, rapid adventitious root formation from the soil surface zone, aerenchyma development in soft tissues, and enhanced glycolysis and fermentation processes. Waterlogging-tolerant legume species that produce adventitious roots include soybean (Morita

et al., 2004; Suematsu et al., 2017), mungbean (Kyu et al., 2021) and common bean (Soltani et al., 2017). Steffens and Rasmussen (2016) revealed that any non-root issue can produce adventitious roots under normal and stressful conditions, including drought, flooding, nutrient deficiency, and wounding. Waterlogging-induced adventitious roots are generally in the form of hypocotyl roots, crown roots, brace roots, nodal roots, and stem roots, depending on the nature of the species (Steffens and Rasmussen, 2016).

Understanding adventitious root and aerenchyma regulation under abiotic stress is essential for sustaining global crop production and creating environmentally resilient crop species. Compared with normoxia, waterlogging stress significantly reduces the gas exchange rate (by up to 10,000-fold), resulting in oxygen deficit and other gaseous accumulation in the rhizosphere (Armstrong et al., 1991; Blom and Voesenek, 1996; Bailey-Serres and Voesenek, 2008). Adventitious roots accelerate the gas exchange rate and nutrient and water uptake from roots to shoots. However, the emergence and formation of adventitious roots vary depending on crop species, genotype, plant growth, water temperature, waterlogging duration and waterlogging depth (Lorbiecke and Sauter, 1999; Dawood et al., 2014; Argus et al., 2015; Zhang et al., 2015). The formation of aerenchyma in adventitious roots and understanding its function under waterlogging are essential for identifying variation in tolerant and intolerant genotypes.

Aerenchyma, enlarged gas spaces interconnected along organs, is found in the roots and shoots of many aquatics and some dryland species in response to abiotic stresses, such as waterlogging, drought and nutrient deficiency (Evans, 2003). There are two types of aerenchyma: lysigenous, resulting from programmed cell death, and schizogenous, resulting from cortical cell separation. Lysigenous aerenchyma are found in many crops, including barley (Arikado and Adachi, 1955), wheat (Trought and Drew, 1980), rice (Armstrong et al., 1991) and maize (He et al., 1996; Gunawardena et al., 2001) and some wetland species, whereas schizogenesis aerenchyma are found only in various wetland species (Evans, 2003).

Aerenchyma formation depends on oxygen concentration, occurring under hypoxic but not anoxic conditions, with oxygen deficiency usually occurring in the stele behind the root tip (Gibbs et al., 1998). He et al. (1996) studied calcium-aided aerenchyma formation in maize using the Ca chelator, ethylene glycol-bis (β-aminoethyl ether) (EGTA), reporting that ethylene and the ethylene signal transduction pathway play a significant role in forming aerenchyma. However, there is little information on aerenchyma formation in mungbean under waterlogging stress.

2.7 GENETIC VARIATION FOR WATERLOGGING TOLERANCE IN CROP SPECIES

Identifying genetic variation for waterlogging tolerance traits present among and within a population is a prerequisite for breeding programs. Considerable variation in waterlogging tolerance exists within and between grain legume species. For example, faba bean (*Vicia faba* L.) produces adventitious roots and aerenchyma (increasing root porosity by 9%); thus, it is more tolerant to short-term waterlogging than yellow lupin (*Lupinus luteus*), grass pea, narrow-leaf lupin (*Lupinus angustifolius*), chickpea (*Cicer arietinum* L.) and lentil (Solaiman et al., 2007). Plant scientists use advanced genomic tools such as genome-wide association analysis based on single-nucleotide polymorphisms (SNPs) to identify genetic variants associated with stress tolerance.

2.8 GWAS: A POWERFUL MAPPING APPROACH FOR EXPLORING THE GENETIC DIVERSITY OF TRAITS

The genetic loci underlying a phenotype are determined using various mapping tools, including quantitative trait locus (QTL) mapping, a powerful method for identifying genomic regions that co-segregate with a given trait (Korte and Farlow, 2013). A drawback of QTL mapping analysis is that it allows polymorphisms only in segregating populations of bi-parental lines or recombinant inbred lines (RILs). The frequency of recombination is limited during the creation of RILs. While the frequency of allelic variation can be increased using advanced generation inter-cross (MAGIC) and Arabidopsis multi-parent RIL (AMPRIL), chromosomal variation cannot be increased as in natural populations (Korte and Farlow, 2013). Nonetheless, GWAS can incorporate greater allelic diversity and map more precisely than QTL. One of the main advantages of GWAS analysis is that it can assess greater genetic diversity for a species using a natural population without crossing rather than using bi-parental populations (Korte and Farlow, 2013). In addition, historical recombination events of the natural population can capture a greater mapping resolution than linkage mapping, provided that low levels of linkage disequilibrium (i.e. small linkage disequilibrium (LD) decay distances) exist in the GWAS mapping population and a large number of genetic markers (saturating the entire genome) are used (Korte and Farlow, 2013).

Hence, plant scientists consider GWAS based on the principle of LD as an alternative approach for detecting associations between DNA markers and target traits or for connecting the genotype–phenotype map (Gupta et al., 2005; Gómez et al., 2011; Korte and Farlow, 2013) to investigate complex traits and polymorphisms within and among populations by testing

genome-wide SNPs across an assembled population (Cortes et al., 2020). In the model crop, *Arabidopsis thaliana*, GWAS analysis identified 30 genetic markers associated with waterlogging tolerance traits in 81 germplasm accessions, indicating that various genetic changes were required for stress tolerance (Vashisht et al., 2016). Similarly, GWAS analyses have been used to identify genetic variants associated with traits of interest for waterlogging tolerance in many crop species, such as rice, maize, barley (Borrego-Benjumea et al., 2021; Manik et al., 2022), soybean (Yu et al., 2019) and common bean (Soltani et al., 2017).

Rice can germinate under flooding, with rapid coleoptile elongation and coleoptile length the major traits of its stress tolerance at the seedling emergence stage. The anoxia-stress-tolerant candidate gene, *LOC_Os06g03520*, controlling coleoptile elongation, has been detected in rice using GWAS (Zhang et al., 2017). In addition, six candidate genes for manganese toxicity tolerance in shoots and a gene (*LOC_Os02g37170*) with unknown function have been identified in rice using 416,741 SNP markers and a mixed linear model under waterlogging stress (Shrestha et al., 2018). Research on maize indicated that the early growth stage is the most sensitive to waterlogging due to its harmful effects on root growth below the soil surface, especially from the second to the seventh leaf stage (Osman et al., 2013).

For *Brassica napus*, the genetic basis of abiotic stress-tolerant traits is complex, with QTL analysis showing that QTL for waterlogging tolerance (assessed via various growth measures: plant height, root length, shoot and root dry weight, total dry weight) overlapped QTL for drought resistance at the germination and seedling stages (Zou et al., 2014).

Several recent studies have identified genetic loci and candidate genes associated with waterlogging tolerance in model legumes. In soybean (*Glycine max* L.), dominant alleles of *qWT_Gm03* controlled waterlogging tolerance at the vegetative stage (Ye et al., 2018). In common bean, genomic regions of Pv08/1.6 Mb and Pv02/41 Mb associated with root weight and germination are involved in waterlogging tolerance (Soltani et al., 2018). In field pea, differences in gene expression associated with waterlogging tolerance have been observed in transcriptomes for different genotypes (Zaman et al., 2018).

In *Phaseolus vulgaris*, GWAS analysis using ~150 K SNPs identified candidate genomic regions at Pv08/1.6 Mb and Pv02/41 Mb relating to physiological responses of germination rate and root weight (Soltani et al., 2017). These two regions were identified in soybean QTL for waterlogging tolerance, indicating that they might control the evolutionary pathway for stress

tolerance in legumes. However, it is not clear which genetic pathways contribute to waterlogging tolerance in legumes (Soltani et al., 2017).

Furthermore, there is a lack of information on the genetic variation for waterlogging tolerance in mungbean. Mungbean is a neglected crop; however, recent efforts have developed genetic and genomic resources for whole-genome studies, such as GWAS. Kang et al. (2014) constructed the first draft genome sequence of mungbean to facilitate genomic research. In addition, the World Vegetable Center – AVRDC harbours more than 6,700 mungbean accessions. Researchers from the World Vegetable Center developed a core collection set of 1,481 accessions based on geographical stratification and traits measured. The core collection reflects the diversity panel of the whole collection. Subsequently, the core collection was genotyped using 20 simple sequence repeat markers to produce a mini core set of 289 accessions covering the core collection's allelic and genotype diversity (Schafleitner et al., 2015). Nowadays, the mungbean mini core collection is available for breeders to access genetic variants of the crop.

Based on a literature review, Tables 2.1 and 2.2 outline the associated traits, controlling genes and physiological pathways for waterlogging tolerance involved in different species. The studies above highlight that the genetic control of tolerance varies across growth stages and further work is needed to understand waterlogging tolerance in mungbean.

TABLE 2.1 ASSOCIATED TRAITS, CONTROLLING GENES AND PHYSIOLOGICAL PATHWAYS FOR WATERLOGGING TOLERANCE IN DIFFERENT PLANT SPECIES

Species	Growth stage	Traits of interest	Gene control	Physiological pathway	Reference
Arabidopsis thaliana	Vegetative	O_2 content in leaf petioles and roots	Gene regulation to carbon metabolism, cell expansion & transcription factors	Anaerobic energy metabolism	Lee et al., 2011
Zea mays	Seedling	Plant height, root length, root, shoot and total dry mass	6 QTLs (ph6-1, rl1-2, sdw4-1, sdw7-1, sdw7- 1, tdw4-1, and tdw7-1) co-located with candidate genes	–	Osman et al., 2013
Glycine max	Early reproductive (R1)	Root system architecture	qWT_Gm03	Auxin-biosynthesis inhibitor	Ye et al., 2018
Vigna radiata	Seedling	Relative water content, MSI & chlorophyll content in leaves	D10266 for SuSy & Z23170 ADH	Carbohydrate metabolism	Sairam et al., 2009
Vigna radiata	Vegetative	Root development	Cytosolic-Cu/Zn-super- oxide dismutase (SOD) and cytosolic-ascorbate peroxidase (APX)	Antioxidant enzymes for scavenging reactive oxygen species	Sairam et al., 2011
Phaseolus vulgaris	Germination and seedling	Germination rate, total weight, leaf weight, root weight, hypocotyl length and SPAD index	Pv08/1.6 Mb and Pv02/41 Mb – associated with root weight and germination	–	Soltani et al., 2017
Phaseolus vulgaris	Germination and seedling	Germination rate	SnRK1.1 (Pv08/3.2 Mb), Pv07/4.7 Mb	Hypoxia response	Soltani et al., 2018
Pisum sativum	Germination	Testa membrane integrity and electro-conductivity	Tyrosine protein kinase	–	Zaman et al., 2018

TABLE 2.2 PHYSIOLOGICAL PATHWAY FOR WATERLOGGING TOLERANCE IN DIFFERENT PLANT SPECIES

Species	Growth stage	Traits of interest	Physiological pathway	Reference
Vigna radiata	Seedlings	Leaf and root dry weights	Net assimilation rate	Musgrave and Vanhoy, 1988
Vigna radiata	Vegetative	Leaf area and dry matter production	Superoxide dismutase, catalase and peroxidase	Prasanna and Ramarao, 2014
Cajanus cajan	Germination and seedling	Seed coat colour, seed size, maturity	–	Sultana et al., 2013
Cajanus cajan	Germination and early vegetative	Plant survival, chlorophyll content	Photorespiration and nutrient mobilisation	Singh et al., 2016
Phaseolus vulgaris	Seedling	Leaf relative water content, total sugar content in leaves and roots	Electrolytic leakage in cell membrane injury	Celik and Turhan, 2011
Brassica napus	Seedling and maturity	Plant height, branch height, primary branch, siliques on main shoots and branches, shoot and root lengths	–	Zou et al., 2014
Lens culinaris	Germination	Biomass, shoot weight, flowering and maturity	–	Wiraguna et al., 2017
Triticum aestivum	Seedling	Total, root and shoot dry weight index	–	Yu and Chen, 2013

2.9 RESEARCH QUESTIONS

This thesis explored the phenotypic and genetic diversity of waterlogging tolerance in mungbean mini core germplasm to expand our understanding of the genetic mechanisms controlling waterlogging tolerance in this species, particularly at the germination and seedling stages, and determine which traits best enable adaptation to the range of physiological challenges presented by waterlogging and identify tolerant mungbean lines for breeding. The following research questions were formulated to meet the overall thesis objective:

- Can mungbean recover from various waterlogging durations at the germination and seedling growth stages (Chapter 3)?
- Is there extensive genotypic variation in response to transient waterlogging among diverse mungbean germplasm to identify sources of tolerance and as a precursor to understanding the genetic control of this complex trait (Chapter 4)?
- Are there significant marker-trait associations for various traits reflecting waterlogging tolerance in mungbean (Chapter 5)?

To sum up, global climate change is threatening world crop production, including mungbean. Therefore, it is essential to develop climate-resilient varieties to increase mungbean productivity and profitability. Exotic germplasm lines and wild species from various agro-climatic areas have exploitable adaptive mechanisms and stress-tolerant traits. Understanding the physiological response of mungbean to waterlogging stress and the effect of waterlogging duration and investigating genotypic and phenotypic variation in mungbean germplasm will help identify waterlogging tolerance traits and their genetic control mechanisms. In this research, the AVRDC mungbean mini core collection genotypes, representing the core collection based on their genetic diversity assessment, will be studied for waterlogging tolerance using effective phenotyping protocols. In addition, GWAS will help discover genetic variants based on single-nucleotide variation and genomic regions for significant candidate genes at the germination and seedling stages in mungbean for use in mungbean breeding programs to develop new lines associated with targeted traits.

CHAPTER 3. RESPONSE OF MUNGBEAN AND BLACKGRAM TO TRANSIENT WATERLOGGING

3.1. ABSTRACT

Mungbean (*Vigna radiata* (L.) Wilczek) and blackgram (*Vigna mungo* (L.) Hepper) are important crops for smallholder farmers in tropical and subtropical regions. Production of both crops is affected by unexpected and increasingly frequent extreme precipitation events, which result in transient soil waterlogging. This study aimed to compare the waterlogging tolerance of mungbean and blackgram genotypes under varying duration of waterlogging stress at the germination and seedling stages. We evaluated the responses to different durations of transient waterlogging in a sandy clay loam under temperature-controlled glasshouse conditions. Waterlogging durations were 0, 1, 2, 3, 4, 5, 6, 7 and 8 days during germination and 0, 2, 4, 8 and 16 days during the seedling stage. We used two mungbean genotypes (green testa), Celera II-AU (small seeded) and Jade-AU (large seeded), contrasting in seed size and hypocotyl pigmentation, and a blackgram genotype (black testa), Onyx-AU. Waterlogging reduced soil redox potential, delayed or even prevented germination, decreased seedling establishment and affected shoot and root development. In the seedlings waterlogged at 15 days after sowing, adventitious root formation and crown nodulation varied between the genotypes and 16 days of waterlogging substantially reduced growth but did not result in plant death. Plants in soil with waterlogging for 8–16 days followed by drainage and sampling at 39 days after sowing had reduced shoot and root dry mass by 60–65% in mungbean and 40% in blackgram compared with continuously drained controls, due at least in part to fewer lateral roots. SPAD chlorophyll content was also reduced. Blackgram Onyx-AU was more tolerant to transient waterlogging than the two mungbeans in both growth stages. Of the two mungbean genotypes, Celera II-AU had greater seedling establishment than Jade-AU post-waterlogging imposed at sowing. In contrast, Jade-AU had more plant biomass and greater recovery growth than Celera II-AU after waterlogging and recovery during the seedling stage. Both species were delayed in emergence in response to the shorter periods of transient waterlogging at germination, and with the longer waterlogging germination and emergence failed, whereas at the seedling stage both showed adaptation by the formation of adventitious roots.

3.2 INTRODUCTION

Mungbean (*Vigna radiata* (L.) Wilczek) and blackgram (*Vigna mungo* (L.) Hepper.) are short-season (sub)tropical grain legumes and important due to their valuable seed nutritional

composition for the human diet and income for growers (Somta and Srinives, 2007). The crops are mainly grown in Asia, with some cultivated in Africa and Oceania. Globally, mungbean covers more than 7.3 million ha, with an annual global production of 5.3 million tons. India and Myanmar each produce about 30% of global output (Nair and Schreinemachers, 2020). Global blackgram production reached 3.2 million tons in 2018, with India producing 1.9 million tons on 3.5 million ha and Myanmar generating 1.24 million tons on 9.78 million ha (Soe et al., 2020). Both crops are predominantly grown in the tropics in rainfed farming systems (Lawn and Ahn, 1985), where yield variation is high due to biotic and abiotic stresses.

Abiotic stresses are a major environmental problem in agricultural crop production (Lesk et al., 2016). Soil waterlogging is an abiotic stress that can affect crop growth and development. This adversely affects crop production and farmers' profits. In waterlogged soil, oxygen deprivation is the major impediment to root growth and functioning. Oxygen is consumed by the respiration of plant roots and soil microorganisms, so the soil becomes anoxic within a few hours to days (Ponnamperuma, 1984; Setter and Waters, 2003). Oxygen deficiency in waterlogged soil can adversely affect nutrient uptake and translocation by roots; as an example, Malik et al. (2002) demonstrated in wheat that shoot N status was reduced during first 7 days of waterlogging that eventually affected growth. Rasaei et al. (2012) reported that soil N content decreases through rapid volatilisation and denitrification processes. Furthermore, prolonged waterlogging can lead to the accumulation of some ions (i.e. Mn^{2+}, Fe^{2+}) to potentially toxic levels (McKee and McKevlin, 1993). In these conditions lacking oxygen, oxidative phosphorylation ceases, yielding low ATP from sugar catabolism and hindering the metabolic functions required for seed germination and then for seedlings for root growth and nutrient acquisition by roots (Yamauchi et al., 2018).

Mungbean and blackgram are both considered highly sensitive to soil waterlogging, mainly during the early stages of growth (Bansal et al., 2019; Douglas et al., 2020). Crops can often be exposed to transient waterlogging during their growth cycle due to extreme weather events (i.e. intense storms bringing rain) and poor soil drainage. Fernandez and Shanmugasundaram (1988) reported that mungbean yields severely declined with annual rainfall >1,000 mm. Flooding restricts the aeration around mungbean roots reducing nodule activity and N fixation (Singh and Singh, 2011). In addition, the 'weakened' plants can be further infected by fungal diseases and suffer from insect pests (Tickoo et al., 2006).

Mungbean and blackgram are widely grown in both upland and lowland ecosystems in Asia. In upland ecosystems of Southeast and South Asia, mungbean is grown as an intercrop with other

legumes, such as pigeonpea (*Cajanus cajan* L.), oilseeds [sesame (*Sesamum indicum* L.) and groundnut (*Arachis hypogaea* L.)] or cereal crops [sorghum (*Sorghum bicolor* L.), maize (*Zea mays* L.)], during pre-monsoon and monsoon seasons (Islam et al., 1993; Herridge et al., 2019) and blackgram is sown as a sole crop during the post-monsoon period. In lowland ecosystems, both legumes are widely grown as relay crops by broadcasting onto the standing rice crop 7–10 days before harvest or dibbling manually after harvest (Gupta et al., 2016). Excess soil moisture immediately before or after rice harvest exposes the seeds of the succeeding crop to waterlogging stress, resulting in reduced germination and/or poor crop establishment, as documented for field and glasshouse experiments for different legumes (Zaman et al., 2018). This type of waterlogging stress has been observed in some grain legumes grown as relay crops after rice and studied for their waterlogging tolerance—for instance, pea (*Pisum sativum* L.), lentil (*Lens culinaris* L.), grasspea (*Lathyrus sativus* L.) and soybean (*Glycine max* L.)—in countries in South, Southeast, and East Asia, including Bangladesh, India, Nepal, Pakistan, and Japan (Samad et al., 2001; Araki, 2006; Malik et al., 2015; Zaman et al., 2018).

To overcome crop yield limitations due to waterlogging stress, it is essential to understand the tolerance mechanism of crops to the stress and develop climate-resilient varieties. Plants adapted to complete submergence, which occurs from deep floods, but also can occur for seeds and seedlings in shallow water and waterlogged soils, have two syndromes (groups of mechanisms) for coping with waterlogging—namely, quiescence and escape (Bailey-Serres and Voesenek, 2008; Colmer and Voesenek, 2009). Recently, both syndromes have been observed in pea genotypes at germination: quiescence was characterised as no germination during several days of waterlogging and following soil drainage, germination occurred and seedlings emerged, whereas escape was germination and seedling emergence during waterlogging (Zaman et al., 2018). Furthermore, testa integrity of seeds under waterlogging is a key tolerance trait for germination (Zaman et al., 2019) because the seed testa serves as a shield for the embryo against adverse environments (Debeaujon et al., 2000). Germinating seeds can tolerate anoxia (absence of oxygen) after imbibition but before the rupture of the testa (Leblovà et al., 1969). Rupturing seed testa due to waterlogging leads to deterioration of membranes and leakage of cellular contents, resulting in failure in germination and/or seed death (Johnson et al., 1989; Zaman et al., 2019). Information on the physiological responses of mungbean to soil waterlogging is scarce, and the types of waterlogging tolerance mechanisms exhibited by mungbean (Douglas et al., 2020) and blackgram remain unknown, especially in early growth. Therefore, this study focused on the waterlogging tolerance of mungbean and

blackgram genotypes under varying durations of waterlogging stress at two critical growth stages: germination and seedling stages.

3.3 MATERIALS AND METHODS

3.3.1 Plant materials

Two mungbean genotypes, Celera II-AU and Jade-AU with contrasting seed size and hypocotyl pigmentation, and blackgram genotype Onyx-AU were used in this study. A single blackgram genotype was used as a benchmark for the genotypes of mungbean, which is of greater economic importance. The genotypes were obtained from the Department of Agriculture and Fisheries, Queensland (Table 3.1).

TABLE 3.1 HYPOCOTYL PIGMENTATION AND TESTA COLOUR, 100-SEED WEIGHT, AND DAYS TO FLOWERING AND MATURITY IN TWO MUNGBEAN GENOTYPES (CELERA II-AU AND JADE-AU) AND A BLACKGRAM GENOTYPE (ONYX-AU).

Cultivar	Species	Hypocotyl pigmentation	Seed testa colour	100-seed weight (g)	Days to	
					Flower	Maturity
Celera II-AU	*V. radiata*	Purple	Shiny green	4.2	42	77
Jade-AU	*V. radiata*	Green	Shiny green	8.1	44	85
Onyx-AU	*V. mungo*	Purple	Black	6.6	45	>80

Source: AMA (2020)

3.3.2 Experimental conditions

The experiments were conducted in a temperature-controlled glasshouse at The University of Western Australia (UWA), Crawley, Western Australia (31° 59’ S, 115° 49’ E) from 15 May to 7 June 2018 (germination stage) and 10 October to 19 November 2018 (seedling stage). The temperature inside the glasshouse ranged from 21±4 (night) to 32±3°C (day) at both growth stages. The day length was approximately 10 h 20 min with maximum PAR of 1,000–1,098 µmol m^{-2} s^{-1} in May, and 12–14 h in Oct–Nov with PAR of 1,400–1,630 µmol m^{-2} s^{-1}. The growing media comprised red-brown sandy clay loam (Calcic Haploxeralf), which had been used for waterlogging studies in pea (Zaman et al., 2018) and grass pea (Wiraguna et al., 2020). The soil was collected from Mukinbudin (30° 78’ S, 118° 31’ E), Western Australia (Kotula et al., 2015), with soil pH ($CaCl_2$) of 7.8, electrical conductivity (EC) 0.64 dS m^{-1} and 1:5 w/v soil/water organic carbon content of 0.26% (Supplementary Table 3.1). The soil was dried for 5 days at 65°C and sieved to 2 mm in diameter. The water content (w/w) at field capacity (i.e. pot capacity when fully drained) was 18%.

3.3.3 Experiment A: Waterlogging at the germination stage

The experimental design was a split plot with three replications. Waterlogging was the main factor, and genotypes were subfactors. The genotypes (Table 3.1) were exposed to nine durations of waterlogging (0, 1, 2, 3, 4, 5, 6, 7 or 8 days). At the end of the waterlogging period, waterlogged pots were drained, and seedling emergence was recorded. The experiment ended 15 days after sowing (DAS).

Seeds were surface sterilised with 1% commercial bleach (active ingredients NaOCl 40 mg L^{-1}) for 1 min and rinsed with deionised water four times. To control seed-borne and seedling root pathogens, P-Pickel T liquid fungicide [Thiram (360 g L^{-1}) + Thiabendazole (200 g L^{-1})] was applied 300 mL 100 kg^{-1} seed. Twenty seeds of each genotype in each of 48 pots were sown either in drained (control) or waterlogged pots at 10 mm depth. The experimental pots were 0.8 L (90 × 90 × 180 mm) with drainage holes (~10 mm) at the bottom. The drainage holes were covered with filter paper to avoid soil loss, and the pots filled with 100 g of gravel followed by 1.0 kg of soil. After potting up, all pots were placed in 60 L plastic tanks and kept at 80% field capacity for 2 days before sowing and experimental layout according to the design. Twelve platinum electrodes: six each for drained and waterlogged pots were placed at 100 mm depth to measure soil redox potential.

The waterlogging treatment was imposed immediately after sowing by adding DI water to the 60 L tanks, as described by Zaman et al. (2018). The water table was maintained at the soil surface for the duration of the waterlogging treatment by adding DI water to the tanks. Drained control pots were kept at 80% field capacity by adding DI water directly to the pots as required throughout the experimental period. At the end of each waterlogging treatment, pots were relocated to free-draining plastic tanks to record emergence and seedling growth during recovery. The 80% field capacity was maintained in the recovery pots by adding additional DI water as required. After 15 days of recovery, the germinated seedlings were gently washed from the soil with tap water for measurements.

3.3.3.1 Measurements for Experiment A

Soil redox potential was measured daily with platinum electrodes (Pt) and an Ag–AgCl reference electrode using a handheld Digital Multimeter (Fluke 114, Everett, Washington, USA). Redox measurements were corrected according to the method developed by Patrick et al. (1996). Seeds with an epicotyl >5 mm were recorded as germinated (i.e., emergence). At

harvest, the percentage of seedling establishment was recorded based on the number of fully grown seedlings.

3.3.4 Experiment B: Waterlogging at the seedling stage

The experiment had a split-plot design with four replications. Duration of waterlogging [0 (WL0), 2 (WL2), 4 (WL4), 8 (WL8) and 16 (WL16) days] was the main factor, and genotype was the subfactor (Table 3.1). After waterlogging, the waterlogged pots were drained to observe plant growth during the recovery. The duration of the recovery period differed for each waterlogging treatment (WL2: 22 days, WL4: 20 days, WL8: 16 days and WL16: 8 days), with the experiment terminated 39 DAS. Six Pt electrodes were placed at 100 mm depth in each waterlogged and drained soil to measure soil redox potential.

Seeds were surface sterilised as described for Experiment A and then inoculated with Group I Rhizobium strain CB 1015 (New Edge Microbial, New South Wales, Australia). Six seeds per pot [free-draining 4 L plastic pots (145 × 145 × 220 mm) with drainage holes (15 mm)] were sown at 30 mm depth in 76 pots for each genotype. Four days after emergence, plants were thinned to two seedlings with similar vigour per pot. The drainage holes inside the pots were covered with filter paper before filling with 500 g of gravel followed by 4 kg of sieved dry soil. The pots were placed in 60 L (310 × 620 × 455 mm) plastic tanks (eight pots per tank) and watered as necessary to keep at 80% field capacity. Each pot was an experimental unit and received 40 mg kg^{-1} of dihydrogen ammonium phosphate [(NH_4) (H_2PO_4)], based on a soil analysis.

Waterlogging treatments were imposed 15 DAS, after the first trifoliate leaf had fully opened. For each genotype, 56 pots were waterlogged to the soil surface, with 20 kept as drained controls watered daily to 80% of field capacity. The harvesting methodology was similar to that used by Malik et al. (2002). Considering the start of the treatment as Day 0 (H1), four pots from each genotype were harvested as initial harvest, with sequential harvesting on Days 2, 4, 8, 16 and 24 (Table 2). On Day 2 (H2), four control pots and four waterlogged pots from each genotype were harvested; meanwhile, 16 waterlogged pots were drained for harvest on Days 4, 8, 16 and 24. Similarly, four control pots and four waterlogged pots were harvested on Day 4 (H3), with 12 pots drained for harvest on Days 8, 16 and 24. On Day 8 (H4), four control pots and four waterlogged pots were harvested, with eight pots drained for harvest on Days 16 and 24. On Day 16 (H5), four control pots and four waterlogged pots were harvested, with four pots drained for harvest on Day 24 (H6). This sequential harvest increased the number of treatments

at each successive harvest, resulting in six experimental treatments at the final harvest on Day 24.

Table 3.2 Harvesting schedule for Experiment B: Initial harvest occurred on the day waterlogging was imposed at 15 days after sowing (DAS). Waterlogging lasted for a maximum of 16 days. Sequential harvests occurred for each waterlogging duration treatment with successive recovery compared with the drained controls.

Treatment	Harvest	DAS
WL0	H1 (Day 0)	15
WL0, WL2	H2 (Day 2)	17
WL0, WL2, WL4	H3 (Day 4)	19
WL0, WL2, WL4, WL8	H4 (Day 8)	23
WL0, WL2, WL4, WL8, WL16	H5 (Day 16)	31
WL0, WL2, WL4, WL8, WL16	H6 (Day 24)	39

3.3.4.1 Measurements for Experiment B

Soil redox potential was measured daily in the waterlogged and drained pots as described for Experiment A. The number of large and small, fully opened trifoliate leaves of one plant per pot was recorded the day before harvest. At harvest, parameters were recorded on two plants per pot. The data for each pot (two plants per pot) were pooled and the mean used as one replicate.

Chlorophyll content was measured using a handheld Minolta SPAD 502 (Konica-Minolta, Japan) on the first trifoliate leaf of each plant on the day of harvest. Twelve independent measurements per genotype were done for each treatment. At harvest, plant height was measured from the collar (point on the stem where roots start to grow) to the leaf base of the youngest fully expanded leaf of the plant. After gently washing the soil from the roots, the maximum taproot length was measured. The number of emerged adventitious roots longer than 5 mm and their length was recorded. Total leaf area per plant was recorded for each plant using a leaf area meter (LI 3000C, Lincoln NE, USA). Nodulation was scored by counting the nodules on the main taproot and lateral roots and using a 0–8 scoring scale according to Yates et al. (2016), where 0 = no nodules, 0.5 = white ineffective nodules, 1 = rare effective, 2 = scarce, 3 = moderate, 4 = adequate, 5 = ample, 6 = abundant, 7 = very abundant and 8 = extremely abundant. Finally, the plants were divided into shoots and roots and dried in paper bags in a 60°C oven for 3 days to record shoot and root dry weights.

3.3.5 Statistical analysis

Analysis of variance (ANOVA) was performed for each waterlogging treatment and compared with the controls using GenStat 19th edition (VSN International, UK). The effect of waterlogging was based on the significance level of main and interaction effects. For all analyses, the means were separated based on their significance levels at 0.05 probability using Tukey's test (Tukey, 1949). The estimated per cent SPAD chlorophyll content was based on the untreated mean. The relative growth rates (RGR) of shoots and roots were calculated for each waterlogging duration and successive recovery period, according to Hunt (1990).

3.4 RESULTS

3.4.1 Waterlogging tolerance at the germination stage

The redox potential in waterlogged pots decreased gradually from 428±18 mV to 236±38 mV, stabilising after 5 days of waterlogging (Supplementary Figure 3.1). The drained control pots remained at 428±18 mV throughout the experimental period.

Waterlogging reduced seedling emergence in all genotypes relative to the drained control (Figure 3.1). The waterlogged seedlings started to emerge 2 days after removing the stress, with full emergence within 10 days after removing the stress on average. Emergence in the drained control pots started 3 DAS, with full emergence completed by 7 DAS in all genotypes. Seedling emergence was significantly ($P<0.001$) reduced by the waterlogging duration.

With 4 days of waterlogged treatment, the largest genotypic differences were observed for the number of seedlings, with Onyx-AU (50% reduction) followed by Celera II-AU (45%) and Jade-AU (25%) (Figure 3.2A). Some waterlogged seeds failed to emerge; for example, only 5% of Onyx-AU seedlings had emerged in the 8 days of waterlogging treatment by the end of the experiment, with none for the other two genotypes (Figure 3.2B).

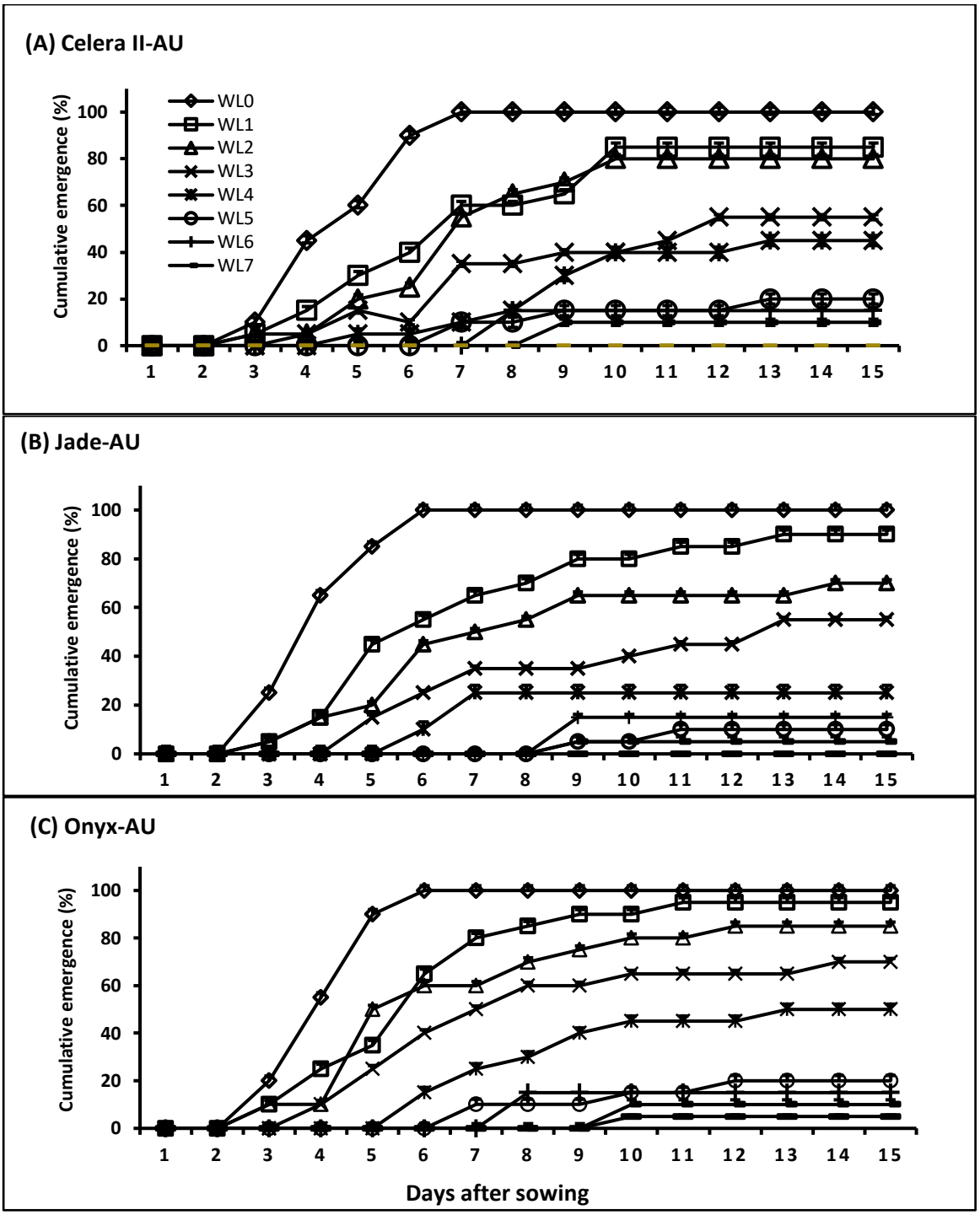


FIGURE 3.1 PERCENTAGE OF CUMULATIVE EMERGENCE IN MUNGBEAN **(A)** CELERA II-AU AND **(B)** JADE-AU AND BLACKGRAM **(C)** ONYX-AU UNDER DIFFERENT DURATIONS OF WATERLOGGING AND SUBSEQUENT RECOVERY. WL0, DRAINED CONTROL; WL1, WATERLOGGING FOR 1 DAY AND RECOVERY FOR 14 DAYS; WL2, WATERLOGGING FOR 2 DAYS AND RECOVERY FOR 13 DAYS; WL3, WATERLOGGING FOR 3 DAYS AND RECOVERY FOR 12 DAYS; WL4, WATERLOGGING FOR 4 DAYS AND RECOVERY FOR 11 DAYS; WL5, WATERLOGGING FOR 5 DAYS AND RECOVERY FOR 10 DAYS; WL6 WATERLOGGING FOR 6 DAYS AND RECOVERY FOR 9 DAYS; WL7, WATERLOGGING FOR 7 DAYS AND RECOVERY FOR 8 DAYS; WL8, WATERLOGGED FOR 8 DAYS AND RECOVERY FOR 7 DAYS. SYMBOLS ARE MEANS ± SE OF THREE REPLICATES.

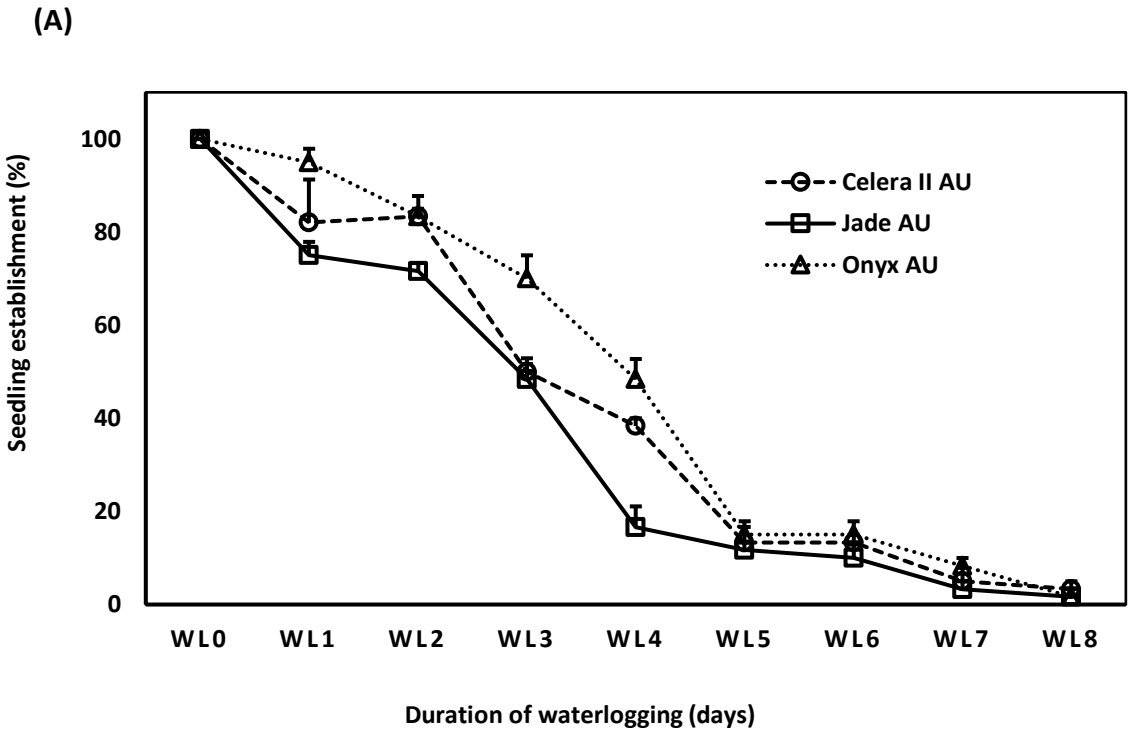


FIGURE 3.2 SEEDLING ESTABLISHMENT (%) OF **(A)** MUNGBEAN (○) CELERA II-AU, (□) JADE-AU AND BLACKGRAM (Δ) ONYX-AU AFTER DIFFERENT DURATIONS OF WATERLOGGING (WL0, DRAINED CONTROL; WL1, WATERLOGGING FOR 1 DAY; WL2, WATERLOGGING FOR 2 DAYS; WL3, WATERLOGGING FOR 3 DAYS; WL4, WATERLOGGING FOR 5 DAYS; WL6 WATERLOGGING FOR 6 DAYS; WL7, WATERLOGGING FOR 7 DAYS; WL8, WATERLOGGING FOR 8 DAYS) AND SUBSEQUENT 15 DAYS OF RECOVERY. SYMBOLS ARE THE MEAN ± SE OF THREE REPLICATES. **(B)** PHOTOGRAPHS OF FULLY EMERGED SEEDLINGS OF THE SAME GENOTYPES AFTER 4 DAYS OF WATERLOGGING AND SUBSEQUENT 8 DAYS OF RECOVERY.

3.4.2 Waterlogging tolerance at the seedling stage

3.4.2.1 Soil redox potential

At the start of the waterlogging treatment, soil redox potential in the drained control pots was 452 ± 9 mV, where it remained throughout the experiment (Figure 3.3). During waterlogging, soil redox potential rapidly decreased to 239±14 mV on Day 2, plateauing at 225±13 mV on Day 3, where it remained until Day 7, before increasing gradually to 330±25 mV by Day 13. This increase in redox potential coincided with an increasing number of adventitious roots (see below). Once the treatment was completed on Day 16, the waterlogged pots were drained to allow the plants to recover. At recovery, the soil redox potential returned to the control value (459±5 mV) within 4 days.

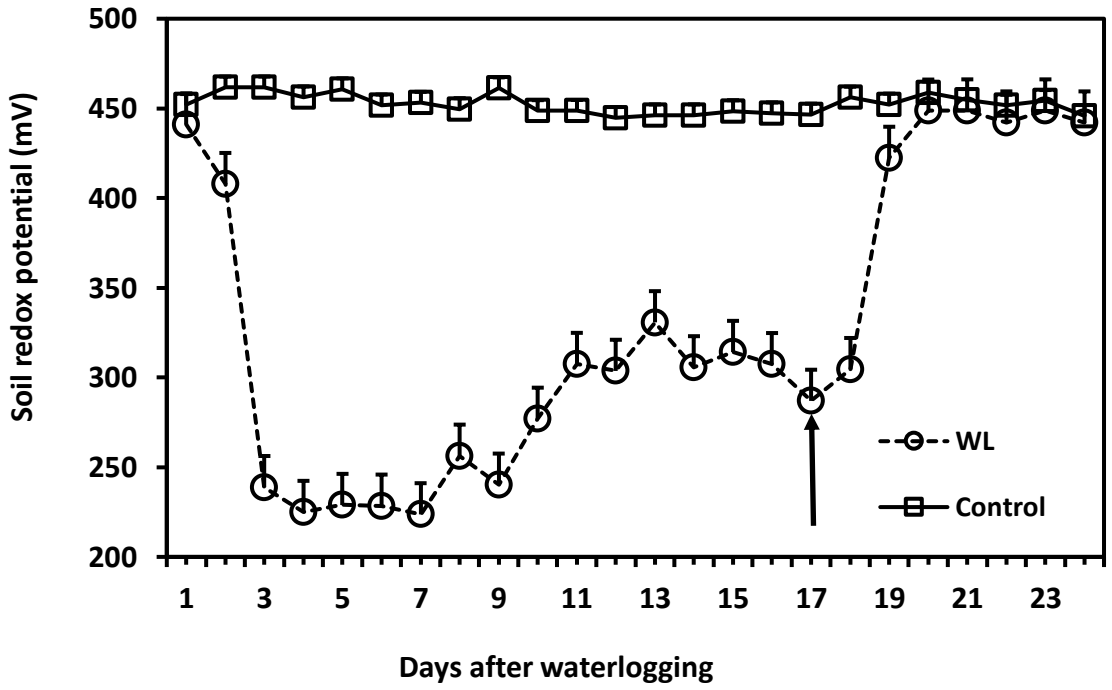


FIGURE 3.3 COMPARISON OF THE SOIL REDOX POTENTIAL OF DRAINED CONTROL AND CONTINUOUS WATERLOGGING FOR 16 DAYS FOLLOWED BY RECOVERY UPON DRAINAGE. THE ARROW REPRESENTS THE FIRST DAY OF RECOVERY AFTER RELEASE OF THE WATERLOGGING TREATMENT. THE VERTICAL BARS REPRESENT THE MEANS ± SE.

3.4.2.2 Shoot growth

Soil waterlogging strongly adversely affected seedling growth. The ANOVA showed that duration of waterlogging had a higher mean square than genotype and the interaction for all parameters except nodulation score and maximum taproot depth (Table 3.3). The effects of waterlogging duration, genotype, and their interaction were significant for all characters.

In the drained control soil (39 DAS), the large-seeded mungbean Jade-AU had 22% more shoot dry mass than the small-seeded mungbean Celera II-AU and 40% more than blackgram Onyx-AU. During waterlogging, shoot growth reductions were similar in both species, with maximum reductions of 47% in Celera II-AU, 40% in Jade-AU and 41% in Onyx-AU, relative to the drained controls after 16 days of waterlogging (Figure 3.4A). Shoot growth declined more during recovery than during waterlogging (Figure 3.5A). At the end of the recovery period (39 DAS), the drained controls had 1–3 times more shoot dry mass than the waterlogged plants. Nevertheless, waterlogging for 2 days had no effect on the plant growth of Jade-AU (Figure 3.5B). Among genotypes, growth of Celera II-AU declined the most for every waterlogging treatment (Figure 3.5A) at final harvest (39 DAS). In contrast, Onyx-AU had the potential to recover its growth from the damage of waterlogging stress during the subsequent period of drainage (Figure 3.5C).

The effect of waterlogging on other growth-related traits, such as plant height and the number of trifoliate leaves, are shown in Tables 3.4 and 3.5. Waterlogging did not significantly reduce plant height in the earlier days of treatment, and 16 days of waterlogging (WL16) treatment produced shorter (by 19–20%) plants than those in the drained control.

Waterlogging also affected total leaf area. The two treatments (WL8 and WL16) reduced total leaf area in Celera II-AU, Jade-AU, Onyx-AU by 40%, 22% by 21%, respectively, relative to the controls. Despite Celera II-AU producing new leaves during waterlogging, it had a smaller total leaf area than the other genotypes, signifying its sensitivity to waterlogging (Supplementary Figure 3.2A). During the recovery, leaf area expansion of Celera II-AU ceased, but slightly increased in Jade-AU and Onyx-AU (Supplementary Figure 3.2B).

TABLE 3.3 DEGREES OF FREEDOM (DF), F VALUES AND PROBABILITIES OF TWO-WAY ANALYSIS OF VARIANCE AT THE SEEDLING STAGE.

Character	Source of variation	Treat (T)	Genotype (G)	G× T
	df	19	2	38
SPAD chlorophyll content	F value	79.1	55.36	2.02
	Probability	<.001	<.001	0.002
Leaf area per plant	F value	75.16	37.48	4.77
	Probability	<.001	0.004	<.001
No. trifoliate leaves	F value	106.04	29.63	3.87
	Probability	<.001	<.001	<.001
Plant height (cm)	F value	65.50	23.45	3.36
	Probability	<.001	<.001	<.001
Shoot dry weight (g)	F value	119.3	51.9	7.05
	Probability	<.001	<.001	<.001
Root dry weight (g plant^{-1})	F value	80.16	47.51	4.32
	Probability	<.001	<.001	<.001
Total dry weight (g plant^{-1})	F value	128.82	51.72	7.50
	Probability	<.001	<.001	<.001
Nodulation score	F value	7.42	20.21	2.3
	Probability	<.001	<.001	<.001
Taproot depth (cm)	F value	5.08	17.79	2.54
	Probability	<.001	<.001	<.001
No. adventitious roots	F value	104.7	13.4	1.95
	Probability	<.001	<.001	<.001

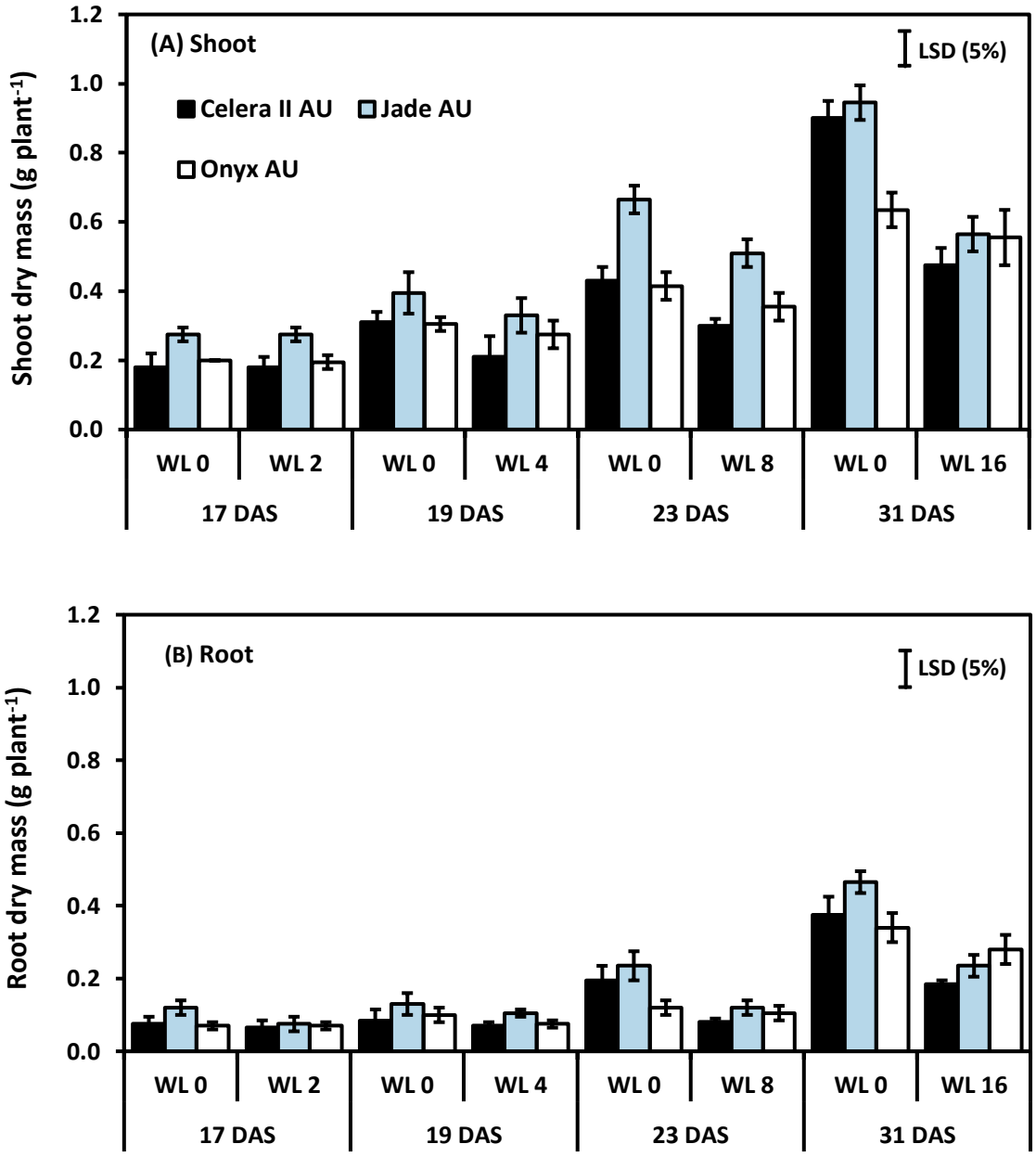


FIGURE 3.4 THE EFFECT OF DIFFERENT WATERLOGGING DURATIONS ON SHOOT **(A)** AND ROOT **(B)** DRY MASS OF THREE GENOTYPES GROWN IN SOIL: WL0, DRAINED CONTROL; WL2, WATERLOGGED FOR 2 DAYS, HARVESTED AT 17 DAS; WL4 WATERLOGGED FOR 4 DAYS, HARVESTED AT 19 DAS; WL8, WATERLOGGED FOR 8 DAYS, HARVESTED AT 23 DAS; WL16, WATERLOGGED FOR 16 DAYS, HARVESTED AT 31 DAS. TREATMENTS WERE IMPOSED AT 15 DAS. BARS ARE MEANS (N=4) ± SE AND LEAST SIGNIFICANT DIFFERENCES (LSD) AT P=0.05 FOR GENOTYPE.

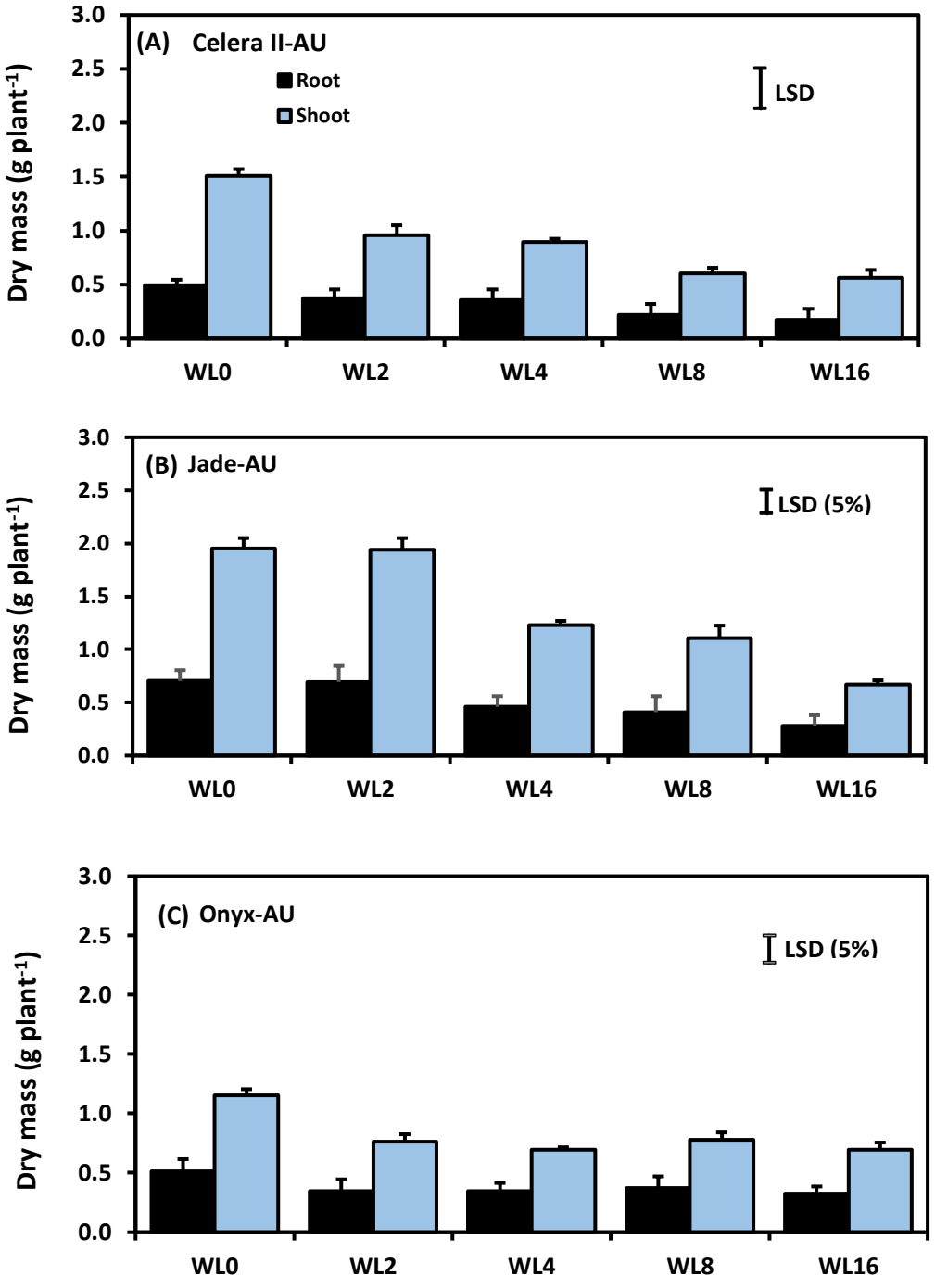


FIGURE 3.5 THE EFFECT OF DIFFERENT WATERLOGGING DURATIONS ON SHOOT AND ROOT DRY MASS AFTER DIFFERENT DURATION OF RECOVERY AT 39 DAS: **(A)** CELERA II-AU, **(B)** JADE-AU, **(C)** ONYX-AU. WL2, WATERLOGGING FOR 2 DAYS, RECOVERY FOR 22 D; WL4, WATERLOGGING FOR 4 DAYS, RECOVERY FOR 20 DAYS; WL8, WATERLOGGING FOR 8 DAYS, RECOVERY FOR 16 DAYS; WL16, WATERLOGGING WL 16 DAYS, RECOVERY FOR 8 DAYS. BARS ARE MEAN (N=4) ± SE AND LEAST SIGNIFICANT DIFFERENCES (LSD) AT P=0.05 FOR GENOTYPE.

3.4.2.3 Root growth

The taproot and lateral roots in the drained soil reached the base of pots at the start of waterlogging treatment [i.e. initial harvest (H1)]. Waterlogging reduced root system size in both species, more so with longer durations of waterlogging. Furthermore, waterlogging duration had a highly significant ($P<0.001$) effect on root dry mass for all three genotypes (Table 3.3). At the end of the waterlogging period, the WL8 treatment had reduced root dry matter in Celera II-AU by 26% and Jade-AU by 23%, relative to the drained controls; the corresponding values in the WL16 treatment were 41% in Celera II-AU and 40% in Jade-AU. For Onyx-AU in WL8, root dry matter decreased by 13% during waterlogging (Figure 3.4B). At the end of the recovery (39 DAS), Jade-AU recovered its root growth, showing similar root dry weight with its control under a shorter duration of waterlogging (WL2) but 24% reduction in Celera II-AU, and 33% in Onyx-AU. Root dry matter declined by 62% in Celera II-AU, 65% in Jade-AU and 40% in Onyx-AU in both WL8 and WL16 treatments (Figure 3.4B).

The reduction in root dry matter resulted from decay and damage to the existing root system. For all genotypes, the maximum depth of the taproot did not significantly differ from the control in the treatments (WL2 and WL4), showing the roots' ability to penetrate waterlogged soil. The taproot even survived 16 days of waterlogging. Jade-AU consistently maintained its maximum taproot length throughout the experiment in all waterlogging treatments (Table 3.4), but the taproot length of Celera II-AU and Onyx-AU significantly decreased ($P<0.001$) in the treatment (WL16) before recovery.

3.4.2.4 Adventitious root formation

There were no adventitious roots on plants in the drained controls. Adventitious roots were initiated near the shoot–root junction (hypocotyl region) and ranged in length from 0.2–0.5 cm after 2 days of waterlogging. The length and number of adventitious roots increased with the duration of waterlogging and varied with genotype. Adventitious root length reached 3–4 cm on average after WL8 to 7–10 cm after WL16. The effect of waterlogging duration, genotype and their interaction significantly differed ($P<0.001$) (Table 3.3). The genotypes differed in 8 days and 16 days of treatments, with Jade-AU and Onyx-AU producing more adventitious roots than Celera II-AU (Figure 3.6). Furthermore, some surface roots were initiated from the lateral roots, while others emanated from adventitious roots near the soil surface after 4 days of waterlogging. However, surface root tips dried up during the recovery stage after draining. By

the end of the recovery period, the adventitious roots had resumed elongation and extended to 15–20 cm (data not shown).

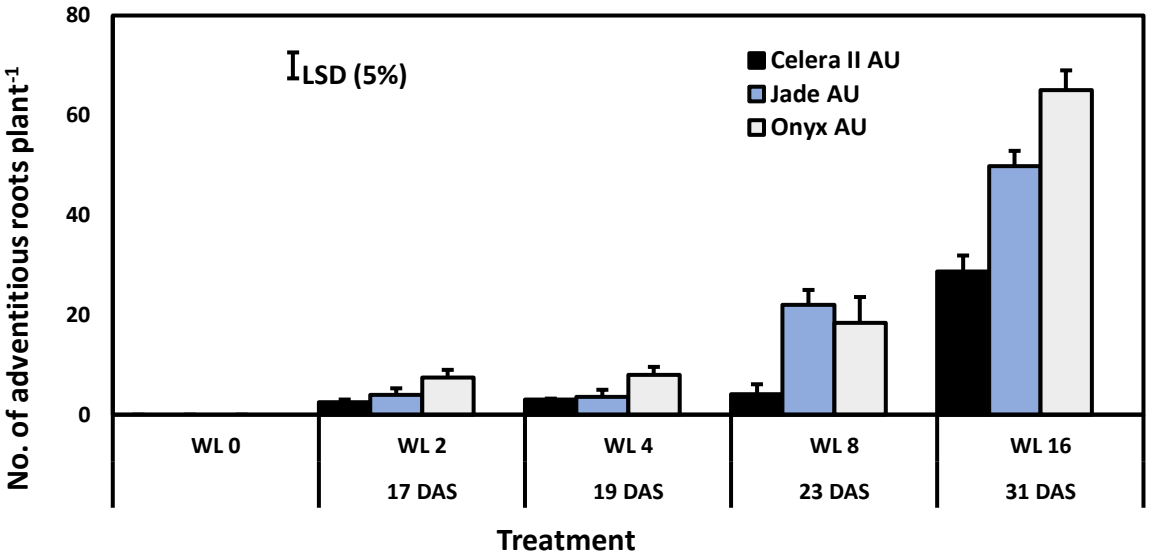


FIGURE 3.6 NUMBER OF ADVENTITIOUS ROOTS AFTER DIFFERENT WATERLOGGING DURATIONS. W0, DRAINED CONTROL; WL2, WATERLOGGING FOR 2 DAYS; WL4, WATERLOGGING FOR 4 DAYS; WL8, WATERLOGGING FOR 8 DAYS; WL16, WATERLOGGING FOR 16 DAYS. ADVENTITIOUS ROOTS DID NOT FORM ON PLANTS IN THE DRAINED CONTROLS. HARVEST OCCURRED AT 17 DAS (WL2), 19 DAS (WL4), 23 DAS (WL8) AND 31 DAS (WL16). BARS ARE THE MEAN (N=4) ± SE AND LEAST SIGNIFICANT DIFFERENCES (LSD) AT P=0.05 FOR THE GENOTYPE.

3.4.2.5 Relative growth rate

All genotypes had 2–3 times higher root RGR than shoot RGR in the drained control after WL2 (17 DAS). After 19 DAS, Celera II-AU quickly increased its shoot RGR from 0.11 to 0.27 g g^{-1} d^{-1} and decreased its root RGR from 0.31 to 0.03 g g^{-1} d^{-1}. Waterlogging stress reduced shoot and root RGRs, relative to the drained controls in all waterlogging treatments, except for Jade-AU in WL2 (Supplementary Figure 3.3). The shoot RGR of Onyx-AU after 8 days of recovery following WL16 was 0.028 g g^{-1} d^{-1}, relative to 0.075 g g^{-1} d^{-1} in the control, resulting in a 40% decline in shoot dry mass. Despite the rapid formation of adventitious roots in the WL8 and WL16 treatments, total root dry matter did not fully recover to the level of the drained control. The roots of Celera II-AU had a negative RGR (–0.007 g g^{-1} d^{-1}) during recovery in the WL16 treatment, compared with 0.036 g g^{-1} d^{-1} in the drained control (Supplementary Figure 3.4).

3.4.2.6 SPAD chlorophyll content

SPAD chlorophyll content values of the first trifoliate leaves were recorded to understand the effect of waterlogging on leaf nitrogen status. Jade-AU had the highest chlorophyll content (SPAD value) in the drained control, followed by Onyx-AU and Celera II-AU. Conversely, waterlogging reduced SPAD chlorophyll content at similar rates for all genotypes. Short-term waterlogging (i.e. up to WL4) had no significant effect on chlorophyll content. The WL8 and WL16 treatments significantly ($P<0.001$) reduced the overall chlorophyll content relative to the drained control for all genotypes (Figure 3.7). The rate of recovery was affected by waterlogging duration (Figure 3.8). The WL2 and WL4 treatments returned to the drained control level for SPAD chlorophyll and visually reverted from a pale yellow to green just 2 days after drainage. Genotypic variation for SPAD value was the greatest at recovery in the WL16 treatment (39 DAS), being 72% of the control in Onyx-AU, 66% in Jade-AU and 61% in Celera II-AU.

3.4.2.7 Nodule formation

In the drained controls, plant nodulation scores increased as nodule number and size increased with crop growth. At the initial harvest (H1) in the drained control, Jade-AU had the highest nodulation score, followed by Onyx-AU, while Celera II-AU had no nodules at this point. Under the different waterlogging durations, all plants produced root nodules near the soil surface (visual observation), which appeared to survive even in the WL16 treatment and continued to grow during the recovery (Tables 3.4 and 3.5). However, for roots in waterlogged soil, the nodules at depth were white, indicating that they were not functional. No nodules were observed on adventitious roots.

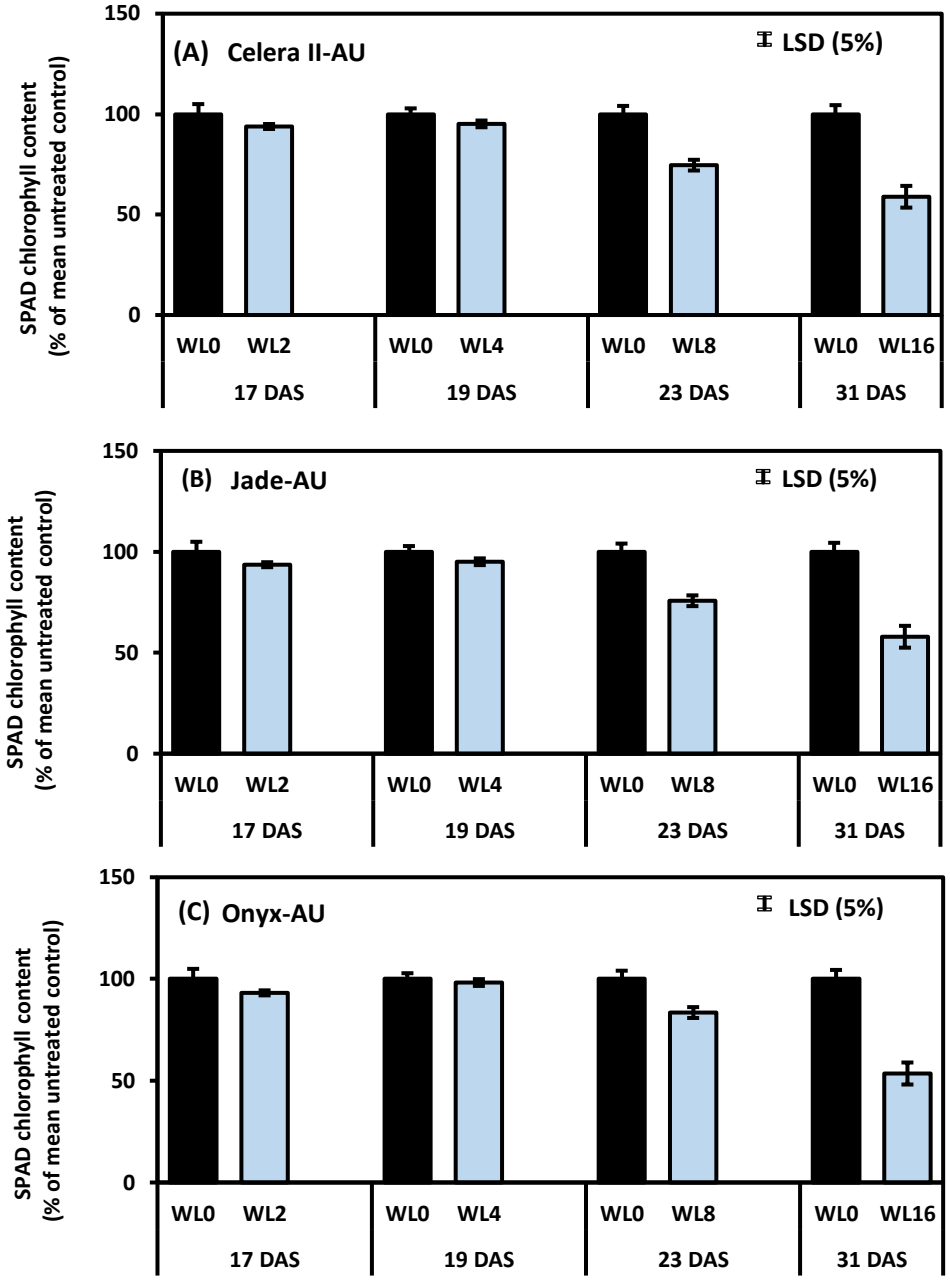


FIGURE 3.7 RELATIVE CHLOROPHYLL CONTENT (SPAD READINGS OF THE FIRST TRIFOLIATE LEAF) UNDER DIFFERENT WATERLOGGING DURATIONS FOR **(A)** CELERA II-AU, **(B)** JADE-AU AND **(C)** ONYX-AU. THE PERCENTAGE OF CHLOROPHYLL CONTENT INDEX (SPAD CCI) FOR WATERLOGGED PLANTS WAS ESTIMATED RELATIVE TO THEIR DRAINED CONTROLS. WL0, DRAINED CONTROL; WL2, WATERLOGGED FOR 2 DAYS, HARVESTED AT 17 DAS; WL4, WATERLOGGED FOR 4 DAYS, HARVESTED AT 19 DAS; WL8, WATERLOGGED FOR 8 DAYS, HARVESTED AT 23 DAS; WL16, WATERLOGGED FOR 16 DAYS, HARVESTED AT 31 DAS. THE AVERAGE SPAD CHLOROPHYLL CONTENT IN THE DRAINED CONTROL IS 41±3 IN CELERA II-AU, 50±3 IN JADE-AU, AND 44±3 IN ONYX-AU. DATA ARE MEANS ± SE OF FOUR REPLICATE POTS. VERTICAL BARS ARE LEAST SIGNIFICANT DIFFERENCES (LSD) AT P=0.05 FOR THE GENOTYPE.

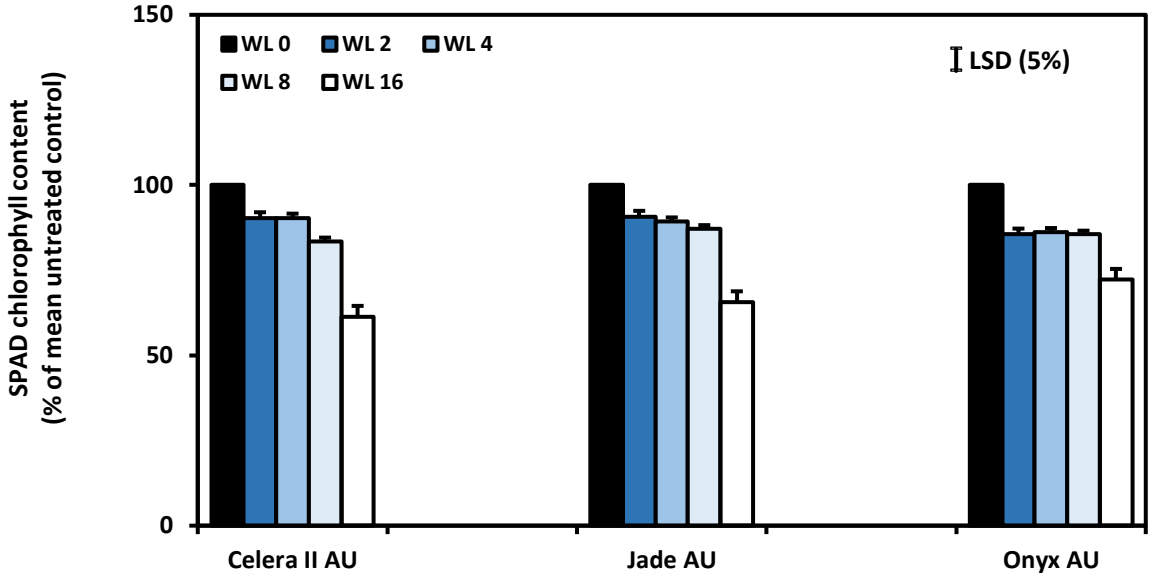


FIGURE 3.8 RELATIVE CHLOROPHYLL CONTENT (SPAD READINGS OF THE FIRST TRIFOLIATE LEAF) OF DIFFERENT GENOTYPES AT THE END OF THE RECOVERY (39 DAS). THE PERCENTAGE OF CHLOROPHYLL CONTENT INDEX (SPAD CCI) FOR WATERLOGGED PLANTS WAS ESTIMATED RELATIVE TO THEIR DRAINED CONTROLS. WL0, DRAINED CONTROL; WL2, WATERLOGGING FOR 2 DAYS, RECOVERY FOR 22 DAYS; WL4, WATERLOGGING FOR 4 DAYS, RECOVERY FOR 20 DAYS; WL8, WATERLOGGING FOR 8 DAYS, RECOVERY FOR 16 DAYS; WL16, WATERLOGGING FOR 16 DAYS, RECOVERY FOR 8 DAYS. DATA ARE MEANS ± SE OF FOUR REPLICATE POTS. VERTICAL BARS ARE LEAST SIGNIFICANT DIFFERENCES (LSD) AT P=0.05 FOR THE GENOTYPE.

TABLE 3.4 PLANT HEIGHT, NUMBER OF TRIFOLIATE LEAVES, TAPROOT DEPTH AND NODULATION SCORE AFTER DIFFERENT WATERLOGGING DURATIONS. W0, DRAINED CONTROL; WL, WATERLOGGING; WL2, WATERLOGGING FOR 2 DAYS; WL4, WATERLOGGING FOR 4 DAYS, WL8, WATERLOGGING FOR 8 DAYS; WL16, WATERLOGGING FOR 16 DAYS. DATA SHOW MEAN OF FOUR REPLICATE POTS AND LEAST SIGNIFICANT DIFFERENCES (LSD) AT P=0.05 FOR THE GENOTYPE (G), TREATMENT (T), AND GENOTYPE × TREATMENT INTERACTION (G×T). ON DAY 0, TWO PLANTS WERE HARVESTED IN EACH REPLICATE. NODULATION WAS SCORED BY COUNTING THE NODULES ON THE MAIN TAPROOT AND LATERAL ROOTS AND USING A 0–8 SCORING SCALE ACCORDING TO YATES ET AL. (2016), WHERE 0 = NO NODULES, 0.5 = WHITE INEFFECTIVE NODULES, 1 = RARE EFFECTIVE, 2 = SCARCE, 3 = MODERATE, 4 = ADEQUATE, 5 = AMPLE, 6 = ABUNDANT, 7 = VERY ABUNDANT, 8 = EXTREMELY ABUNDANT.

Waterlogging treatment	**Plant height (cm)**		**No. trifoliate leaves**		**Max. depth of tap root (cm)**		**Nodulation score**	
	WL0	**WL**	**WL0**	**WL**	**WL0**	**WL**	**WL0**	**WL**
WL2 (17 DAS)								
Celera II-AU	15	15	1	1	28	27	2	1
Jade-AU	15	14	1	1	32	31	4	4
Onyx-AU	12	12	1	1	31	31	4	4
WL4 (19 DAS)								
Celera II-AU	18	17	2	2	32	32	4	3
Jade-AU	15	14	2	1	35	32	5	4
Onyx-AU	15	15	2	1	35	32	6	4
WL8 (23 DAS)								
Celera II-AU	20	18	2	2	33	32	4	3
Jade-AU	17	17	2	2	37	32	6	4
Onyx-AU	18	16	2	2	28	28	6	5
WL16 (31 DAS)								
Celera II-AU	23	20	4	3	36	28	6	4
Jade-AU	21	18	4	3	37	36	6	4
Onyx-AU	20	17	4	3	37	30	6	5
LSD (5%) G		0.7		0.1		1.4		0.5
LSD (5%) T		1.2		0.1		3.0		0.7
LSD (5%) G×T		2.1		0.2		4.3		1.3

TABLE 3.5 PLANT HEIGHT, NUMBER OF TRIFOLIATE LEAVES, MAXIMUM DEPTH TAPROOT DEPTH AND NODULATION SCORE AT THE END OF THE RECOVERY, AS AFFECTED BY THE DURATION OF TRANSIENT WATERLOGGING. WL0, DRAINED CONTROL; WL2, WATERLOGGING FOR 2 DAYS, RECOVERY FOR 22 DAYS; WL4, WATERLOGGING FOR 4 DAYS, RECOVERY FOR 20 DAYS; WL8, WATERLOGGING FOR 8 DAYS, RECOVERY FOR 16 DAYS; WL16, WATERLOGGING FOR 16 DAYS, RECOVERY FOR 8 DAYS. DATA SHOW MEAN OF FOUR REPLICATE POTS AND LEAST SIGNIFICANT DIFFERENCES (LSD) AT P=0.05 FOR THE GENOTYPE (G), TREATMENT (T), AND GENOTYPE × TREATMENT INTERACTION (G×T). ON DAY 0, TWO PLANTS WERE HARVESTED IN EACH REPLICATE.

Waterlogging treatment	Plant height	No. trifoliate leaves	Max. depth of tap root (cm)	Nodulation score
Celera II-AU				
W0	25	4	39	6
WL2	22	4	33	5
WL4	23	3	33	5
WL8	21	3	31	4
WL16	21	3	28	4
Jade-AU				
W0	29	4	38	7
WL2	27	3	33	5
WL4	24	3	30	5
WL8	23	3	33	4
WL16	21	3	36	5
Onyx-AU				
W0	25	6	38	7
WL2	20	5	33	4
WL4	20	5	30	5
WL8	23	5	32	5
WL16	23	4	37	4
LSD (5%) G	0.51	0.12	14.62	0.3
LSD (5%) T	1.32	0.31	6.98	0.8
LSD (5%) G×T	2.30	0.54	2.09	1.3

3.5 DISCUSSION

Waterlogging is a destructive abiotic stress, the occurrence of which is increasing in some parts of the world due to a high frequency of unseasonal rainfall caused by global climate change. This is the first study to compare mungbean and blackgram genotypes under different waterlogging durations at two critical growth stages: germination and seedling stages. The current study found that waterlogging delayed germination and longer durations reduced seed germination, seedling emergence and establishment. Waterlogging at the seedling stage reduced shoot and root dry mass, which was proportional to the waterlogging duration. At the

end of the experiment, plants exposed to 16 days of waterlogging and then allowed 8 days recovery had 60% less total dry mass in mungbean and 40% less in blackgram than those of drained control plants. One unanticipated finding was that no seedlings died even after 16 days of waterlogging (almost one-fourth of their life cycle). The plants exhibited adaptation to transient waterlogging through extensive adventitious root formation. The effect of soil waterlogging on seed germination and seedling growth varied between the genotypes, as discussed below.

At germination, the drained controls had rapid seedling emergence starting at 3 DAS and completed full emergence (100%) at 6–7 DAS. During soil waterlogging, all three genotypes exhibited no seedling emergence. These seeds in waterlogged soil presumably could not germinate due to a lack of sufficient oxygen. When the pots with shorter durations of waterlogging were drained, some seedlings then emerged, but with longer durations of waterlogging, the seeds lost viability, as evidenced by the failure to germinate and establish seedlings upon drainage of the previously waterlogged pots. Some seeds grew hypocotyls out of their testa, but no growth beyond this was observed, and the seeds failed to emerge after 8 days of waterlogging. Hence, the longer the waterlogging duration, the greater the reduction in germination and seedling establishment. Previous research indicates that in other legumes [i.e. common bean (*Phaseolus vulgaris* L.) and soybean], there is a failure to emerge under soil-waterlogged conditions because low oxygen levels restrict the respiration process required for germination (Morinaga, 1926; Cardwell, 1984; Hou and Thseng, 1992; Tian et al., 2005; Rajashekar and Baek, 2014). The prolonged period of anaerobiosis by soil waterlogging also results in the death of germinating seeds in waterlogging-sensitive peas (Zaman et al., 2018). Similarly, other dryland crops such as wheat and barley could not germinate under waterlogged conditions because the lack of amylolytic enzymes and rapid uptake of excessive water by seeds led to membrane damage and solute leakage (Powell and Matthews, 1978).

Tolerance of crops to waterlogging may vary depending on plant species. Recently, two contrasting tolerance mechanisms were studied in response to transient waterlogging in field pea: ‘escape’ (germination under waterlogging) and ‘quiescence’ (germination/emergence only after the removal of the stress) (Zaman et al., 2018). Another important finding in the seeds of soybean is that the aleurone layer helps to block the abrupt water entry in to the embryo, as it covers the surface of the embryo, absorbing water slowly and maintaining membrane integrity under transient waterlogging (Tian et al., 2005). Furthermore, seed testa colour appears to be related to waterlogging tolerance, with dark testa genotypes more tolerant than light testa

genotypes in wheat (Ueno and Takahashi, 1997), soybean (Hou and Thseng, 1991) and field peas (Zaman et al., 2019). In the present study, blackgram (dark testa and hypocotyl pigmentation) had higher seedling emergence upon drainage following a period of waterlogging than mungbean (green testa) (Figure 3.2). Of the two mungbean genotypes, Celera II-AU (small seeded and hypocotyl pigmentation) had better seedling establishment than Jade-AU (large seeded and no hypocotyl pigmentation) under waterlogging. To develop a full picture of waterlogging tolerance at the germination stage, additional studies on diverse germplasm are needed to reveal possible tolerance mechanisms in both species.

At the seedling stage, the formation of adventitious roots probably played an important role in waterlogging tolerance. Sauter (2013) reported that adventitious roots with aerenchyma, which facilitates oxygen movement within these roots, enable water and nutrient uptake through these roots, and thus plant survival and even growth. Adventitious roots also influenced the soil redox potential in this study. At the start of waterlogging treatment, the soil redox potential under waterlogging at the seedling stage declined as in the germination trial, stabilising after 5 days. However, by Day 7, the soil redox potential started to trend upwards, presumably because the seedlings started to produce adventitious roots under waterlogging, and there would likely have been some radial oxygen loss from those roots to the soil (Armstrong, 1979). No such roots were observed in the drained controls. Hence, adventitious roots formed at the seedling stage under waterlogging are regarded as an adaptation in both mung bean and blackgram under waterlogging.

Adventitious root formation is a quantitative, heritable trait controlled by multiple factors, including species, genotype, growth stage, water temperature, and waterlogging duration and depth (Lorbiecke and Sauter, 1999; Sorin et al., 2006; Dawood et al., 2014; Argus et al., 2015; Zhang et al., 2015). Various studies have assessed the efficacy of waterlogging to stimulate adventitious roots containing aerenchyma as an adaptive response (Visser and Voesenek, 2004; Sauter, 2013; Steffens and Rasmussen, 2016; Shimamura et al., 2003; Thomas et al., 2005). In addition, root growth-regulating hormones might be important in the formation of aerenchymatous adventitious roots (e.g. rice (Lin and Sauter, 2020), Rumex (Visser et al., 1996), wheat (Nguyen et al., 2018), and Arabidopsis (Verstraeten et al., 2014)). Nonetheless, the formation of aerenchyma in response to waterlogging varies depending on plant species (Smirnoff and Crawford, 1983; Justin and Armstrong, 1987; Visser et al., 2000; McDonald et al., 2002; Grimoldi et al., 2005). Yamauchi et al. (2013) reported the role of primary aerenchyma in the adventitious roots of cereals (rice, maize, barley and wheat) and secondary

aerenchyma in the stem, hypocotyl, taproot, adventitious roots and root nodules of legumes such as soybean. Further study is required to understand the role of aerenchyma inside adventitious roots and root porosity in mungbean and blackgram under soil waterlogging stress.

Waterlogging reduced root and shoot dry matter by damaging the existing root systems of mungbean and blackgram (current study), as shown also for some other crop species (wheat, Malik et al., 2002; chickpea and fababean, Munir et al., 2019). Although seedlings responded to transient waterlogging by producing adventitious roots and large nodules on the primary root near the soil surface; however, that did not compensate for the role of the roots that formed at the initial stage. Damage to the root systems under waterlogging had consequences for the shoots, such as reduced number of leaves and smaller leaf area. Limitation of nutrient uptake, as likely demonstrated for N by reduced chlorophyll content (SPAD value) following a longer duration of waterlogging (WL8 and WL16) could be one cause of the reduction in shoot growth. Adverse effects of waterlogging on nutrient acquisition have been reported previously (e.g. soybean (Bacanamwo and Pucell, 1999); wheat (Malik et al., 2002); soybean (Board, 2008); pea, lentil and grasspea (Malik et al., 2015); cotton (Najeeb et al., 2015)). The reasons for these reductions could be due to the death and decay of most lateral roots and the incomplete capacity of the adventitious roots to fully compensate for the loss of the other roots.

Another important consideration is the role of nodules housing rhizobia in the roots of legumes in waterlogged soils. In legumes, symbiotic nitrogen fixation through rhizobia nodules is the main source of nitrogen acquisition. In the present study, mungbean and blackgram in the drained controls produced more nodules than the waterlogged treatments (Tables 3.4 and 3.5). Nodules below 5 cm of waterlogged soil might have become ineffective after 4 days of waterlogging as their colour changed from pink to white (observed by cutting open nodules). Nodules at the plant crown continued to grow under different waterlogging durations and presumably were able to function into the recovery stage, as these were always pink. Similar nodulation was observed in a tropical forage legume, American jointvetch (*Aeschynomene americana* L.), which maintained nitrogenase activity and net assimilation rate for growth under waterlogged conditions (Tobisa et al., 2014). Previous research indicated that legumes could change the pathway of oxygen diffusion to nodules (Roberts et al., 2010). In soybean, studies have shown that the aerenchyma connects to the outer cortex of nodules, presumably enabling their functioning for roots in waterlogged soils (Shimamura et al., 2003; Thomas et al., 2005).

The genotypes differed in responses to the waterlogging treatments at both growth stages (significant statistical interaction in ANOVA; Table 3.3). Genotypic variation in waterlogging

tolerance was greatest at the germination stage after 4 days of waterlogging and at the seedling stage after 8 days of waterlogging. Blackgram, Onyx-AU had a higher germination rate and seedling establishment than the mungbean genotypes for all waterlogging durations. Genotypic variation was evident for adventitious root formation in plants waterlogged at the seedling stage. Onyx-AU and Jade-AU rapidly produced many adventitious after 8 days of waterlogging, with less extensive growth in Celera II-AU (Supplementary Figure 3.4). Onyx-AU (blackgram) was more waterlogging-tolerant than mungbean at both growth stages. Between the two mungbeans, Celera II-AU had a higher germination per cent than Jade-AU. In contrast, Jade-AU grew more and recovered quicker after waterlogging at the seedling stage than Celera II-AU. Further research should be undertaken to identify the linkage between waterlogging tolerance and morphological traits, e.g. seed size, hypocotyl pigmentation and testa colour in the two species.

The results of this research have significant implications for understanding waterlogging tolerance in mungbean and blackgram. As genetic tolerance enhances yield and its stability, there is a need for systematic screening of a wide range of germplasm to identify and exploit the genetic variation in both species. The methodology developed in this study can be used for designing extensive screening of germplasm to identify waterlogging tolerance and understand its genetic basis.

3.6 CONCLUSION

The research compared mungbean and blackgram cultivars under different waterlogging durations at two critical growth stages: (i) germination and (ii) early vegetative growth. All waterlogging treatments significantly reduced germination and retarded seedling growth; adverse effects were greater with longer waterlogging duration. Prolonged waterlogging duration adversely affected the germination rate, plant establishment, and shoot and root growth and development. Blackgram has the ability to cope with the low-oxygen environment more than mungbean at both growth stages. To follow an adaptive strategy at the seedling stage, waterlogging for 16 days reduced growth but did not result in plant death due to plant adaptations to stress, such as producing adventitious roots and rhizobia nodules near the crown.

CHAPTER 4 SCREENING A MINI CORE COLLECTION OF MUNGBEAN FOR WATERLOGGING TOLERANCE AT THE GERMINATION AND SEEDLING STAGES

4.1 ABSTRACT

Mungbean is sensitive to transient waterlogging at the germination and seedling stages. This study investigated phenotypic variation in waterlogging traits in a mungbean mini core germplasm population. The screening methodology created hypoxic soil conditions to identify sources of waterlogging tolerance at both growth stages. At the germination stage, waterlogging tolerance was related to maintaining seed viability under hypoxia and subsequent emergence on the release of hypoxia. At the seedling stage, waterlogging tolerance significantly correlated with the rapid formation of adventitious roots. Frequency distributions of related traits revealed leptokurtic (positive kurtosis) and platykurtic (negative kurtosis) distributions, suggesting polygenic control of waterlogging tolerance with duplicate gene epistasis and dominant-based complementary epistatic gene action. The highest broad-sense heritability estimates occurred for seed emergence (81%) at the germination stage and shoot (81%) and root (79%) dry mass at the seedling stage. The estimated broad-sense heritability for adventitious root formation was 56%, and SPAD chlorophyll content was 56–71% at the seedling stage. Such heritability estimates combined with identifying sources of waterlogging tolerance demonstrate the possibility of selecting for these traits and accelerating mungbean breeding for climate-resilient cultivars.

4.2 INTRODUCTION

Mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) is a major subtropical crop in Asia and other parts of the world. It is native to the Indo-Burma region (Jain and Mehra, 1978), with India and Myanmar each supplying about 30% of global production, followed by China (16%) and Indonesia (5%) (Nair and Schreinemachers, 2020). Mungbean is a short-season legume, completing its life cycle of 50–90 days. It requires 600–1,000 mm annual rainfall with 28–30°C optimal temperatures for vegetative growth. Mungbean has been transformed from a marginal to a major crop in Asia. It provides farming families with income or food, as well as other benefits such as improving the nutrition of poor and anaemic women and children and contributing to soil fertility (Nair et al., 2012). Globally, the area sown to mungbean has steadily increased to 7.3 million ha, with average yields of 721 kg ha^{-1} (Nobel et al., 2018).

Mungbean can be grown in upland and lowland areas due to its adaptability and plasticity (Islam et al., 1993; Herridge et al., 2019). Adopting mungbean as a cash crop can increase the productivity of the subsequent rice crop by 8% because mungbean fixes soil nitrogen and helps break pest and disease cycles (Weinberger, 2003). However, excess soil moisture immediately after rice harvest exposes the seeds of the succeeding crop in rice–legume cropping systems to waterlogging stress (Zaman et al., 2018). In addition, global warming exacerbates climate variability and creates extreme weather conditions, such as precipitation resulting in flooding that affects agricultural food production (Rötter et al., 2012, 2013). Furthermore, waterlogging adversely impacts the growth of most plants (Dwivedi et al., 2016), preventing crops from achieving their genetic yield potential (Douglas et al., 2020).

Mungbean is susceptible to soil waterlogging (Nair et al., 2012), mainly during early growth (Tickoo et al., 2006; Douglas et al., 2020). Islam et al. (2008) reported that waterlogging adversely impacts mungbean growth and leaf chlorophyll content and damages roots, decreasing grain yield. In addition, oxygen deprivation in the rhizosphere due to soil waterlogging reduces the N_2 fixation activity of root nodules in mungbean (Singh and Singh, 2011; Kyu et al., 2021) and soybean (Suematsu et al., 2017). Varietal differences in mungbean response to transient waterlogging at the late vegetative stage have been reported (Bagga et al., 1984; Ahmed et al., 2002; Islam et al., 2007, 2008). However, these studies comprised a restricted range of genetic diversity and only measured final yield, yield components and SPAD chlorophyll content, but not tolerance-related traits, such as adventitious root formation.

Waterlogging tolerance in mungbean can be improved by understanding the genetic basis of responses to stress. The degree of tolerance to waterlogging depends on crop growth stage (VanToai et al., 1994), growth habit and waterlogging duration (Greenway et al., 1994). Recently, two Australian mungbean cultivars—investigated for their transient waterlogging tolerance under different waterlogging durations at the germination and early seedling stages and their subsequent recovery upon drainage—failed to germinate during waterlogging, and waterlogged seedlings formed adventitious roots in response to waterlogging (Kyu et al., 2021). Therefore, systematic screening of a wide range of germplasm is needed to identify sources of waterlogging tolerance and understand the genetic basis of the tolerance.

The genetic basis of waterlogging tolerance has been studied in some leguminous crop species, including soybean (*Glycine max* L.) (Shannon et al., 2005; Suematsu et al., 2016), common bean (*Phaseolus vulgaris* L.) (Soltani et al., 2017) and pigeonpea (*Cajanus cajan* L.) (Krishnamurthy et al., 2012; Sultana et al., 2012) using large sets of germplasm from different

origins, including core and mini core collections. Such collections contain genetically diverse germplasm that can be exploited to develop climate-resilient genotypes with biotic and abiotic stress tolerance (Upadhyaya et al., 2008).

In 2015, the World Vegetable Center (AVRDC) created a mungbean mini core germplasm collection, representing genetic resources from more than 6,700 accessions based on genotypic and phenotypic traits (Schafleitner et al., 2015). From this mini core collection, several candidate genes have been identified, such as hypocotyl pigmentation and maturation under abnormally hot weather and different photoperiods (Sokolkova et al., 2020), seed coat lustre (Breria et al., 2019), salinity tolerance at germination (Breria et al., 2020), seed size (Akhtar et al., 2021) and favourable root traits for heat and drought stress resistance (Aski et al., 2021). However, there is a lack of information on the genetic basis of waterlogging tolerance in mungbean.

Screening germplasm lines using the robust phenotyping methodology that Kyu et al. (2021) developed will help identify specific traits for developing climate-resilient genotypes to meet mungbean's yield potential. We hypothesised that the mungbean mini core collection contains functional genotypic variation for transient waterlogging tolerance. Therefore, we assayed genotypic variation in response to transient waterlogging at the germination and seedling stages to identify sources of tolerance and as a precursor to understanding its genetic control.

4.3 MATERIALS AND METHODS

4.3.1 Plant material

A set of 292 mini core collection genotypes from nine regions was studied to identify phenotypic responses to transient waterlogging at two critical growth stages: germination and seedling. The mini core collection genotypes came from the World Vegetable Center (AVRDC) (Schafleitner et al., 2015); Table 4.1 lists their original distribution regions. The Department of Agriculture and Fisheries, Queensland, supplied the seed. For practicality, the genotypes were divided randomly into two cohorts for the germination screening and three cohorts for the seedling stage screening. Eight check genotypes (VI64209, VI64210, VI002173, VI002537 AG, VI004069 BG, VI004954 BG, VI064518 and VI064521) were replicated five times in each cohort at the germination stage, but only once in each cohort at the seedling stage due to limited space.

TABLE 4.1 DISTRIBUTION OF REGIONAL ORIGINS OF MUNGBEAN MINI CORE COLLECTION GENOTYPES

Variable	Region		No. genotypes	Percentage (%)
Region	Africa	(AFR)	2	0.7
	Mexico	(MA)	1	0.3
	East Asia	(EA)	5	1.7
	Europe	(EUR)	2	0.7
	Oceanic Pacific	(OP)	7	2.4
	South Asia	(SA)	186	63.7
	Southeast Asia	(SEA)	20	6.8
	South America	(SM)	1	0.3
	Southwest Asia	(SWA)	57	19.5
	Not available	(NA)	11	3.8

4.3.2 Methods

4.3.2.1 Experimental conditions

The experiments were conducted in a temperature-controlled glasshouse at The University of Western Australia (UWA), Crawley, Western Australia (31° 59’ S, 115° 49’ E) from May to June 2019 (germination stage) and September to December 2019 (seedling stage). The temperature inside the glasshouse ranged from 21±4°C (night) to 32±3°C (day), with 10 h 45 min of daylight (1,150–1,627 µmol m^{-2} s^{-1}) on average. The seeds were grown on the same red-brown sandy clay loam (Calcic Haploxeralf) used for waterlogging studies in pea (Zaman et al., 2018), grass pea (Wiraguna et al., 2020) and mungbean and blackgram (Kyu et al., 2021). Oven-dried soil was sieved in a 2 mm soil sieving machine (see Chapter 3). The field capacity of the soil (i.e. pot capacity when fully drained) was 18% of water content (w/w).

4.3.2.2 Experimental design

The experiments had a split-plot design with three replications. A spatial row–column blocking design was used to control variation within replicates and improve the precision of treatment comparisons. Treatment (waterlogging vs. drained control) was the main factor, with genotype as the subfactor. Based on Kyu et al. (2021), the optimal soil waterlogging duration for identifying variation in waterlogging responses was 4 days at the germination stage and 8 days at the seedling stage. A pot was considered an experimental unit.

4.3.2.3 Screening at the germination stage

Screening procedures for waterlogging and data recording followed Kyu et al. (2021). Prior to sowing, the seeds were surface sterilised with 1% commercial bleach (active ingredients NaOCl 40 mg L^{-1}) for 1 min and rinsed with deionised water four times. P-Pickel T liquid fungicide [Thiram (360 g L^{-1}) + Thiabendazole (200 g L^{-1})] was applied at 300 mL 100 kg^{-1} seed. Twenty seeds of each genotype were sown per pot at 10 mm depth and covered with soil. The pots were 0.8 L (90 × 90 × 180 mm) with drainage holes (~10 mm) at the base. The drainage holes were covered with filter paper to avoid soil loss before filling the pots with 100 g gravel, followed by 1.0 kg soil. After potting up, all pots were placed in 60 L plastic tanks and kept at 80% field capacity for 2 days before sowing. Twelve platinum electrodes—six each for drained and waterlogged pots—were placed at 100 mm depth to monitor soil redox potential (Zaman et al., 2018).

Immediately after sowing, the waterlogging treatment was imposed by adding DI water to the 60 L tanks. The soil water table was retained at the soil surface during the waterlogging treatment by adding DI water to the tanks. Drained control pots were kept at 80% field capacity by adding DI water directly to the pots as required. After 4 days of waterlogging, treated pots were relocated to free-draining plastic tanks to record emergence and seedling growth during the recovery. The 80% field capacity was maintained in the recovery pots by adding additional DI water as required.

Hundred-seed weight (g) was measured before sowing. Soil redox potential was measured daily with platinum electrodes (Pt) and an Ag–AgCl reference electrode using a handheld Digital Multimeter (Fluke 114, Everett, Washington, USA). Redox values were calculated according to the method by Patrick et al. (1996). After 7 days of recovery, germinated seedlings were gently washed from the soil with tap water for measurements. Waterlogged and drained control pots were harvested on the same day.

At harvest, seedlings with at least one trifoliate leaf were counted as fully grown emerged plants. After washing the plants, the harvested plants were separated into shoots and roots and oven-dried at 60°C for 3 days to measure dry mass.

4.3.2.4 Screening at the seedling stage

For seedling screening, the experimental pots were free-draining 4 L plastic pots (145 × 145 × 220 mm); the drainage holes (15 mm) were covered with filter paper before filling with 500 g gravel followed by 4 kg sieved dry soil. The pots were placed in 60 L plastic tanks (10 per tank)

and retained at 80% field capacity two days prior to sowing. Each pot received 40 mg kg^{-1} dihydrogen ammonium phosphate [(NH_4) (H_2PO_4)] based on the soil analysis (Supplementary Table 4.1). The sterilised seeds were placed on 55 mm diameter Grade 1 Whatman filter paper in a 55 mm diameter Petri dish and incubated overnight in a temperature-controlled room (25°C). The next day, six germinated seeds were placed at 30 mm depth. The seeds were inoculated with Group I mungbean Rhizobium strain CB 1015 (New Edge Microbial, New South Wales, Australia) and then covered with 2 mm sieved dry soil. Each pot was thinned to two seedlings with similar vigour four days after emergence. Waterlogging was imposed 15 days after sowing (DAS), when the first trifoliate leaf had fully opened (Figure 4.1), maintaining the soil water table 10 mm above of topsoil surface layer. The drained controlled pots were maintained at 80% field water capacity. Twelve 12 Pt electrodes—six in waterlogged and six in drained control pots—were placed at 100 mm depth in randomly selected pots of each waterlogged and drained soil to measure soil redox potential. After 8 days of waterlogging, the waterlogged pots were drained to observe plant growth during 7 days of recovery, with the experiment terminating at 30 DAS.

FIGURE 4.1 SCREENING MUNGBEAN GERMPLASM COLLECTION FOR TRANSIENT WATERLOGGING TOLERANCE AT THE SEEDLING STAGE IN A TEMPERATURE-CONTROLLED GLASSHOUSE.

SPAD chlorophyll content (SPAD unit_1) was measured immediately after removing the waterlogging (23 DAS) on the first trifoliate leaves and at the end of recovery (30 DAS) on the first (SPAD unit_2) and second (SPAD unit_3) trifoliate leaves of waterlogged and drained control plants with a handheld Minolta SPAD 502 (Konica-Minolta, Japan). At harvest, the soil was removed from the roots of each plant with tap water before phenotyping for four agronomic traits: shoot, root and total dry mass and adventitious root formation. The number of

adventitious roots was counted. Shoots and roots were separated, oven-dried at 60°C for 3 days and weighed.

4.3.3 Statistical analysis

Analyses of variance (ANOVA) of check genotypes and mini core collection genotypes was undertaken in a split-split plot design to understand the homogeneity among cohorts and the effect of different cohorts on check genotypes, treatments and their interactions. Further, best linear unbiased predictors were determined for block effects using GENSTAT 21st edition (VSN International, UK). Tables were constructed using spatial analysis of row–column design according to linear mixed models (REML: restricted maximum likelihood) at each growth stage. The predicted means of REML were analysed to estimate genetic diversity parameters: mean, minimum, maximum, standard deviation, skewness and kurtosis. Broad-sense heritability was expressed as the proportion of phenotypic variation (V_p) due to genetic values (V_g), which may include effects due to dominance and epistasis (Falconer and Mackay, 2005).

$$H^2 = \frac{V_g}{V_p}$$

The variance components due to genotype (σ^2_g), and error (σ^2_e) for each trait were estimated using the following formula on RStudio 4.1 (Schmidt et al., 2019).

$$\partial_p^2 = \partial_g^2 + \frac{\partial_e^2}{n_r}$$

The frequency distribution of variation for the traits of interest (% of control) was measured, and a normality test was performed using the Kolmogorov–Smirnov test in RStudio 4.1. A one-way ANOVA was undertaken based on origin. Pearson's correlation, regression and principal component analyses of quantitative trait data were carried out in GENSTAT 21st edition to explore the relationships between variables under waterlogging and control conditions. Biplots were constructed to visualise the principal component analysis (PCA) results and understand the loading vectors.

4.4 RESULTS

The data of the mini core collection of 292 genotypes were summarised to understand transient waterlogging tolerance in mungbean at two critical growth stages: germination and seedling. Supplementary Table 2 lists the phenotypic data for the 292 genotypes.

4.4.1 Effect of transient waterlogging on soil redox potential

Transient soil waterlogging reduced soil redox potential. At the germination stage, drained pots had a stable soil redox potential (400±32 mV) throughout the experimental period. In contrast, the soil redox potential of the waterlogged pots gradually declined to 208±10 mV after 4 days of waterlogging, where it remained for 3 days before gradually increasing during the recovery, reaching the drained control value at 10 DAS (Figure 4.2).

At the seedling stage, the drained pots had a stable soil redox potential (440±19 mV) from the first day of waterlogging (15 DAS) to harvest (30 DAS). In contrast, the soil redox potential of the waterlogged pots gradually decreased, reaching its lowest point 220±15 mV after 3 days of waterlogging (18 DAS) before gradually increasing to 250±35 mV by the end of the waterlogging treatment (23 DAS) and reaching the control level after 6 days of recovery (Figure 4.3).

4.4.2 Screening at the germination stage

4.4.2.1 Check genotypes

ANOVA was only done for the check genotypes in the two cohorts [Cohort I (C-I) and Cohort II (C-II)] to understand the cohort effect on the checks (Table 4.2). Significant differences occurred between the check genotypes (G). The transient waterlogging treatment (Treat) effect on genotype and their interaction (Treat × Gen and Treat × Gen × Cohort) significantly differed for emergence (%) and shoot, root and total dry mass in both cohorts. Further testing was undertaken for those traits with a significant Treat × Gen × Cohort interaction to understand whether the genotype response to waterlogging was greater than the cohort effect for this interaction. The results showed that the genotypic response to waterlogging was significantly greater than the cohort effect for this interaction; thus, the data from the two cohorts were combined for analysis. Among the control genotypes, BI4069 had the highest emergence (62%), followed by Celera II-AU (46%), VI 4954 (45%), VI 2173 (38%) and VI 2537 (46%), while BARIM3, BARIM6 and Jade-AU were the most susceptible (~20%) to waterlogging (Figure 4.4).

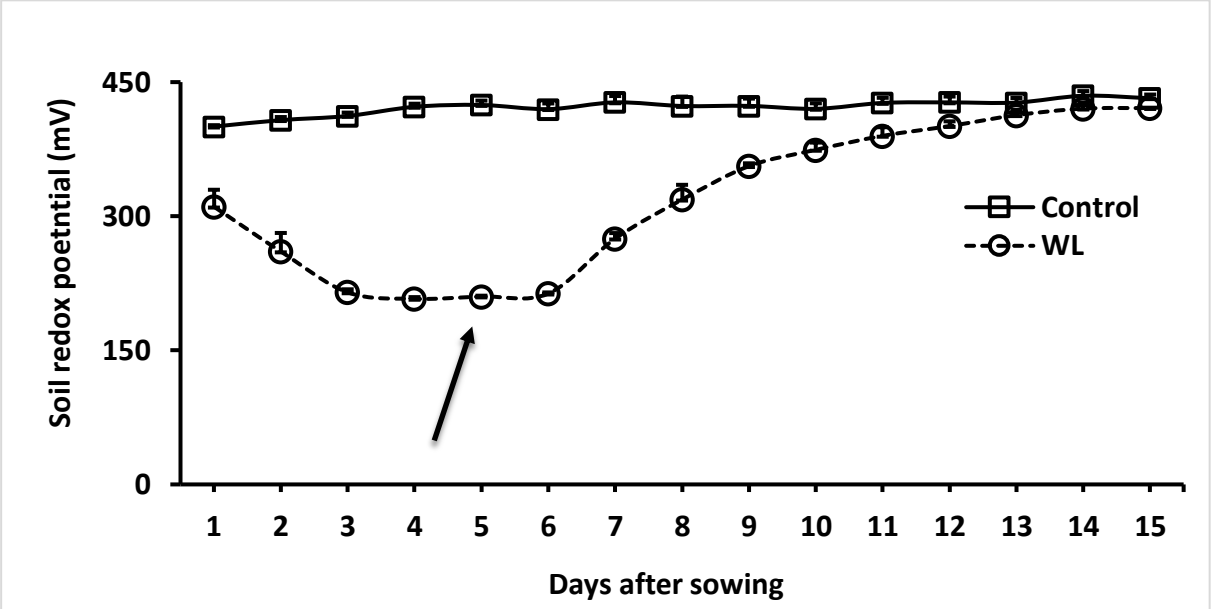


FIGURE 4.2 SOIL REDOX POTENTIAL DURING THE GERMINATION STAGE IN MUNGBEAN UNDER WATERLOGGED (WL) AND DRAINED (CONTROL) CONDITIONS. WL WAS IMPOSED IMMEDIATELY AFTER SOWING FOR 4 DAYS, FOLLOWED BY 7 DAYS OF RECOVERY, WITH THE EXPERIMENT TERMINATING AT 11 DAS. THE ARROW INDICATES THE FIRST DAY OF RECOVERY AFTER THE END OF THE WL TREATMENT. THE DATA ARE MEANS OF SIX SOIL REDOX POTENTIAL PROBES FOR EACH TREATMENT IN EACH COHORT. VERTICAL BARS REPRESENT STANDARD ERRORS (±SE) OF THE MEAN.

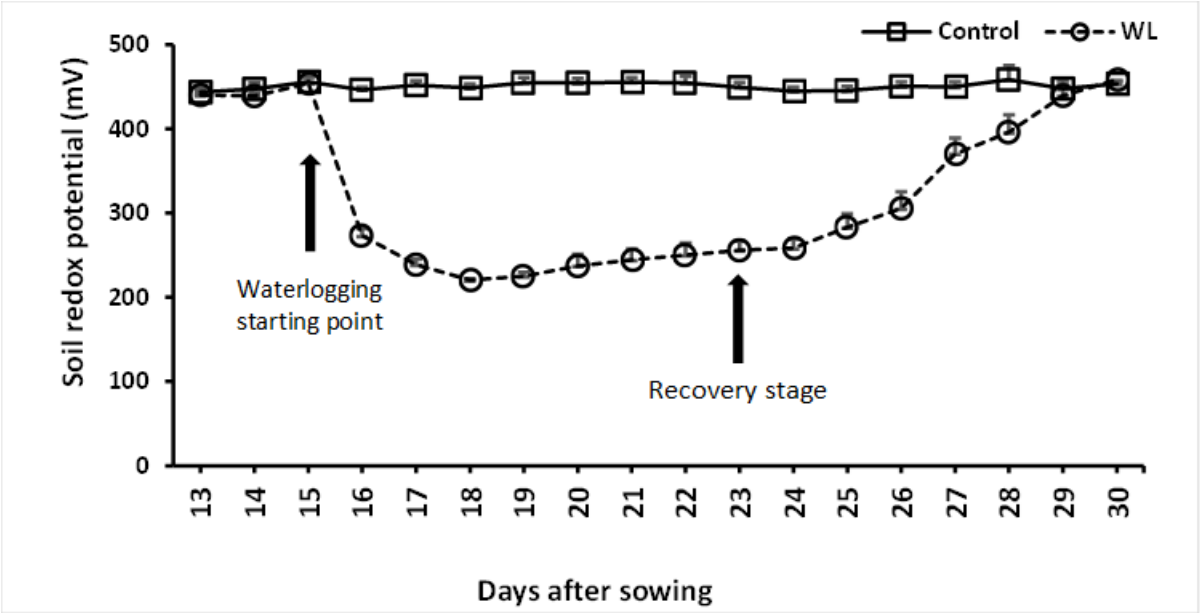


FIGURE 4.3 SOIL REDOX POTENTIAL DURING THE SEEDLING STAGE IN MUNGBEAN UNDER WATERLOGGED (WL) AND DRAINED (CONTROL) CONDITIONS. WL WAS IMPOSED AT 15 DAS FOR 8 DAYS, FOLLOWED BY 7 DAYS OF RECOVERY, WITH THE EXPERIMENT TERMINATING AT 30 DAS. THE ARROWS INDICATE THE FIRST DAY OF WATERLOGGING AND THE FIRST DAY OF RECOVERY AFTER THE END OF THE WL TREATMENT. THE DATA ARE MEANS OF SIX SOIL REDOX POTENTIAL FOR EACH TREATMENT IN EACH COHORT. VERTICAL BARS REPRESENT STANDARD ERRORS (±SE) OF THE MEAN.

TABLE 4.2 DEGREES OF FREEDOM (DF), F VALUES AND PROBABILITIES OF ANALYSIS OF VARIANCE FOR COHORT, CHECK GENOTYPES, TREATMENTS, AND THEIR INTERACTIONS IN MUNGBEAN MINI CORE COLLECTION GENOTYPES SCREENED IN TWO COHORTS AT THE GERMINATION STAGE

Trait	Source of Variation	Cohort	Treat	Gen	Gen × Cohort	Treat × Cohort	Treat × Gen	Treat × Gen × Cohort	Treat × Gen/ Treat × Gen × Cohort*
Emergence (%)	df	1	1	7	7	1	7	7	7/7
	F value	7.12	3450.33	47.51	17.65	3.09	32.41	4.03	8.04
	Probability	0.11	<.001	<.001	0.01	0.01	<.001	0.001	0.01
Shoot dry mass (g)	F value	10.58	1789.09	54.81	8.55	4.02	28.78	2.93	9.82
	Probability	0.08	<.001	<.001	0.04	0.001	<.001	0.011	0.004
Root dry mass (g)	F value	51.47	4265.91	39.31	18.05	3.05	14.48	2.63	5.05
	Probability	0.02	<.001	<.001	0.01	0.01	<.001	0.02	0.02
Total dry mass (g)	F value	14.95	2272.43	68.06	10.39	5.06	31.97	3.85	8.30
	Probability	0.06	<.001	<.001	0.04	<.001	<.001	0.002	0.01

*Note: Further analysis (Treat × Gen/Treat × Gen × Cohort) was undertaken to understand whether the genotype response to waterlogging was greater than the cohort effect for this interaction.

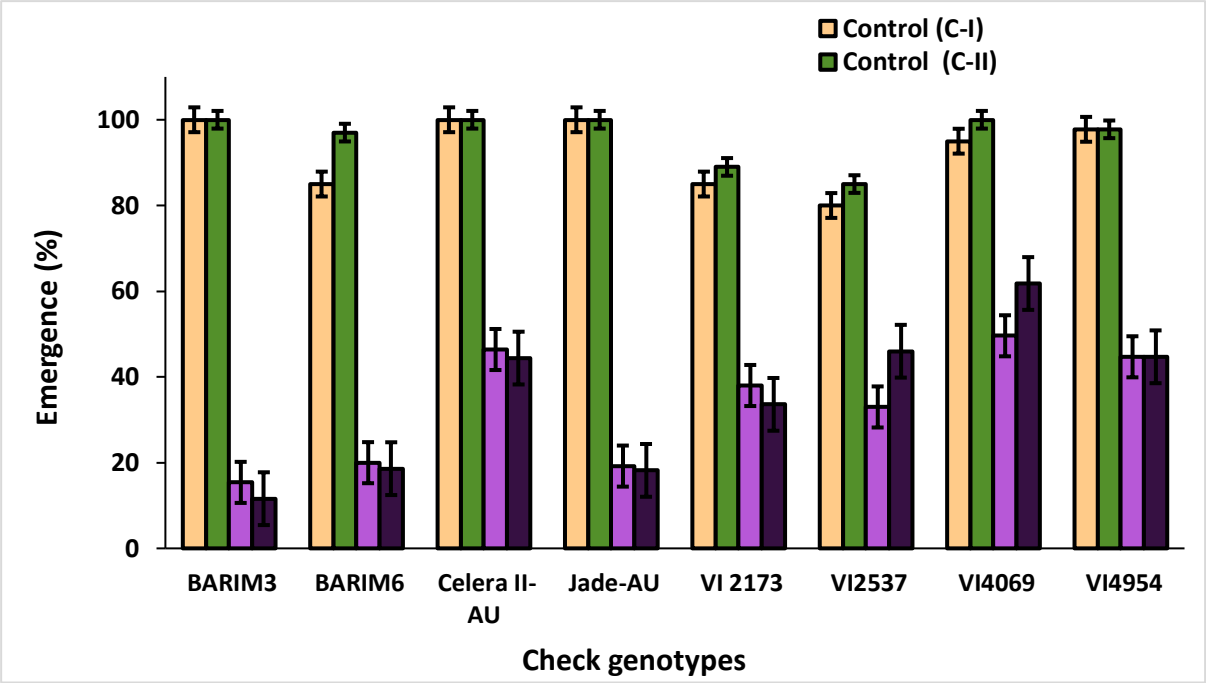


FIGURE 4.4 EFFECT OF TRANSIENT WATERLOGGING (WL) ON THE EMERGENCE (%) OF EIGHT CHECK GENOTYPES FROM THE MUNGBEAN MINI CORE COLLECTION SCREENED IN TWO COHORTS [COHORT I (C-I) AND COHORT II (C-II)]. THE CHECK GENOTYPES WERE REPLICATED FIVE TIMES IN EACH COHORT. WL WAS IMPOSED IMMEDIATELY AFTER SOWING FOR 4 DAYS, FOLLOWED BY 7 DAYS OF RECOVERY, WITH THE EXPERIMENT TERMINATING AT 15 DAS. THE DATA ARE MEANS FOR EACH GENOTYPE IN EACH TREATMENT; VERTICAL BARS REPRESENT STANDARD ERRORS (±SE) OF THE MEAN.

4.4.2.2 Mini core collection genotypes

A wide range of genotypic variation was observed for emergence (%) and shoot and root dry mass. The REML ANOVA table shows significant genotypic, treatment and Treat × Gen interaction effects at P<0.001 for all traits, with the treatment effect higher than the genotypic and interaction effects (Table 4.4). Based on the comparative analysis of the quantitative data, 4 days of soil waterlogging reduced emergence by an average of 52% relative to the drained control (Table 4.6). Highly tolerant lines had 90% emergence, while some highly susceptible lines failed to emerge after 4 days of WL. Transient waterlogging also adversely affected seedling establishment and reduced shoot and root dry mass by 50% and 75% on average (Table 4.6).

According to the Kolmogorov–Smirnov normality test, all traits followed a platykurtic distribution at the germination stage. The skewness, kurtosis and frequency distribution (% of control) for all traits are in Table 4.6 and Figure 4.7. The waterlogging treatment had negative skewness (–0.2) and negative kurtosis (–0.6) for emergence, and positive values for shoot dry mass (1.0, 0.9), root dry mass (1.2, 2.4) and total dry mass (1.0, 1.0). The frequency distribution for emergence revealed three genotypes highly tolerant to transient waterlogging (>90% emergence) compared with 11 genotypes highly susceptible to transient waterlogging (<20% emergence). Moreover, the traits exhibited high broad-sense heritability: 81% for emergence, 83% for shoot dry mass, and 71% for root dry mass (Table 4.6).

A one-way ANOVA was undertaken to understand the relationship between origin and waterlogging tolerance, with the results showing significant variation between regions (Table 4.7). However, this analysis was highly unbalanced, with 64% of genotypes from South Asia, 20% from Southwest Asia and 6.8% from Southeast Asia (Table 4.1). Nonetheless, genotypes from South Asia and Oceanic Pacific had the highest emergence (51%, 50%) compared with those from Europe (29%) and Africa (35%).

According to Pearson's correlation and regression analysis, seedling emergence under transient waterlogging correlated highly with shoot, root and total dry mass but not 100-seed weight (Figure 4.8). The PCA produced similar results (Figure 4.9) and explained the Eigenvalues, percentage variability and cumulative variance component (Table 4.9). The three principal components had Eigenvalues of 3.14 (PC1), 1.05 (PC2) and 0.57 (PC3), accounting for 63% (PC1), 21% (PC2) and 11% (PC3) of total variability at germination. PC1 had a high positive loading on shoot, root and total dry mass, while PC2 had a high positive loading for 100-seed

weight. The PCA biplot shows that seedling emergence positively correlated with shoot, root and total dry mass after 4 days of WL but not 100-seed weight or emergence (Figure 4.8).

4.4.3 Screening at the seedling stage

4.4.3.1. Check genotypes

The eight check genotypes were randomised spatially in each of the three cohorts [cohort I (C-I), cohort II (C-II) and cohort III (C-III)] and analysed as per the germination stage to gauge homogeneity between the cohorts. Significant differences occurred between check genotypes (Gen), treatments (Treat), cohorts (Cohort), and the Treat × Gen and Treat × Cohort interactions for all traits, but not Gen × Cohort or Treat × Gen × Cohort interactions (except for SPAD unit_1 and SPAD unit_2) (Table 4.3). Further testing was undertaken for those traits with a significant Treat × Gen × Cohort interaction to understand whether the genotype response to waterlogging was greater than the cohort effect on this interaction. The results showed that the genotypic response to waterlogging was significantly greater than the cohort effect for this interaction; thus, the data from the three cohorts were combined for further analysis.

The mean performance of check genotypes for shoot and root dry mass (Figure 4.5A, B) and adventitious root number (Figure 4.6) were graphed. Transient waterlogging at the seedling stage affected roots more than shoots. The check genotypes had different reduction patterns. For example, while Celera II-AU, Jade-AU, VI 4954 and BARIM-6 had similar shoot and root dry mass in the drained control, waterlogging reduced shoot and root growth by more than 50% in Celera II-AU, compared with 32% and 29% in Jade-AU, 36% and 60% in VI4954 and 25% and 35% in VI2173, respectively. Under waterlogging, Jade-AU produced the most adventitious roots, including surface roots, followed by VI4954, BARIM-6 and Celera II-AU, while BARIM-3 produced the least.

TABLE 4.3 ANALYSIS OF RESTRICTED MAXIMUM LIKELIHOOD (REML) OF THE MUNGBEAN MINI CORE POPULATION SCREENED AT THE GERMINATION STAGE FOR EMERGENCE (%) AND SHOOT, ROOT AND TOTAL DRY MASS. WATERLOGGING (WL) WAS IMPOSED IMMEDIATELY AFTER SOWING FOR 4 DAYS, FOLLOWED BY 7 DAYS OF RECOVERY, WITH THE EXPERIMENT TERMINATING AT 15 DAS. THE SCREENING WAS UNDERTAKEN IN TWO COHORTS, WITH THE DATA COMBINED AND ANALYSED TO EXPLORE GENETIC VARIATION IN MUNGBEAN.

Variable	Source of variation	Genotype	Treatment	Treatment × Genotype
Emergence (%)	n.d.f*	291	1	291
	d.d.f**	1384	1384	1384
	Wald statistic	2760.63	9671.48	1803.99
	F statistic	9.49	9671.48	6.2
	F pr	<0.001	<0.001	<0.001
Total dry mass (g)	Wald statistic	3710.12	8570.59	2463.24
	F statistic	12.75	8570.59	8.46
	F pr	<0.001	<0.001	<0.001
Shoot dry mass (g)	Wald statistic	2757.35	9565.41	1826.80
	F statistic	9.48	9565.41	6.28
	F pr	<0.001	<0.001	<0.001
Root dry mass (g)	Wald statistic	1659.37	3497.50	1330.06
	F statistic	5.7	3497.5	4.57
	F pr	<0.001	<0.001	<0.001

*numerator degrees of freedom
**denominator degrees of freedom

TABLE 4.4 DEGREES OF FREEDOM (DF), F VALUES AND PROBABILITIES OF ANALYSIS OF VARIANCE FOR COHORT, CHECK GENOTYPES, TREATMENTS, AND THEIR INTERACTIONS IN MUNGBEAN MINI CORE COLLECTION GENOTYPES IN THREE COHORTS SCREENED AT THE SEEDLING STAGE

Trait	Source of variation	Cohort	Gen	Treat	Gen × Cohort	Treat × Cohort	Treat × Gen	Treat × Gen × Cohort	Treat × Gen/ Treat × Gen × Cohort
Total dry mass (g)	df	2	7	1	14	2	7	14	7/14
	F value	32.1	13.43	122.21	0.98	6.55	2.35	0.91	2.58
	Probability	0.003	<0.001	<0.001	0.48	0.03	0.03	0.55	0.06
Shoot dry mass (g)	F value	24.02	10.81	111.26	0.68	5.34	2.58	0.68	3.76
	Probability	0.01	<0.001	<0.001	0.79	0.05	0.02	0.78	0.02
Root dry mass (g)	F value	26.94	14.1	110.08	2.42	6.69	2.12	1.11	1.92
	Probability	0.01	<0.001	<0.001	0.01	0.03	0.05	0.36	0.14
Adventitious root numbers	F value	19.09	6.27	2420.66	1.09	18.99	6.27	1.09	5.75
	Probability	0.01	<0.001	<0.001	0.378	0.003	<0.001	0.378	0.002
SPAD unit _1	F value	44.36	7.05	301.01	3.12	4.96	2.85	2.14	0.67
	Probability	0.002	<0.001	<0.001	<0.001	0.05	0.01	0.02	0.69
SPAD unit_2	F value	22.45	11.23	1249.48	2.55	0.12	6.72	2.53	2.65
	Probability	0.01	<0.001	<0.001	0.004	0.88	<0.001	0.01	0.05
SPAD unit_3	F value	2.92	1.18	1555.15	0.66	27.95	1.53	0.96	1.59
	Probability	0.16	0.32	<0.001	0.80	<0.001	0.17	0.5	0.22

*Note: Further analysis (Treat × Gen/Treat × Gen × Cohort) was undertaken to understand whether the genotype response to waterlogging was greater than the cohort effect for this interaction.

4.4.3.2 Mini core collection genotypes

The REML analysis showed that genotypic (Gen), treatment (Treat), and their interaction (Gen × Treat) effects significantly differed for all traits at $P<0.001$ (Table 4.5). The Gen effect had a higher Wald statistic than the Treat or Treat × Gen effect for shoot, root and total dry mass. The Treat effect was the highest for adventitious root formation and SPAD chlorophyll content (Table 4.5).

The data revealed genotypic and phenotypic variability among the mini core collection genotypes for all traits. Waterlogging decreased shoot, root and total dry mass by 50% after 8 days and SPAD chlorophyll content on the first and second trifoliate leaves at the end of recovery (i.e. 30 DAS) by 71% and 67%, respectively, relative to the drained control (Table 4.6). Similar to the germination stage, the Kolmogorov–Smirnov normality test verified that all traits followed a platykurtic distribution. Shoot, root and total dry mass and 100-seed weight had positive skewness and kurtosis values, while all SPAD chlorophyll measurements on the first and second trifoliate leaves had negative skewness and positive kurtosis values (Table 4.6). The frequency distribution pattern of all traits of interest (based on % of control) is in Figure 4.7. Among the 292 genotypes, waterlogging reduced root dry mass by 7–8% of their controls in only two genotypes compared with 70–93% in 22 genotypes.

Some genotypes rapidly produced adventitious roots in the hypocotyl region after 2–4 days of transient waterlogging, including some which grew along the surface of the soil. No adventitious roots were observed in any of the drained control pots. Considerable variation for adventitious root number existed among genotypes, with an average of 12 adventitious roots produced and some genotypes not producing any (Table 4.6 and Figure 4.7). In the mini core collection, 133 genotypes produced 11–15 adventitious roots and seven produced >20 adventitious roots. Visual observation identified nodules on some roots near the soil surface, but these were not measured. A wide range of variation existed for SPAD chlorophyll content in the first and second trifoliate leaves (Table 4.6). High broad-sense heritability (H^2) values were estimated for shoot and root dry mass (81% and 78%), but a lower value occurred for the number of adventitious roots per plant (56%). Heritability for SPAD chlorophyll content was 70% in SPAD unit_1 (i.e. first trifoliate leaves at 23 DAS), 61% in SPAD unit_2 (i.e. first trifoliate leaves at 30 DAS) and 56% in SPAD unit_3 (i.e. second trifoliate leaves at 30 DAS). SPAD chlorophyll content had higher heritability immediately after waterlogging than during recovery (i.e. SPAD unit_2 and SPAD unit_3).

The ANOVA based on origin revealed significant differences among regions for the measured traits (Table 4.7). As mentioned earlier, 64% of the total population originated in South Asia, and the most susceptible and tolerant genotypes came from South Asia, followed by Southwest Asia, Southeast Asia and Oceanic Pacific. In total, Asia contributed about 92% of the genotypes (Table 4.1). In contrast, other regions had limited numbers of genotypes; hence, the genotypic variation in those regions was negligible.

Pearson's correlation and regression analysis showed a high correlation between shoot and root growth under transient waterlogging (Figure 4.9). Adventitious root formation significantly correlated with shoot dry mass (0.52), root dry mass (0.77) and total dry mass (0.97). Likewise, 100-seed weight significantly correlated with shoot dry mass (0.56), root dry mass (0.50), adventitious root number (0.34) and total plant dry mass (0.30).

In the PCA, the first three components accounted for 87% of the total variation in the data (39% PC1, 30% PC2 and 17% PC3) (Table 4.8). The individual contributions to total variance were highest for adventitious root numbers in PC1 and PC2 (0.62 and 0.76). However, for PC1, SPAD unit_1 had a negative contribution (–0.68), while SPAD unit_3 had a positive contribution (0.68). The biplot analysis explained 39% of PC1 and 30% of PC2 (Figure 4.9). At the seedling stage, shoot, root and total dry mass and 100-seed weight positively correlated with adventitious root formation but negatively correlated with SPAD chlorophyll content.

TABLE 4.5 ANALYSIS OF RESTRICTED MAXIMUM LIKELIHOOD (REML) OF THE MINI CORE COLLECTION GENOTYPES SCREENED IN THREE COHORTS AT THE SEEDLING STAGE FOR SHOOT, ROOT AND TOTAL DRY MASS, ADVENTITIOUS ROOT NUMBER AND SPAD CHLOROPHYLL CONTENT. WATERLOGGING (WL) WAS IMPOSED 15 DAS FOR 8 DAYS, FOLLOWED BY 7 DAYS OF RECOVERY, WITH THE EXPERIMENT TERMINATING AT 30 DAS. THE COHORT DATA WERE COMBINED AND ANALYSED TO EXPLORE THE GENETIC VARIATION.

Variable	Source of variation	Genotype	Treatment	Treatment × Genotype
Total dry mass (g)	n.d.f*	291	1	291
	d.d.f**	1258	1258	1258
	Wald statistic	1409.1	1302.8	296.3
	F statistic	4.8	1302.8	1.0
	F pr	<0.001	<0.001	<0.001
Shoot dry mass (g)	Wald statistic	1211.26	1032.57	270.72
	F statistic	4.1	1032.6	0.9
	F pr	<0.001	<0.001	<0.001
Root dry mass (g)	Wald statistic	1651.04	1320.04	354.3
	F statistic	5.7	1320.0	1.2
	F pr	<0.001	<0.001	<0.001
Adventitious root number	Wald statistic	662.06	6513.08	664.34
	F statistic	2.3	6513.1	2.3
	F pr	<0.001	<0.001	<0.001
SPAD unit_1	Wald statistic	942.58	4532.3	538.6
	F statistic	3.2	4532.3	538.6
	F pr	<0.001	<0.001	<0.001
SPAD unit_2	Wald statistic	1440.9	8219.2	796.3
	F statistic	4.9	8219.2	2.7
	F pr	<0.001	<0.001	<0.001
SPAD unit_3	Wald statistic	607.9	3504.3	460.5
	F statistic	2.1	3504.3	1.6
	F pr	<0.001	<0.001	<0.001

*numerator degrees of freedom
**denominator degrees of freedom

TABLE 4.6 SUMMARY STATISTICS FOR MUNGBEAN MINI CORE COLLECTION GENOTYPES SCREENED AT THE GERMINATION AND SEEDLING STAGES UNDER CONTROL AND WATERLOGGING (WL CONDITIONS. PLANTS WL FOR 4 DAYS AT THE GERMINATION STAGE TO MEASURE EMERGENCE (%) AND SHOOT, ROOT AND TOTAL DRY MASS, AND 8 DAYS AT THE SEEDLING STAGE TO MEASURE SHOOT, ROOT AND TOTAL DRY MASS AND SPAD CHLOROPHYLL CONTENT.

Variable	Mean		Minimum		Maximum		SD		Skewness		Kurtosis		Broad-sense heritability (%)
	Control	WL	Control	WL	Control	WL	Control	WL	Control	WL	Control	WL	
Germination stage													
Emergence (%)	85	48	91	0.0	100	90	0.6	1.2	–0.3	–0.2	–1.1	–0.6	81
Shoot dry mass (g)	0.4	0.2	0.1	0.0	0.9	0.1	0.3	0.1	0.2	1.0	–0.3	0.9	83
Root dry mass (g)	0.3	0.1	0.1	0.0	0.6	0.4	0.1	0.1	1.8	1.2	5.2	2.4	71
Total dry mass (g)	0.9	0.2	0.3	0.0	2.3	1.1	0.4	0.2	0.4	1.0	0.0	1.0	83
Seedling stage													
Total dry mass (g)	1.2	0.6	0.4	0.2	2.8	1.5	0.4	0.2	0.8	0.9	0.8	1.1	79
Shoot dry mass (g)	0.8	0.4	0.2	0.1	1.8	1.0	0.3	0.2	0.7	0.9	0.4	0.9	81
Root dry mass (g)	0.4	0.2	0.1	0.02	1.1	0.6	0.2	0.1	1.1	1.1	1.7	2.3	78
Adventitious root number	0.0	12	0.0	0.0	0.0	25	0.0	4.9		0.1		0.1	56
SPAD unit_1*	38.4	27.1	31.0	15.1	46.9	37.8	2.6	3.9	0.3	–0.1	0.2	0.4	70
SPAD unit_2**	42.7	30.1	34.4	22.4	54.2	38.6	3.7	2.8	0.9	–0.01	0.9	0.1	61
SPAD unit_3***	34.9	23.3	24	10.3	50.1	29.4	3.5	3.1	0.02	–0.6	1.6	1.0	56
100-seed weight (g)	36.7	36.7	21.1	21.1	74.8	74.8	9.2	1.2	1.9	1.9	3.9	3.9	

*SPAD chlorophyll content on the 1st trifoliate leaf at 23 DAS

**SPAD chlorophyll content on the 2nd trifoliate leaf at 30 DAS

***SPAD chlorophyll content on the 3rd trifoliate leaf at 30 DAS

TABLE 4.7 ANALYSIS OF VARIANCE (ANOVA) BASED ON THE ORIGIN OF THE MUNGBEAN MINI CORE COLLECTION GENOTYPES SCREENED AT THE GERMINATION AND SEEDLING STAGES UNDER CONTROL AND WATERLOGGING (WL) CONDITIONS. PLANTS WL FOR 4 DAYS AT THE GERMINATION STAGE TO MEASURE EMERGENCE (%) AND SHOOT, ROOT AND TOTAL DRY MASS, AND 8 DAYS AT THE SEEDLING STAGE TO MEASURE SHOOT, ROOT AND TOTAL DRY MASS AND SPAD CHLOROPHYLL CONTENT.

Source of variation	Germination stage				Seedling stage						
	Emergence (%)	Shoot dry mass (g)	Root dry mass (g)	Total dry mass (g)	Shoot dry mass (g)	Root dry mass (g)	Total dry mass (g)	Adventitious root number	SPAD unit_1	SPAD unit_2	SPAD unit_3
df	9	9	9	9	9	9	9	9	9	9	9
F value	4.19	2.32	1.72	2.26	9.60	11.88	11.25	2.88	2.20	1.04	0.60
Probability	<0.001	0.01	0.08	0.02	<.001	<.001	<.001	0.002	0.02	0.40	0.80

TABLE 4.8 PRINCIPAL COMPONENT LOADINGS OF GERMINATION TRAITS [EMERGENCE (%) AND SHOOT, ROOT AND TOTAL DRY MASS] AND SEEDLING TRAITS [SHOOT, ROOT AND TOTAL DRY MASS, ADVENTITIOUS ROOT NUMBER, SPAD CHLOROPHYLL CONTENT AND 100-SEED WEIGHT] IN THE MUNGBEAN MINI CORE POPULATION SHOWING EIGENVALUES AND CONTRIBUTIONS OF PC1, PC2 AND PC3 TO TOTAL VARIANCE AND CUMULATIVE (%). THE DATA WERE NORMALISED FOR ANALYSIS.

Growth stage	**Waterlogging**		
	PC1	**PC2**	**PC3**
Germination stage			
Emergence (%)	0.39	–0.32	0.85
Shoot dry mass (g)	**0.54**	0.05	–0.13
Root dry mass (g)	**0.50**	0.07	–0.33
Total dry mass (g)	**0.55**	0.05	–0.20
100-seed weight (g)	0.04	**0.94**	0.33
Eigenvalues	3.14	1.05	0.57
Variability (%)	63.00	21.00	11.34
Cumulative (%)			95.34
Seedling stage			
Shoot dry mass (g)	0.013	0.018	0.004
Root dry mass (g)	0.010	0.007	–0.002
Total dry mass (g)	0.023	0.025	0.002
Adventitious root number	**0.620**	**0.761**	0.153
SPAD unit_1	**–0.688**	0.433	**0.573**
SPAD unit_2	–0.243	0.324	–0.415
SPAD unit_3	–0.286	0.345	**0.686**
100-seed weight (g)	0.027	0.090	0.066
Eigenvalues	19.92	15.24	8.78
Variability (%)	39.48	30.2	17.41
Cumulative (%)			87.09

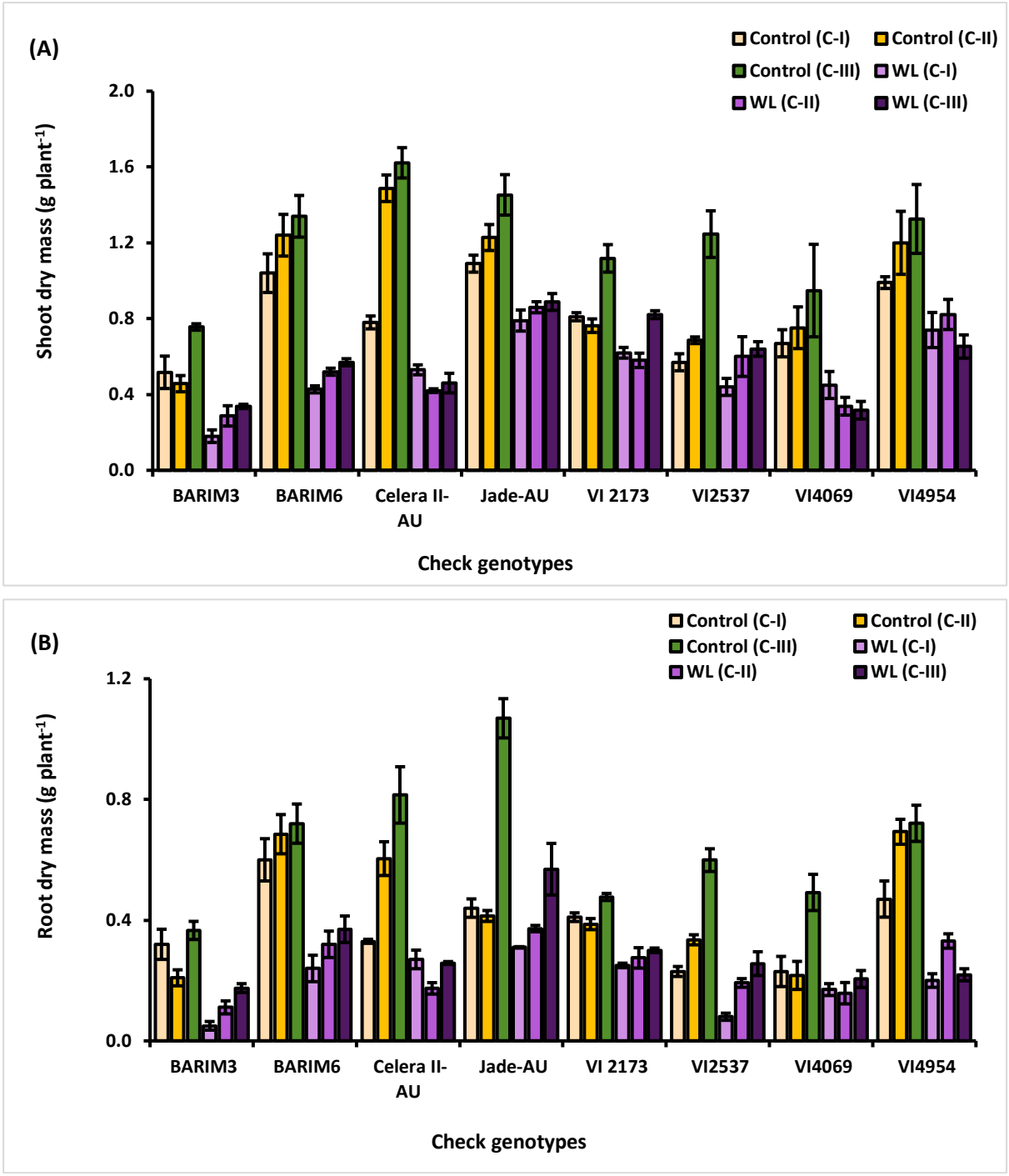


FIGURE 4.5 EFFECT OF TRANSIENT WATERLOGGING (WL) ON SHOOT (A) AND ROOT (B) DRY MASS OF EIGHT CHECK GENOTYPES IN THE MUNGBEAN MINI CORE COLLECTION SCREENED IN THREE COHORTS [C-I, C-II, C-III] AT THE SEEDLING STAGE. WL WAS IMPOSED 15 DAS FOR 8 DAYS, FOLLOWED BY 7 DAYS OF RECOVERY, WITH THE EXPERIMENT TERMINATING AT 30 DAS. THE DATA ARE MEANS FOR EACH GENOTYPE IN EACH TREATMENT; VERTICAL BARS REPRESENT STANDARD ERRORS (±SE) OF THE MEAN.

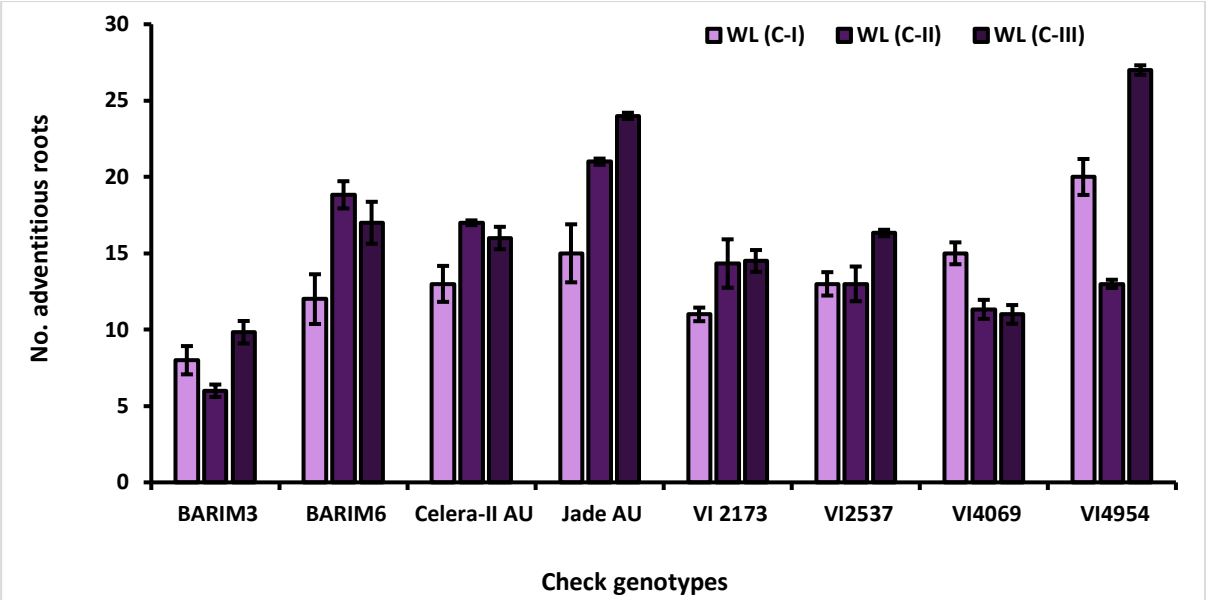


FIGURE 4.6 ADVENTITIOUS ROOT FORMATION OF EIGHT CHECK GENOTYPES FROM A MUNGBEAN MINI CORE SELECTION SCREENED UNDER TRANSIENT WATERLOGGING IN THREE COHORTS (C-I, C-II, C-III) AT THE SEEDLING STAGE. WL WAS IMPOSED 15 DAS FOR 8 DAYS, FOLLOWED BY 7 DAYS OF RECOVERY, WITH THE EXPERIMENT TERMINATING AT 30 DAS. THE NUMBER OF ADVENTITIOUS WAS COUNTED AT 30 DAS. CONTROL PLANTS DID NOT PRODUCE ADVENTITIOUS ROOTS, SO NO DATA IS SHOWN. THE DATA ARE MEANS FOR EACH GENOTYPE IN EACH COHORT; VERTICAL BARS REPRESENT STANDARD ERRORS (±SE) OF THE MEAN.

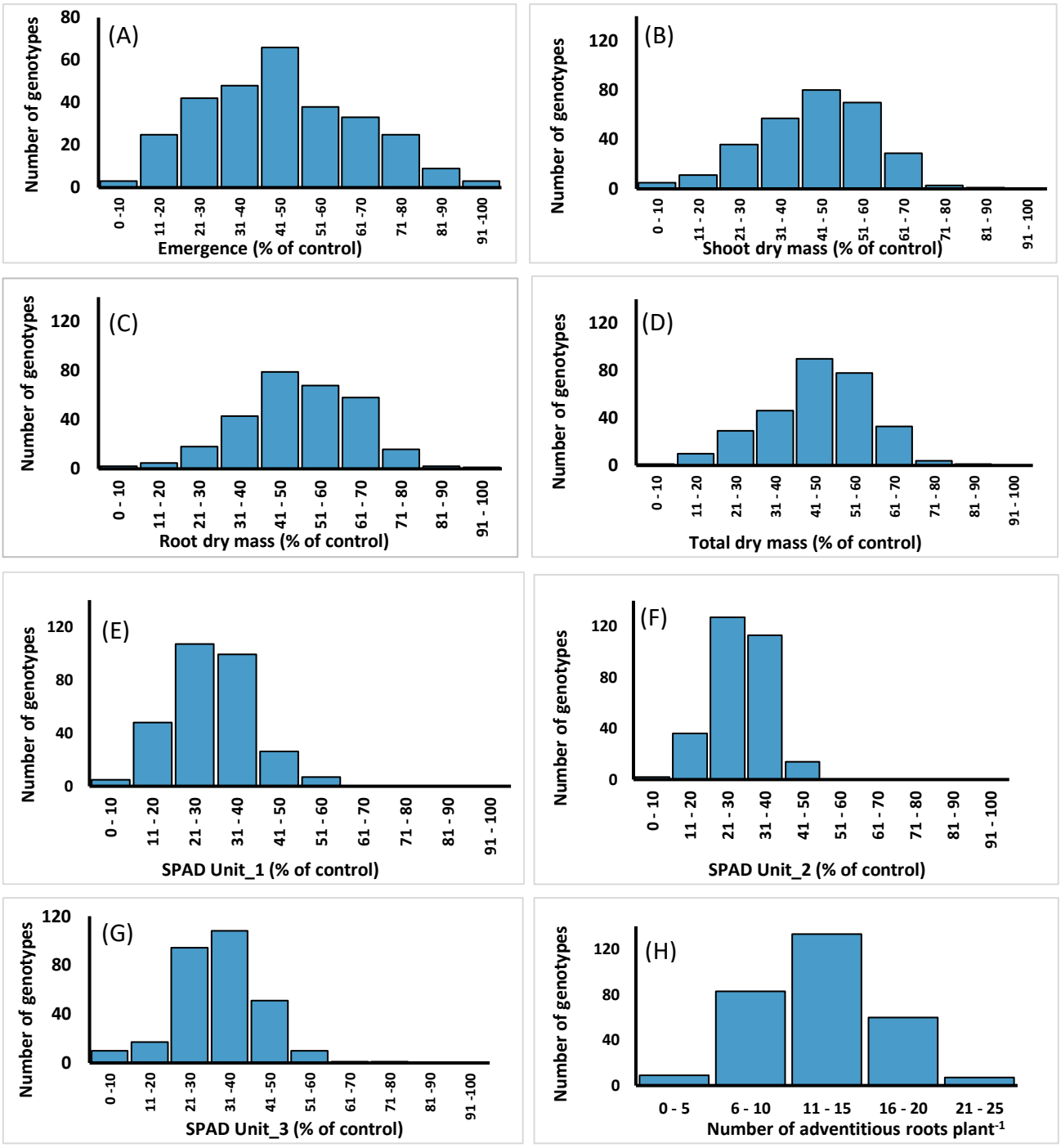
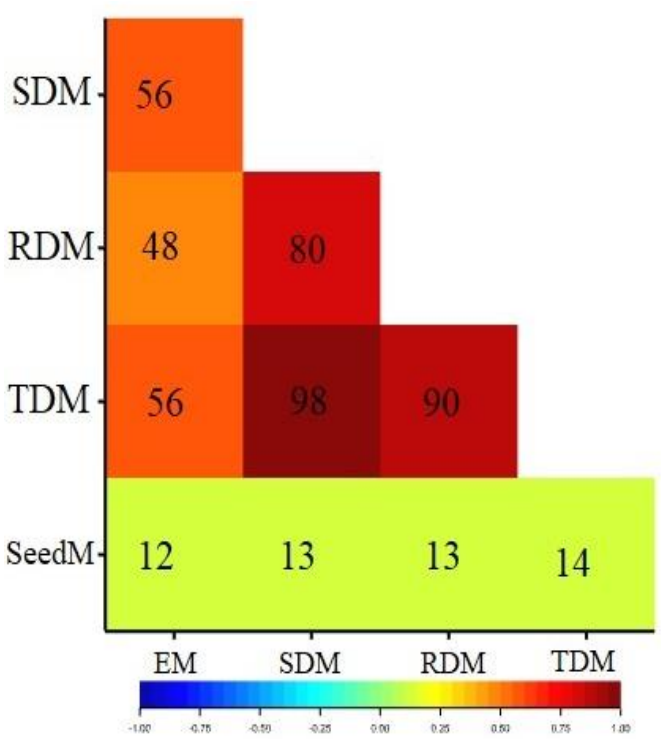
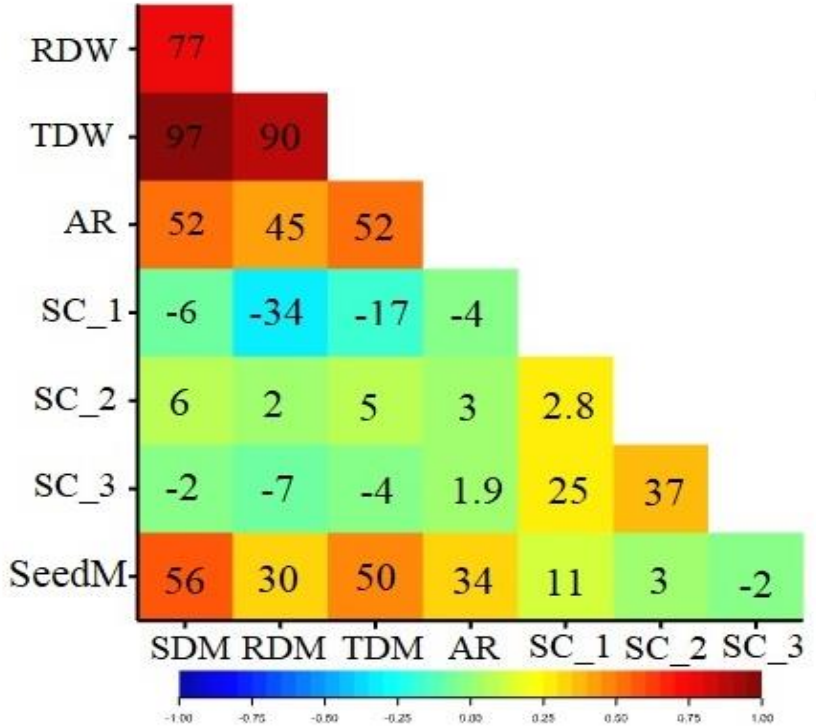


FIGURE 4.7 FREQUENCY DISTRIBUTION FOR THE VARIATION IN EIGHT TRAITS IN 292 MUNGBEAN MINI CORE COLLECTION GENOTYPES. DATA REPRESENT THE PER CENT REDUCTION COMPARED WITH ITS CONTROL. AT THE GERMINATION STAGE: (A) SEEDLING EMERGENCE, AND AT THE SEEDLING STAGE: (B) SHOOT DRY MASS, (C) ROOT DRY MASS, (D) TOTAL DRY MASS, (E) SPAD UNIT_1, (F) SPAD UNIT_2, (G) SPAD UNIT_3 AND (H) ADVENTITIOUS ROOT NUMBER. DATA FOR SEEDLING EMERGENCE WERE RECORDED FOR SEEDS EXPOSED TO 4 DAYS OF SOIL WATERLOGGING FOLLOWED BY 7 DAYS OF RECOVERY. SHOOT, ROOT AND TOTAL DRY MASS, SPAD UNIT_1, SPAD UNIT_2, SPAD UNIT_3, AND ADVENTITIOUS ROOT NUMBER WERE RECORDED FOR SEEDLINGS (15 DAS) EXPOSED TO WATERLOGGING FOR 8 DAYS FOLLOWED BY 7 DAYS OF RECOVERY.



(A) Germination stage



(B) Seedling stage

FIGURE 4.8 CORRELATION HEATMAPS FOR TRAITS OF INTEREST. (A) GERMINATION STAGE: EVALUATED AFTER 4 DAYS OF WATERLOGGING AND 7 DAYS OF RECOVERY, (B) SEEDLING STAGE: EVALUATED AFTER 8 DAYS OF WATERLOGGING FORM 15 DAYS AND 7 DAYS OF RECOVERY. EVALUATED TRAITS: EMERGENCE (EM), SHOOT DRY MASS (SDM), ROOT DRY MASS (RDM), TOTAL DRY MASS (TDM), 100-SEED WEIGHT (SEEDM), NO. ADVENTITIOUS ROOTS (AR), SPAD UNIT_1 (SC_1), SPAD UNIT_2 (SC_2), SPAD UNIT_3 (SC_3). VALUES WITHIN EACH CELL ARE PEARSON'S CORRELATION COEFFICIENT × 100. NON-SIGNIFICANT CORRELATIONS ARE INDICATED BY BLUE CELLS AND SIGNIFICANT CORRELATIONS BY ORANGE AND RED CELLS.

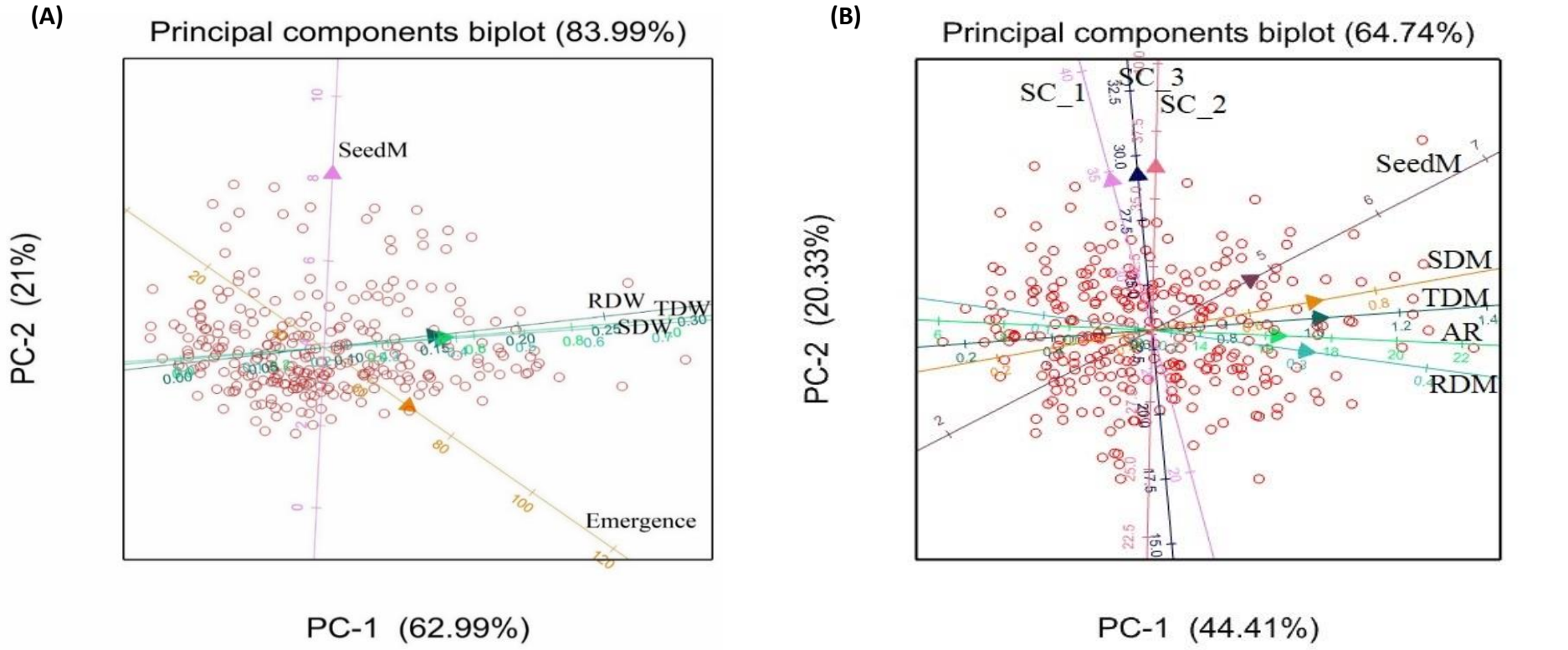


FIGURE 4.9 PRINCIPAL COMPONENT ANALYSIS EXPLAINED BY BIPLOT OF MUNGBEAN EXPOSED TO WATERLOGGING (WL) AT THE GERMINATION STAGE FOR 4 DAYS (A) AND SEEDLING STAGE FOR 8 DAYS (B). AT THE GERMINATION STAGE, THE CONTRIBUTION OF VARIABLES AS VECTORS (ARROWS) WAS 62.99% FOR PC1 AND 21% FOR PC2. SEEDLING EMERGENCE (%) UNDER WATERLOGGING AND SHOOT, ROOT AND TOTAL PLANT DRY MASS UNDER CONTROL CONTRIBUTE TO PC1, AND 100-SEED WEIGHT CONTRIBUTES TO PC2. THE ANGLES BETWEEN THE VECTOR FOR EMERGENCE (%) POSITIVELY CORRELATED WITH SHOOT, ROOT AND TOTAL DRY MASS BUT NEGATIVELY CORRELATED WITH 100-SEED WEIGHT. AT THE SEEDLING STAGE, PC1 EXPLAINS 44.41% AND PC2 EXPLAINS 20.33% OF TOTAL VARIATION. SHOOT, ROOT AND TOTAL DRY MASS, ADVENTITIOUS ROOT NUMBER AND 100-SEED WEIGHT POSITIVELY CORRELATED WITH EACH OTHER AND CONTRIBUTED TO PC1. SPAD CHLOROPHYLL CONTENT OF THE FIRST TRIFOLIATE LEAVES [SPAD UNIT_1 (SC_1), SPAD UNIT_2 (SC_2)] AT 23 DAS AND 30 DAS, AND SPAD UNIT_3 (SC_3) ON THE SECOND TRIFOLIATE LEAVES AT 30 DAS POSITIVELY CORRELATED WITH EACH OTHER BUT NEGATIVELY CORRELATED WITH PLANT DRY MASS.

4.5 DISCUSSION

This is the first report to estimate the overall genetic variation for waterlogging tolerance in mungbean by sampling the mini core collection at the germination and seedling stages. The screening methodology created hypoxic conditions in soil pots to identify genetic variation in waterlogging tolerance. All traits of interest, emergence (%), adventitious root formation, shoot, root and total dry mass, and SPAD chlorophyll content of leaves significantly varied at the 1% significant level, demonstrating a wide range of genotypic variation in recovery from waterlogging stress. The frequency distribution patterns of the traits of interest followed leptokurtic and platykurtic distributions, suggesting the involvement of many genes governing waterlogging tolerance with duplicate gene epistasis and dominant-based complementary epistasis gene action (Pooni et al., 1977; Kapur, 1981). High broad-sense heritability for all traits of interest will help capture the genetic factors influencing waterlogging tolerance.

Genetic variation in waterlogging tolerance among the mini core collection genotypes occurred at both growth stages. At the germination stage, tolerant genotypes (i.e. VI001284AG, VI002999AG, VI003658BG) had similar emergence percentages to the drained controls after removing the stress, but sensitive genotypes (i.e. VI001406BG, VI001993BG, VI000589B-BR) failed to emerge and died, similar to barley germplasm screened for waterlogging tolerance (Takeda and Fukuyama, 1987). The current study confirmed that mungbean seeds do not germinate during waterlogging, and the variation in waterlogging tolerance was related to maintaining seed viability under hypoxia and subsequent emergence on the release of hypoxia. In other legumes, such as soybean, waterlogging tolerance is related to the thickness of the seed aleurone layer, with tolerant genotypes absorbing water more slowly than sensitive genotypes (Tian et al., 2005; Sato et al., 2019). Moreover, Powell and Matthews (1979) reported that pea seeds rapidly absorbed water under soil waterlogging, but the inhibited respiration rate decreased the formation of adenosine triphosphate (ATP) (Johnson et al., 1989), resulting in poor seed viability and germination. In this study, shoot and root growth depended on emergence after removing the waterlogging stress. Nonetheless, some genotypes had a low emergence percentage but rapid seedling growth. Further studies are needed to understand the metabolic traits that enable survival during waterlogging and subsequent recovery.

At the seedling stage, transient waterlogging affected the mini core collection genotypes by reducing shoot and root relative growth rates (% of control) and SPAD chlorophyll content. Mungbean produced adventitious roots to acclimate to the soil O_2 deprivation and associated

energy crisis; hence, adventitious root formation played a major role in stress tolerance. Genotypes that formed adventitious roots in response to waterlogging had higher root and shoot dry mass and SPAD chlorophyll content than genotypes that did not form adventitious roots (Supplementary Table 4.3). Soil O_2 deprivation damages root meristematic tissues during waterlogging stress (Kozlowski, 1984; Valliyodan et al., 2017). Damaged root systems lead to an energy crisis, impairing growth, reducing nutrient ion uptake, preventing cell maintenance, and even causing death (Pedersen et al., 2021). Newly formed adventitious roots, generally with high porosity (i.e. internal gas spaces), are important for carrying O_2 to the active region of roots for nutrient uptake during waterlogging (Voesenek and Bailey-Serres, 2013; Steffens and Rasmussen, 2016). With adventitious root formation, mungbean plants could maintain root function under hypoxic conditions. Tolerant mungbean genotypes (i.e. VI001733BG, VI002532AG, VI003251A-BL, VI004789) produced many adventitious roots, while others delayed or failed to produce any adventitious roots. In addition to differences in adventitious roots, a range of phenotypic variation was observed more generally for root systems; some genotypes had short taproots and a limited number of lateral roots (i.e. VI000170B-BR, VI000212A-BLM, VI000317BG). Similarly, adventitious root formation is a key factor for waterlogging tolerance in soybean (Valliyodan et al., 2014; Y. Kim et al., 2015), barley (Zhang et al., 2015) and wild maize (teosinte) (Mano et al., 2005). In this study, waterlogging tolerance of mungbean positively and significantly correlated with adventitious root formation (Figure 4.8). Notably, genotypes AGG325732 and AGG325650 tolerated transient waterlogging at the germination and seedling stages (Supplementary Tables 4.1 and 4.2).

The highest broad-sense heritability estimates occurred for emergence (81%) at the germination stage and shoot (81%) and root (79%) dry mass at the seedling stage. The estimated broad-sense heritability for adventitious root formation was 56%, and SPAD chlorophyll content ranged from 56–71% at the seedling stage. The results demonstrate the possibility of successfully selecting those traits for mungbean breeding. The nature of gene action governing the traits of interest was studied in terms of the distribution pattern (% of control), skewness and kurtosis of the mini core population. A negatively skewed platykurtic distribution suggests that many genes with duplicate epistasis control the trait; for example, emergence (%) at the germination stage in the present study. At the seedling stage, shoot, root and total dry mass follow leptokurtic distribution suggesting few genes control these traits with dominant-based complementary epistasis (Fisher, 1932; Robson, 1956). Adventitious root formation had a positively skewed leptokurtic distribution with very low skewness and kurtosis (0.1, 0.1),

assuming that it follows dominant-based complementary epistatic gene action. SPAD chlorophyll content on the first (SPAD_unit_1) and second trifoliate leaves (SPAD_unit_2) had a negatively skewed leptokurtic distribution, suggesting that few genes with duplicate epistasis control the trait. Heritability estimates combined with identifying sources of waterlogging tolerance demonstrate the possibility of selecting these traits and accelerating mungbean breeding for climate-resilient cultivars.

4.6 CONCLUSION

This study will guide the genetic improvement of waterlogging tolerance in mungbean. Understanding the genetic variability of waterlogging tolerance is the first step towards varietal improvement. In mungbean, waterlogging tolerance is based on specific morphological traits, such as emergence (%) under hypoxia and rapid adventitious root formation that can supply water and nutrients to the whole plant. Genetic variance analysis revealed the polygenic control of waterlogging tolerance with duplicate gene epistasis and dominant-based complementary epistatic gene action. The candidate genes associated with the waterlogging-tolerant traits will be identified using GWAS analysis in the next chapter.

CHAPTER 5 GENOME-WIDE ASSOCIATION STUDIES OF MUNGBEAN MINI CORE COLLECTION FOR WATERLOGGING TOLERANCE AT THE GERMINATION AND SEEDLING STAGES

5.1 ABSTRACT

Mungbean (*Vigna radiata* var. *radiata* (L.) R. Wilczek) is a fast-growing crop and important for the millions of smallholder farmers in tropical developing countries. Recent developments in genotyping mungbean mini core lines using Diversity Arrays Technology sequencing technologies have facilitated genome research to accelerate molecular breeding related to biotic and abiotic stresses. The current study used the mungbean mini core collection genotypes to predict the specific loci that underlie the phenotypic traits of interest using genome-wide association studies and understand the genetic basis of transient waterlogging tolerance at germination and seedling stages. The genotypes were divided randomly into two cohorts for germination screening (4 days of waterlogging followed by 7 days of recovery) and three cohorts for seedling stage screening (8 days of waterlogging followed by 7 days of recovery), each with its respective drained control. At the germination stage, zinc finger protein (*ZFP8*) could be a suitable candidate gene influencing emergence after four days of waterlogging. At the seedling stage, FGGY carbohydrate kinase domain-containing protein was associated with adventitious root formation. Significantly, transcription factor HHO5 was a pleiotropic gene related to three traits: shoot, root and total dry mass. The SPAD chlorophyll content was associated with a 3-oxoacyl-[acyl-carrier-protein] synthase, mitochondrial. Seed weight was associated with many genes, with lipopolysaccharide-binding protein/bactericidal permeability-increasing protein (LBP/BPI), trehalose-6-phosphate synthase (TPS), and pentatricopeptide repeat-containing protein (PCMP-E1) as promising candidate genes for seed weight in this study. These findings strengthen the understanding of the genetic mechanisms underlying transient waterlogging tolerance in mungbean and will be useful for marker-assisted breeding or gene cloning to develop waterlogging-tolerant mungbean lines. Further studies are needed to validate the associated candidate genes and develop markers based on associated single-nucleotide polymorphism.

5.2. INTRODUCTION

Plant genetic resources play a pivotal role in plant genomics and phenomics studies, boosting major scientific discoveries in advanced agricultural systems (Srivastava et al., 2020). For example, the genetic basis of waterlogging tolerance has been studied by screening various genetic resources, such as worldwide germplasm collection of *Hordeum vulgare* (Borrego-Benjumea et al., 2021), breeding germplasm of *Phaseolus vulgaris* and *Glycine max* (Soltani et al., 2017; Yu et al., 2019), recombinant inbred lines of *Glycine max*, *Pisum sativum* and *Brassica napus* (Ye et al., 2018; Zaman et al., 2019; Wang et al., 2020), and a nested-association mapping population of *Glycine max* (Ali et al., 2020) to identify small haplotypes associated with traits of interest. Among the genetic resources, a limited amount of variation and recombination occurred during the creation of the RIL or NAM populations (Kover et al., 2009; Huang et al., 2011; Weigel, 2012). In contrast, plant germplasm collections harbour a much greater range of natural genetic diversity, comprising landraces or old crop cultivars that serve as potential donors of useful genes to identify alleles for enhancing yield and abiotic stress adaptation (Dwivedi et al., 2016).

Generally, specific loci underlying a phenotype are identified via various mapping approaches, including quantitative trait locus (QTL) mapping (Korte and Farlow, 2013), a powerful method for identifying genomic regions co-segregate with a given trait. However, it can only be used to assay allelic diversity that segregates between the parents of the particular F_2 or within the RIL population, limiting the mapping resolution (Kover et al., 2009; Huang et al., 2011; Weigel, 2012). Hence, plant scientists consider genome-wide association studies (GWAS) based on the principle of linkage disequilibrium (LD) as an alternative approach for detecting substantial associations between DNA marker and target trait or connecting the genotype–phenotype map (Gupta et al., 2005; Gómez et al., 2011; Korte and Farlow, 2013) to investigate complex traits and polymorphisms within and among populations by testing genome-wide SNPs across an assembled population (Cortes et al., 2020). GWAS has a superior resolution mapping power using mass recombination events from numerous meiotic events throughout the germplasm's evolutionary history to characterise several alleles concurrently in diploid (Zhao et al., 2007) and polyploid (Breseghello and Sorrells, 2006) crops. Recently, GWAS has been used widely to identify common genetic factors responsible for expected variations in complex traits, such as waterlogging tolerance in soybean (Ye et al., 2018; Yu et al., 2019) and common bean (Soltani et al., 2017). Conceptually, the genome-wide complex trait analysis estimates the variance explained by all genotyped SNPs for a given trait, which is typically less than the

estimated heritability of that trait and provides an upper boundary for the expected variance capture for a given SNP set and trait (Yang et al., 2011).

Mungbean is an orphan crop; however, recent efforts have developed genetic and genomic resources for whole-genome scan studies, such as GWAS. Kang et al. (2014) constructed the first draft genome sequence of mungbean to facilitate genomic research. In addition, the World Vegetable Center, Taiwan, developed a mungbean mini core collection comprising 296 accessions based on phenotypic and molecular characterisation using 20 SSR markers (Schafleitner et al., 2015).

Mungbean is a predominantly self-pollinated crop, making it particularly well suited to GWAS. The crop can be maintained as inbred lines continuing self-fertilisation; thus, it is possible to repeatedly phenotype genetically identical individuals (Korte and Farlow, 2013). Likewise, the mungbean mini core genotypes have been studied using GWAS technologies for several important trait associations such as hypocotyl colour (Sokolkova et al., 2020), seed coat lustre (Breria et al., 2020), 100-seed weight (Akhtar et al., 2021) and root traits (Aski et al., 2021). However, in mungbean, there is a lack of information on the genetic basis of stress tolerance especially waterlogging tolerance, despite the crop's high sensitivity to transient waterlogging.

The increased frequency of soil waterlogging due to global warming is concerning. Mitigating the adverse effects of waterlogging will help farmers increase productivity and profit certainty. This study used the mungbean mini core collection to predict specific loci underlying the phenotypic traits of interest using GWAS and understand the genetic basis of transient waterlogging tolerance at two growth stages: germination and seedling.

Breeding waterlogging-tolerant crop varieties is a crucial component of an integrated approach to decreasing the adverse effects of this abiotic stress (Manik et al., 2019). Understanding the molecular mechanism and genetic basis underlying waterlogging tolerance is important in mungbean breeding. This study aimed to answer the research question, 'Are there significant marker-trait associations for traits measured for waterlogging tolerance?'. We used existing data on 10,224 high-quality SNPs developed from DArT sequencing (Schafleitner et al., 2015; Kang et al., 2014) together with phenotype data (Chapter 4) to identify significant loci associated with waterlogging tolerance traits and possible underlying candidate genes.

5.3 MATERIALS AND METHODS

5.3.1 Genetic materials

The study used 292 mini core collection genotypes from a core collection of 1,481 genotypes representing the collection of 5,234 genotypes at the World Vegetable Centre (AVRDC), formerly the Asian Vegetable Research and Development Center (Schafleitner et al., 2015).

5.3.2 Phenotyping

Chapter 4 details the waterlogging tolerance screening of the mini core genotypes at the germination and seedling stages. Briefly, mini core genotypes were planted in the experimental unit pots in a temperature-controlled glasshouse at The University of Western Australia, Crawley, Western Australia (31° 59' S and 115° 49' E) from May to June 2019 (germination stage) and September to December 2019 (seedling stage). The temperature inside the glasshouse ranged from 21±4°C to 32±3°C, with 10 h 45 min daylight (1,150–1,627 µmol m^{-2} s^{-1}). The treatments received four days of waterlogging for the germination stage and eight days for the seedling stage. Each treatment had a respective drained control. The mini core collection genotypes were divided randomly into two cohorts for germination stage screening and three cohorts for seedling stage screening. Eight check genotypes (VI64209, VI64210, VI002173, VI002537 AG, VI004069 BG, VI004954 BG, VI064518, VI064521) were replicated five times at the germination stage, but only once at the seedling stage due to limited space. There were three replications for each growth stage. The waterlogging treatment was mimicked by adding DI water to the plastic tanks. The soil water table was retained at the soil surface during the treatment. The drained control pots were maintained at 80% field capacity by adding DI water directly to the pots as required throughout the experimental period. After waterlogging, the pots were relocated to free-draining plastic tanks to observe plant responses during a 7-day recovery period. The experiments were terminated 11 days after sowing (DAS) for the germination stage screening and 30 DAS for the seedling stage screening.

The germination stage experiment measured four traits: emergence (%), shoot dry mass, root dry mass and total dry mass. The seedling stage experiment measured seven traits: shoot dry mass, root dry mass, total dry mass, number of adventitious roots, SPAD chlorophyll content on the first trifoliate leaves (i.e., at the end of waterlogging and at the end of recovery) and SPAD chlorophyll content on the second trifoliate leaves (i.e., end of recovery). The seed weight of each mini core genotype was measured before starting the experiment. The

phenotypic data of mini core collection genotypes for each growth stage were analysed using maximum likelihood in GENSTAT 21st edition (VSN International, UK).

5.3.3 Genomic DNA sequencing

The genomic DNA sequencing data were generated by DArT (Diversity Array Technology P/L, Australia http://www.diversityarrays.com/) and accessed by the World Vegetable Center (Schafleitner et al., 2015). Schafleitner and his group aligned the sequence data to the mungbean reference genome sequence, Vardi_ver6 (Kang et al., 2014), and identified 24,870 single-nucleotide polymorphisms (SNPs) as the first DArT sequencing data in mungbean. The selection of molecular markers and genotyping methods are detailed in Schafleitner et al. (2015).

5.3.4 SNPs filtering and genome-wide linkage LD

The genome-wide SNPs across the mini core genotypes were filtered based on the missing proportion and minor allelic frequency (MAF). SNPs were removed for those with no name on the mungbean chromosomes and SNPs on the scaffold followed by SNP loci <0.02 MAF and >20% missing across the mini core population. The pairwise LD between SNPs genome-wide across the mini core genotypes was estimated by allele frequency correlations (r^2) using TASSEL software (V5.1.0) (Bradbury et al., 2007; Tao et al., 2013). An LD decay graph (Figure 5.1) was drawn by fitting a smooth spline of averaged r^2 over physical distance in RStudio 4.1.3. The LD decay was calculated when the squared correlations of allele frequencies r^2 decreased to half their maximum value (Hill and Weir, 1988; Remington et al., 2001).

5.3.5 Population structure analysis

The population structure of the mini core genotypes was analysed using the filtered SNPs in the STRUCTURE program 2.3.4 (Pritchard et al., 2010). STRUCTURE performs a Bayesian model-based clustering approach applying Markov Chain Monte Carlo (MCMC) estimation (Porras-Hurtado et al., 2013). Nine K values (K=2:10) with five replicated runs each were analysed. The burn-in period was set at 5,000 with 50,000 MCMC replications. The admixture model was chosen as the ancestry model assumption. The K values obtained from STRUCTURE mined by STRUCTURE Harvester (Earl and von Holdt, 2011). The most likely number of subpopulations (K) in structure was determined using the non-parametric Wilcoxon test. A threshold of non-admixed individuals was set at a Q matrix value ≥70%. Admixed individuals were classified as having Q matrix values <70%.

The evolutionary history was inferred using the neighbour-joining method (Saitou and Nei, 1987). The bootstrap consensus phylogenetic tree inferred from 100 replicates (Felsenstein, 1985a) represents the evolutionary history of the taxa analysed (Felsenstein, 1985b). Branches corresponding to partitions reproduced in <50% bootstrap replicates were collapsed. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004), with the number of base substitutions per site as units. The analysis involved 292 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option) of the final data had 29,125 positions. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

5.3.6 GWAS analyses and candidate gene prediction

GWAS analysis for the traits of interest was undertaken using the R package Genomic Association and Prediction Integrated Tool, GAPIT version 3 (Lipka et al., 2012). Analyses were performed by marrying the filtered SNPs and phenotypic reduction (per cent of control) for each targeted trait. Multiple models were tested per trait: (i) general linear model (GLM, Price et al., 2006), (ii) mixed linear model (MLM, Yu et al., 2006), (iii) compression MLM (CMLM, Zhang et al., 2010), (iv) fixed and random model circulating probability unification (FarmCPU; Liu et al., 2016) and (v) Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK; Huang et al., 2019).

The most appropriate GWAS method was identified based on inspection of Q-Q plots and Manhattan plots for evidence of P-value inflation. Significant marker-trait associations were identified using different criteria, such as GWAS threshold P-value, FDR threshold (false discovery rate) and Bonferroni threshold. The GWAS threshold P-value was determined based on the equation $\alpha = 1/m$, where m is the number of markers [$-\log_{10}$ (P-value) <4.5] (Wang et al., 2012; Borrego-Benjumea et al., 2021). The FDR threshold was determined based on the proportion of false positive and false negative associations in a simulated dataset. The significant Bonferroni threshold was $p < 0.01/n$, where n represents the number of SNP markers across the entire genome.

The significantly associated markers were assigned to QTL regions of the mungbean reference genome assembly (Vradiata_ver6) using the NCBI genome data viewer using NCBI *Vigna radiata* Annotation Release 101 (Kang et al., 2014) based on the trait, their chromosomal positions, and the estimated LD decay (~190 Kb). The candidate genes were extracted and

checked. Annotations were downloaded from Ensembl (http://plants.ensembl.org), AmiGO gene Ontology (amigo.geneontology.org) and UniProtKB (https://www.uniprot.org).

5.4 RESULTS

5.4.1 Genetic diversity, population structure and LD analysis

The mini core DArTseq data comprised 24,870 raw SNP markers on 11 chromosomes with an average size of 33.5 Mb. The unidentified SNPs (2,026) to chromosomes and scaffolds (4,304) of the reference sequence were excluded (Kang et al., 2014; Breria et al., 2019). Of the remaining 18,540 SNPs, 44.85% were filtered out (MAF $\leq$ 0.02), leaving 10,224 SNPs with MAF $\geq$ 0.02 for the genetic diversity analysis of 292 mini core collection genotypes (Table 5.1). The filtered SNPs were distributed unevenly on the 11 mungbean chromosomes. The average number of SNPs on each chromosome was 1,704, with the fewest SNPs (613) on chromosome 3. The filtered SNPs had an average heterozygosity proportion of 0.034.

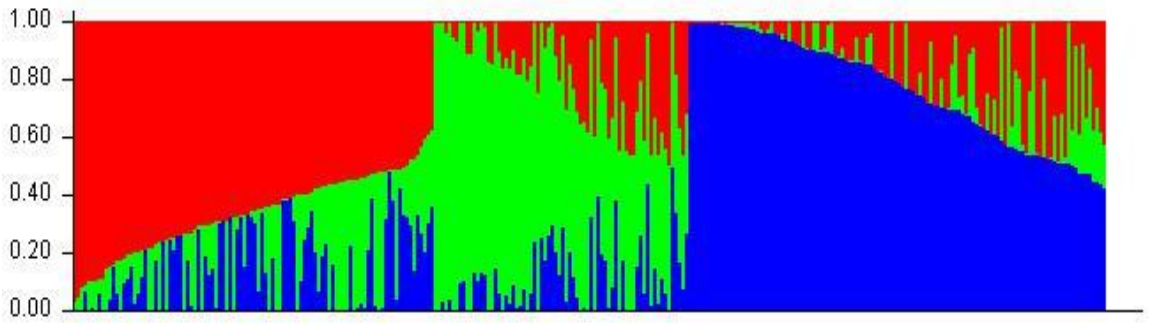
The population structure of the 292 mini core collection genotypes was inferred initially using STRUCTURE 2.3.4 (Pritchard et al., 2000). The Bayesian approach implemented in STRUCTURE revealed the presence of three subpopulations with the highest likelihood for K=3 (Figure 1), partitioning the 292 genotypes into three principal groups comprising 104, 72 and 116 genotypes. The principal component analysis (PCA) produced consistent results, confirming the existence of three subpopulations (Figure 5.2). Furthermore, the phylogenetic tree, constructed by the neighbour-joining method, also identified three major clusters. The results indicated that the three clusters do not correspond to the regions of origin; South Asian genotypes were found across the entire population but predominantly in subpopulation 1. Genotypes from Africa, East Asia, Europe, Mexico, Oceanic Pacific, Southeast Asia, and Southwest Asia were in subpopulation 3 (Figure 5.2).

Squared correlation coefficient (r^2) values among the marker pairs were used to estimate LD decay across the 11 chromosomes (Figure 5.3). The arbitrary baseline r^2 value was 0.1. The genome-wide LD decay analysis revealed that chromosomes decayed at 190,764 bp (Figure 5.4). The LD decay results also revealed that 10,244 SNPs (MAF $\leq$ 0.02) cover the entire genome, adequate for GWAS in the current study. The window size of the QTL determined in the mini core genotype was ±190,764 bp from the highest peak of the significant marker-trait association.

TABLE 5.1 COVERAGE OF SNPS PER INDIVIDUAL MUNGBEAN CHROMOSOME BEFORE AND AFTER FILTERING

Chromosome	Before filtering			After filtering		
	Size (Mb)	Total SNPs	Distance between SNPs (kb)	Size (Mb)	Total SNPs	Distance between SNPs (kb)
1	36.50	1,695	21.5	36.39	1,177	30.9
2	25.36	1,491	17.0	25.07	778	32.2
3	12.95	1,095	11.8	12.87	613	21.0
4	20.81	1,113	18.7	20.54	663	30.9
5	37.18	2,253	16.5	37.05	975	38.0
6	37.44	1,806	20.7	37.29	1,143	32.6
7	55.60	2,716	20.4	55.50	1,255	44.2
8	45.73	2,584	17.7	45.30	1,461	31.0
9	21.01	1,268	16.5	20.98	829	25.3
10	21.00	1,243	16.8	20.92	634	33.0
11	19.73	1,276	15.4	19.49	696	28.0
Contigs	68.6	4,304	15.9	–	–	
Total	401.9	22,844	208.9	331.4	10,224	347.1
Average	36.5	2,076.7	17.4	30.13	929.4	31.5

(A) Population structure



(B) Genetic association of mini core collection

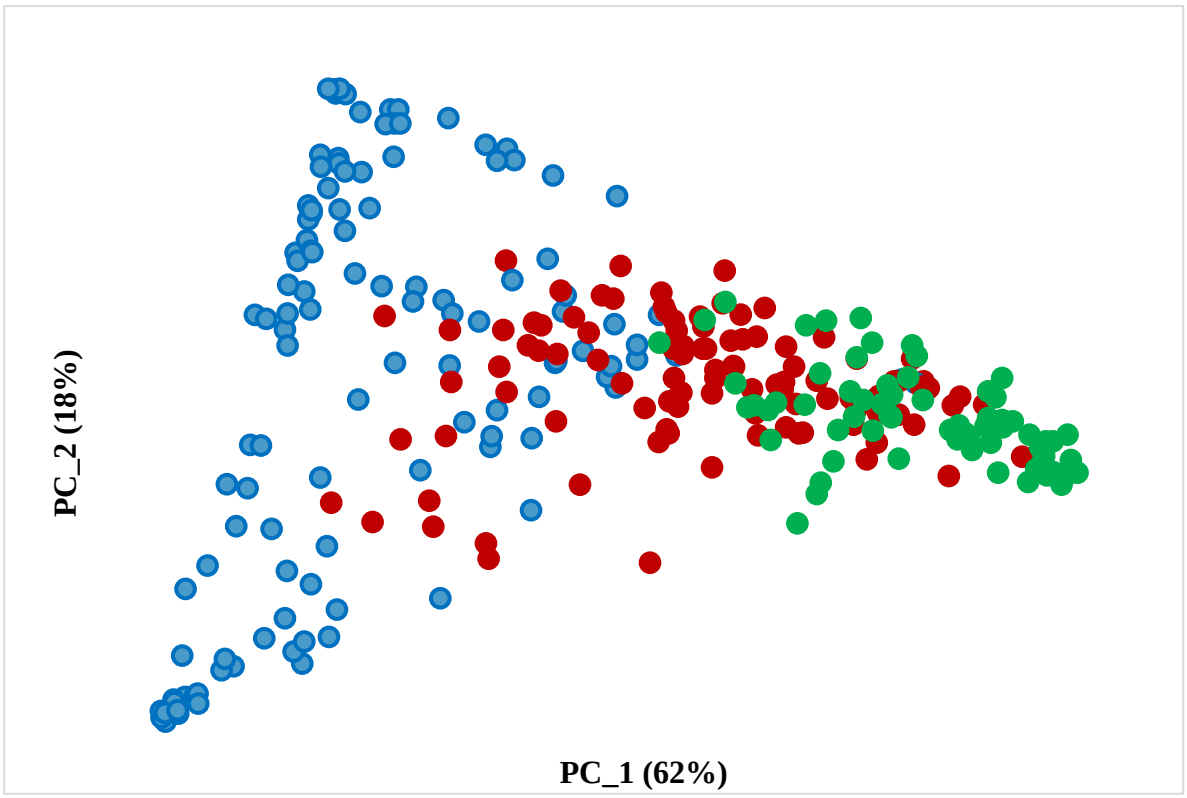


FIGURE 5.1 POPULATION STRUCTURE OF 292 MUNGBEAN MINI CORE GENOTYPES. (A) CLASSIFICATION OF THREE POPULATIONS USING STRUCTURE 2.3.4. THE OPTIMAL NUMBER OF CLUSTERS (K=3) IS ESTIMATED BASED ON THE ABSOLUTE VALUE OF THE SECOND-ORDER RATE OF CHANGE IN THE LIKELIHOOD DISTRIBUTION. EACH VERTICAL BAR REPRESENTS A SINGLE ACCESSION, AND THE LENGTH OF EACH BAR REPRESENTS THE PROPORTION CONTRIBUTED BY EACH POPULATION. (B) GENETIC ASSOCIATION BETWEEN 292 MUNGBEAN MINI CORE COLLECTION GENOTYPES BASED ON 10,244 MARKERS DEVELOPED FROM DART SEQUENCING, REVEALED BY A PRINCIPAL COMPONENT ANALYSIS (PCA). THE COLOUR CODE INDICATES THE DISTRIBUTION OF MINI CORE GENOTYPES TO DIFFERENT POPULATIONS: SUBPOPULATION 1 (RED), SUBPOPULATION 2 (GREEN) AND SUBPOPULATION 3 (BLUE).

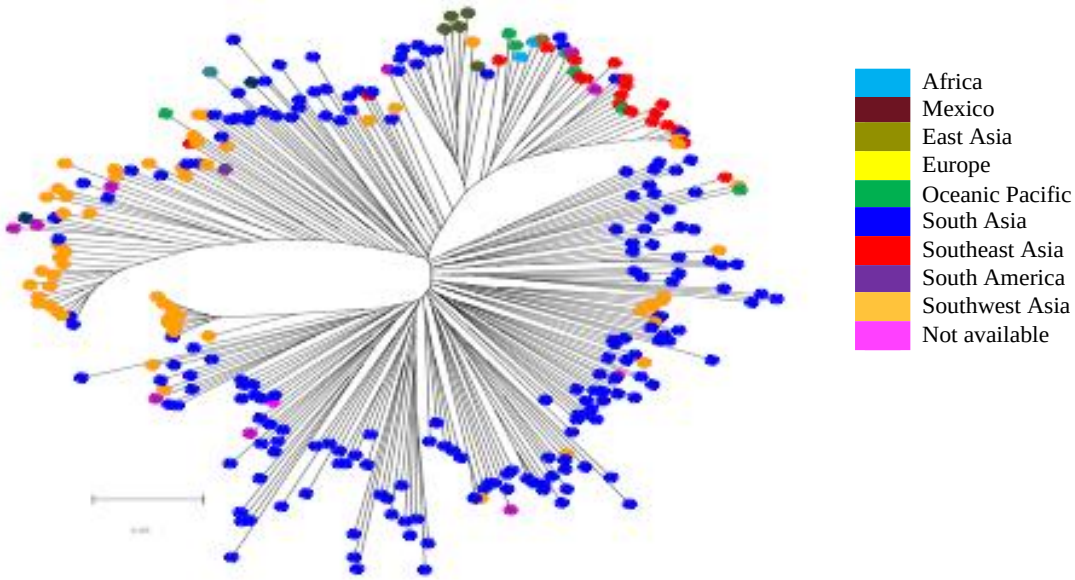


FIGURE 5.2 DIVERSITY OF MUNGBEAN MINI CORE COLLECTION GENOTYPES. NEIGHBOUR-JOINING TREE ANALYSIS OF 292 MUNGBEAN MINI CORE GENOTYPES: 186 FROM SOUTH ASIA, 57 FROM SOUTHWEST ASIA, 20 FROM SOUTHEAST ASIA, SEVEN FROM OCEAN PACIFIC, FIVE FROM EUROPE, TWO FROM AFRICA, TWO FROM SOUTH AMERICA AND 11 FROM UNKNOWN REGIONS. THE ANALYSIS WAS BASED ON A BOOTSTRAP CONSENSUS PHYLOGENETIC TREE INFERRED FROM 100 REPLICATES TO REPRESENT THE EVOLUTIONARY HISTORY OF THE TAXA ANALYSED.

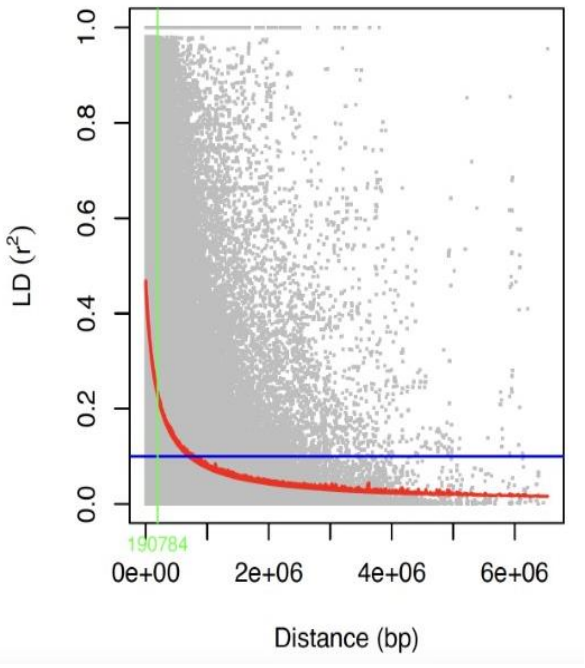


FIGURE 5.3 THE LD DECAY WAS ESTIMATED FROM SINGLE-NUCLEOTIDE POLYMORPHISM (SNP) GENOTYPES OF 292 MUNGBEAN MINI CORE COLLECTION GENOTYPES. THE CURVE REPRESENTS THE AVERAGE LD OF 11 CHROMOSOMES OF THE MINI CORE POPULATION. THE LD DECAY AT R^2 = 0.1 WITH ~190 KB.

5.4.2 GWAS and SNP discovery

A GWAS analysis was performed using the filtered 10,224 SNPs markers (with MAF ≤ 0.02) and phenotypic data, expressed as reduction rate (i.e. per cent (%) of control) for emergence, shoot, root and total dry mass, and SPAD chlorophyll content. However, unadjusted data were used for adventitious root formation and seed weight. Five GAPIT (version 3) models were run to identify marker-trait associations. The BLINK model was the most reliable based on the Q-Q plot results (Figures 5.4–5.8). Manhattan plots showed the significance of markers associated with the measured traits (Figures 5.4–5.8). The Q-Q plots revealed that the expected and observed P-values matched and deviated towards a reasonable positive.

5.4.2.1 Germination stage

At the germination stage, GWAS identified a SNP (Vrad_SNP01783) on chromosome 8 for emergence (% control) that exceeded the GWAS P-value and 5% of significant FDR test and Bonferroni threshold level of $-\log_{10}$ *P*-value= 0.5 (Figure 5.5; Table 5.2). Based on chromosome position, the SNP marker was mapped to the QTL region to reveal a biological protein function. The SNP had a total length of 1,188,000 bp, located in the candidate gene LOC106771057 (XM_014656948.2) region between 255,839 and 255,706 bp with a total exon length of 278 bp (Table 5.2). The gene is involved in regulating *ZFP8* (Zinc Finger Protein 8).

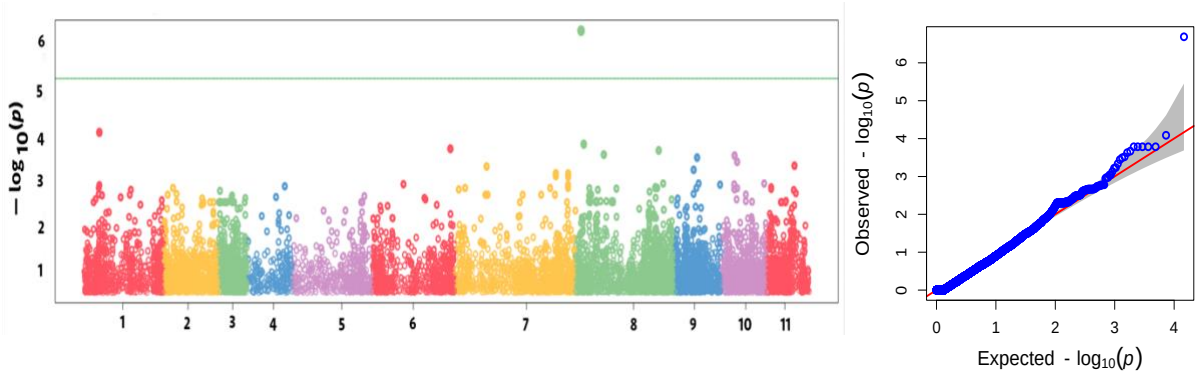


FIGURE 5.4 MANHATTAN DIAGRAM OF GENOME-WIDE ASSOCIATION MAPPING RESULTS FOR EMERGENCE (% OF CONTROL) IN THE MUNGBEAN MINI CORE COLLECTION AT THE GERMINATION STAGE. THE X-AXIS INDICATES THE SNP LOCATION ALONG THE 11 MUNGBEAN CHROMOSOMES, WHILE THE Y-AXIS REPRESENTS $-\text{LOG}_{10}$(P) FOR THE P-VALUE OF THE MARKER-TRAIT ASSOCIATION. SNP12690 WAS SIGNIFICANTLY ASSOCIATED WITH EMERGENCE LOCATED ON CHROMOSOME 8. IT IS THE HOMOLOG OF THE ZFP8 GENE, LOCATED ON CHROMOSOME 2 BETWEEN POSITION 17501629 AND 17519303 IN *ARABIDOPSIS THALIANA*, CHROMOSOME 9 BETWEEN POSITION 19073150 AND 19074807 IN *GLYCINE MAX*, AND CHROMOSOME 7 BETWEEN POSITION 2027287 AND 2028749 IN *VIGNA UNGUICULATA*.

5.4.2.2 Seedling stage

Overall, the GWAS analysis identified seven significant SNPs on chromosomes 1, 2, 5, 7, 8 and 11 (Table 5.2) associated with adventitious root formation (Vrad_SNP07282), total dry mass (Vrad_SNP17065), shoot dry mass (Vrad_SNP17065, Vrad_SNP087467), root dry mass (Vrad_SNP17065), SPAD chlorophyll content [SPAD unit_1 (Vrad_SNP13382)] and seed weight (Vrad_SNP04151, Vrad_SNP01775, Vrad_SNP10377).

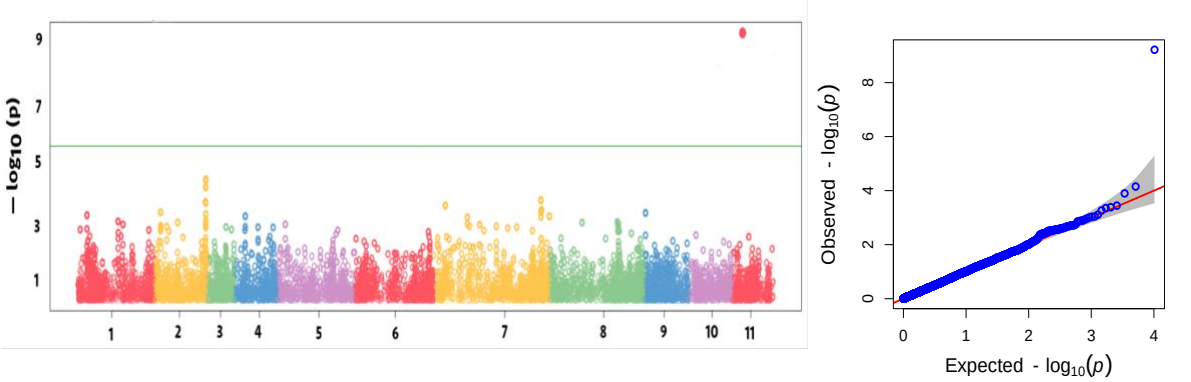
Adventitious root formation was associated with a genomic region of the FGGY carbohydrate kinase domain-containing protein (length 2,300,000 bp) located on chromosome 7 with a total exon length of 459 bp and six transcription factors (Table 5.2; Figure 5.5).

Significantly, a genomic region on chromosome 11 with 396 bp exon length was associated with three traits—shoot dry mass, root dry mass and total dry mass—for transient waterlogging tolerance in mungbean seedlings (Figure 5.6), characterised as transcription factor HHO5 (Table 5.2). In addition, shoot dry mass was associated with a genomic region on Chr_1 with a total exon length of 112 bp, known as uncharacterised protein LOC106780119 (Table 5.2).

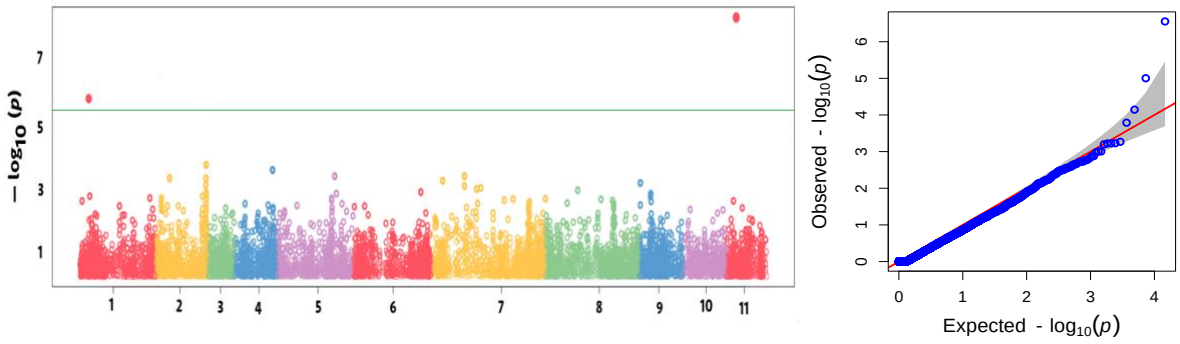
For SPAD_unit 1 (SPAD chlorophyll content on the first trifoliate leaves at 23 DAS), a genomic region located on Chr_8 was detected, characterised as 3-oxoacyl-[acyl-carrier-protein] synthase, mitochondrial. No significant SNP markers were found for SPAD unit_2 or SPAD unit_3.

Seed weight was associated with three significant markers (Table 5.2). The SNP, Vrad_SNP04151, on chromosome 6 was highly associated with the candidate gene region of *LOC106763092* (Figure 5.8), characterised as LOW-QUALITY PROTEIN: putative BPI/LBP family protein (*At1g04970*). Another SNP, Vrad_SNP01775, on chromosome 5 was identified as probable alpha, alpha-trehalose-phosphate synthase [UDP-forming] 9 proteins, while Vrad_SNP10377 on chromosome 2 was pentatricopeptide repeat-containing protein *At5g59600*-like protein.

(A) Total dry mass



(B) Shoot dry mass



(C) Root dry mass

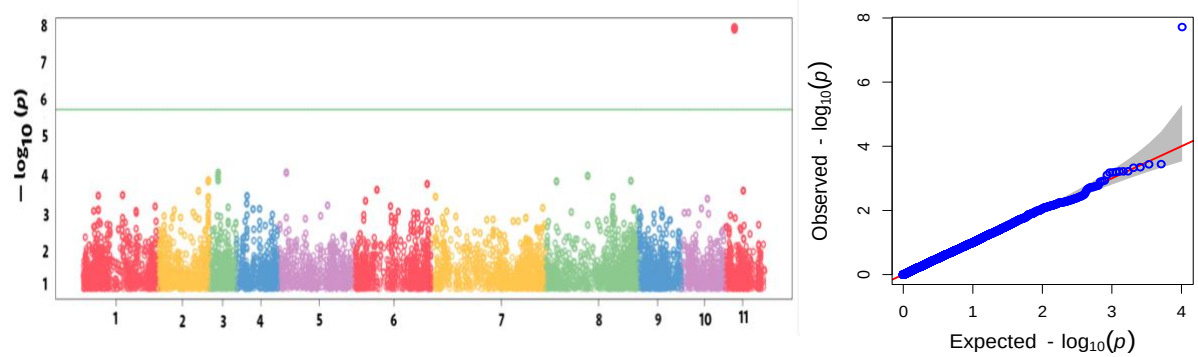


FIGURE 5.5 MANHATTAN DIAGRAMS OF GENOME-WIDE ASSOCIATION MAPPING RESULTS FOR (A) TOTAL PLANT DRY MASS, (B) SHOOT DRY MASS AND (C) ROOT DRY MASS IN THE MUNGBEAN MINI CORE COLLECTION GENOTYPES AT THE SEEDLING STAGE. THE X-AXIS INDICATES THE SNP LOCATION ALONG THE 11 MUNGBEAN CHROMOSOMES, WHILE THE Y-AXIS REPRESENTS –LOG10(P) FOR THE P-VALUE OF THE MARKER-TRAIT ASSOCIATION. VRAD_SNP17065 ON CHROMOSOME 11 WAS IDENTIFIED AS A SIGNIFICANTLY ASSOCIATED SNP WITH SHOOT, ROOT AND TOTAL DRY MASS. THE SNP IS THE HOMOLOG OF THE GENE FOR TRANSCRIPTION FACTOR HHO5, SITUATED ON CHROMOSOME 1 BETWEEN POSITIONS 53015504 AND 53017654 IN *GLYCINE MAX* AND CHROMOSOME 2 BETWEEN POSITIONS 28976695 AND 28978986 IN *VIGNA UNGUICULATA*. FOR SHOOT DRY MASS, VRAD_SNP08467 WAS IDENTIFIED AS A SIGNIFICANT SNP, BEING UNCHARACTERISED PROTEIN LOC100805422 ON CHROMOSOME 4 BETWEEN POSITION 14906745 AND 14916549 IN *GLYCINE MAX* AND UNCHARACTERISED PROTEIN LOC114181812 BETWEEN POSITIONS 42685893 AND 42695112 IN *VIGNA UNGUICULATA*.

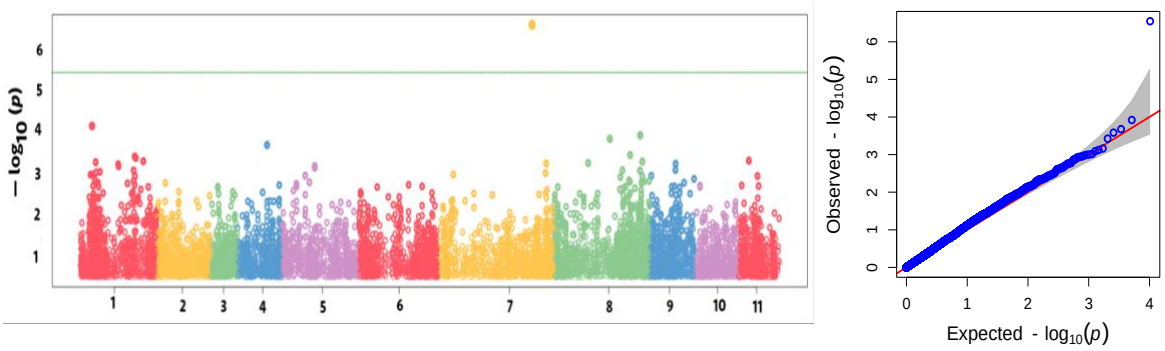
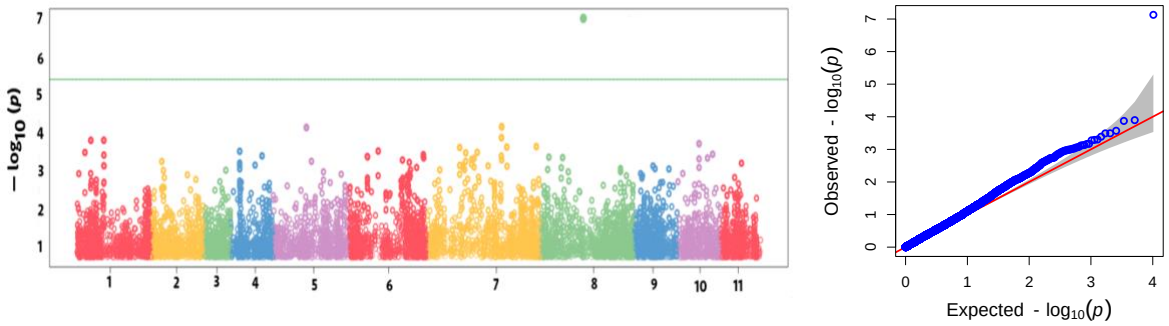
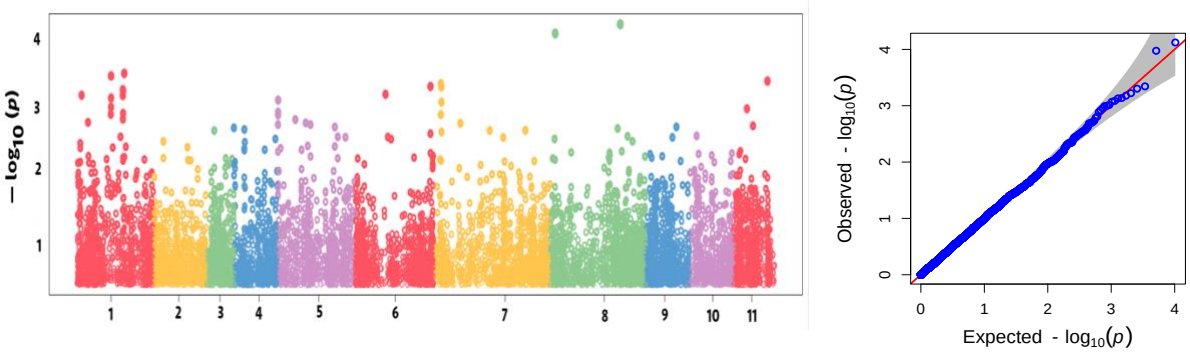


FIGURE 5.6 MANHATTAN DIAGRAM OF GENOME-WIDE ASSOCIATION MAPPING RESULTS FOR ADVENTITIOUS ROOT FORMATION (WL8 DAYS) IN THE MUNGBEAN MINI CORE COLLECTION AT THE SEEDLING STAGE. THE X-AXIS INDICATES THE SNP LOCATION ALONG THE 11 MUNGBEAN CHROMOSOMES, WHILE THE Y-AXIS REPRESENTS $-LOG_{10}(P)$ FOR THE P-VALUE OF THE MARKER-TRAIT ASSOCIATION. VRAD_SNP07282 WAS IDENTIFIED AS A SIGNIFICANTLY ASSOCIATED SNP LOCATED ON CHROMOSOME 7. IT IS A CANDIDATE GENE FGGY CARBOHYDRATE KINASE DOMAIN-CONTAINING PROTEIN, LOCATED ON CHROMOSOME 17 BETWEEN POSITIONS 5061591 AND 5070997 IN *GLYCINE MAX*, AND CHROMOSOME 3 BETWEEN POSITIONS 58517354 AND 58529016 IN *VIGNA UNGUICULATA*.

(A) SPAD unit_1



(B) SPAD unit_2



(C) SPAD unit_3

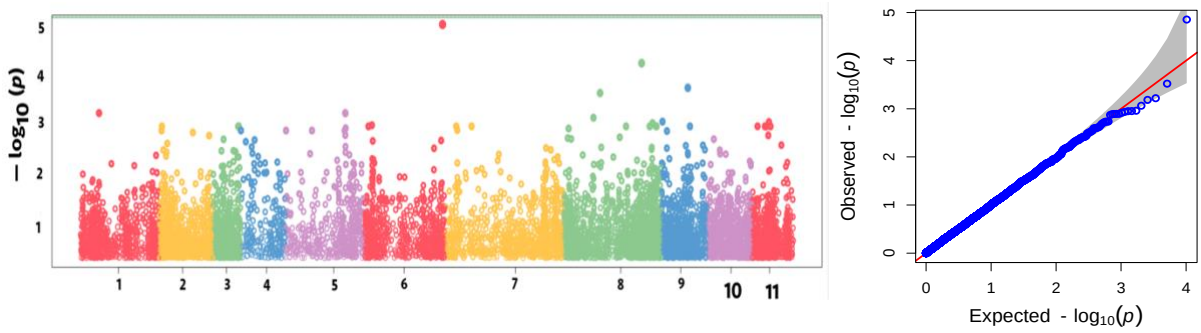


FIGURE 5.7 MANHATTAN DIAGRAMS OF GENOME-WIDE ASSOCIATION MAPPING RESULTS FOR SPAD CHLOROPHYLL CONTENT (WL8 DAYS) IN THE MUNGBEAN MINI CORE COLLECTION AT THE SEEDLING STAGE: (A) SPAD UNIT_1 (SPAD CHLOROPHYLL CONTENT ON THE FIRST TRIFOLIATE LEAVES, 23 DAS) (B) SPAD UNIT_2 (SPAD CHLOROPHYLL CONTENT ON THE FIRST TRIFOLIATE LEAVES, 30 DAS) AND (C) SPAD UNIT_3 (SPAD CHLOROPHYLL CONTENT ON THE SECOND TRIFOLIATE LEAVES, 30 DAS). THE X-AXIS INDICATES THE SNP LOCATION ALONG THE 11 MUNGBEAN CHROMOSOMES, WHILE THE Y-AXIS REPRESENTS $-\text{LOG}_{10}(\text{P})$ FOR THE P-VALUE OF THE MARKER-TRAIT ASSOCIATION. THE GREEN LINE DENOTES THE 5% BONFERRONI-CORRECTED THRESHOLD FOR 10,224 MARKERS. FOR SPAD UNIT_1, VRAD_SNP13382 WAS IDENTIFIED AS A SIGNIFICANTLY ASSOCIATED SNP LOCATED ON CHROMOSOME 8, 3-OXOACYL-[ACYL-CARRIER-PROTEIN] SYNTHASE, MITOCHONDRIAL, LOCATED ON CHROMOSOME 13 BETWEEN POSITIONS 23179939 AND 23184985 IN *GLYCINE MAX* AND CHROMOSOME 7 BETWEEN POSITIONS 13877254 AND 13881154 IN *VIGNA UNGUICULATA*. NO SIGNIFICANT SNP OCCURRED FOR SPAD UNIT_2 OR SPAD UNIT_3.

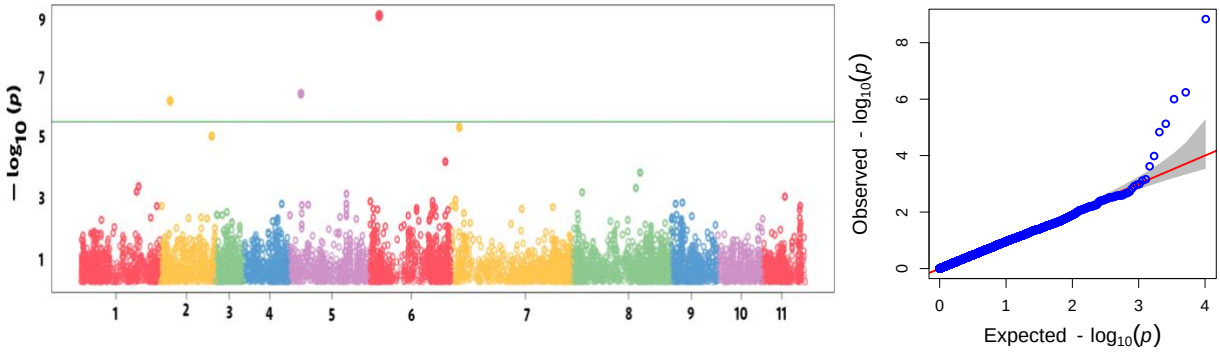


FIGURE 5.8 MANHATTAN DIAGRAM OF GENOME-WIDE ASSOCIATION MAPPING RESULTS FOR SEED WEIGHT IN THE MUNGBEAN MINI CORE COLLECTION GENOTYPES AT THE SEEDLING STAGE. THE X-AXIS INDICATES THE SNP LOCATION ALONG THE 11 MUNGBEAN CHROMOSOMES, WHILE THE Y-AXIS REPRESENTS $-\text{LOG}_{10}(\text{P})$ FOR THE P-VALUE OF THE MARKER-TRAIT ASSOCIATION. THREE SNPS, VRAD_SNP04151, VRAD_SNP01775 AND VRAD_SNP10377, WERE SIGNIFICANTLY ASSOCIATED WITH LOCATIONS ON CHROMOSOMES 2, 5 AND 6. VRAD_SNP04151 IS THE HOMOLOG OF A GENE LOCATED ON CHROMOSOME 14 BETWEEN POSITIONS 4012518 AND 4017757 IN *GLYCINE MAX* AND CHROMOSOME 8 BETWEEN POSITIONS 34055552 AND 34057972 IN *VIGNA UNGUICULATA*. VRAD_SNP01775 IS THE HOMOLOG OF A GENE LOCATED ON CHROMOSOME 6 BETWEEN POSITIONS 15888831 AND 15896074 IN *GLYCINE MAX* AND CHROMOSOME 8 BETWEEN POSITIONS 10703929 AND 10709738 IN *VIGNA UNGUICULATA*. VRAD_SNP10377 IS THE HOMOLOG OF A GENE LOCATED ON CHROMOSOME 11 BETWEEN POSITIONS 10336574 AND 10338643 IN *GLYCINE MAX* AND CHROMOSOME 11 BETWEEN POSITIONS 36634904 AND 36637192 IN *VIGNA UNGUICULATA*.

TABLE 5.2 SIGNIFICANT SNPS AND CANDIDATE GENES FOR WATERLOGGING-TOLERANT TRAITS BASED ON NCBI (NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION) (KANG ET AL., 2014)

SNP/Trait	Chrom*	Position (bp)	P-value	FDR** P-values	MAF***	Gene ID	Allele	Length (nt)	Protein	Length (aa)	*Glycine max*	*Vigna unguiculata*	*Arabidopsis thaliana*
Germination stage													
Emergence													
Vrad_SNP12690	8	2,556,631	2.05E-07	0.003	0.05	LOC106771057	T/A	1188	XP_014512434.1	278	KAH1232985.1	XP_02793568.1	At2G41940
Seedling stage													
Total plant dry mass													
Vrad_SNP17065	11	4,785,849	6.11E-10	6.24E-06	0.17	LOC106776956	G/C	1753	XP_014519923.2	396	XP_003517284.2	XP_027908273.1	At4g37180
Shoot dry mass													
Vrad_SNP17065	11	4,785,849	5.35E-09	5.47E-05	0.17	LOC106776956	G/C	1753	XP_014519923.2	396	XP_003517284.2	XP_027908273.1	At4g37180
Vrad_SNP08467	1	4,402,474	2.10E-06	0.0107	0.38	LOC106780119	G/C	569	XP_022636712.1	112	NP_001242052.1	XP_027924183.1	
Root dry mass													
Vrad_SNP17065	11	4,785,849	1.93E-08	0.0002	0.19	LOC106776956	G/C	1753	XP_014519923.2	396	XP_003517284.2	XP_027908273.1	At4g37180
Adventitious roots													
Vrad_SNP07282	7	44,922,869	2.83E-07	0.0028	0.24	LOC106768660	G/C	2300	XP_022639606.1	459	XP_006593550.1	XP_027919931.1	AT4G30310
						LOC106768660			XP_014509400.1	594	XP_006600499.1		
						LOC106768660			XP_014509402.1	459			
						LOC106768660			XP_014509401.1	459			
SPAD unit_1													
Vrad_SNP13382	8	20,831,249	7.44E-08	0.0029	0.25	LOC106770822	C/G	1997	XP_014512122.1	469	XP_025980579.1	XP_027936764.1	At1g62640
100-seed weight													
Vrad_SNP04151	6	4,401,882	1.47E-09	1.50E-05		LOC106763092	G/C	1793	XP_014502755.1	510	NP_001241365.3	XP_027940688.1	At1g04970
Vrad_SNP01775	5	5,632,636	5.73E-07	0.0029		LOC106762884	G/C	3392	XP_022636254.1	861	XP_003527027.1	XP_027902951.1	At1g23870
Vrad_SNP10377	2	4,586,517	1.00E-06	0.0034	0.003	LOC106778626	C/G	1280	XP_014522097.2	324	XP_003539074.1	XP_027910348.1	At5g59600

Note: *Chrom represents Chromosome, **FDR represents False Discovery Rate and ***MAF represents Minor Allelic Frequency

5.5 DISCUSSION

This study was designed to understand the links between transient waterlogging-stress-tolerance traits and regions of the genome and associated candidate genes at the germination and seedling stages in mungbean. Five GAPIT models were compared to assess their ability to map QTL and identify SNPs associated with waterlogging tolerance. The BLINK model produced the most informative analysis by optimising statistical power using the LD method and approximating the maximum likelihood using Bayesian Information Criterion (BIC) in a fixed-effect model to eliminate the computational burden (Wang and Zhang, 2021). GWAS identified genetic loci associated with the tolerance traits using phenotypic data (% of control) evaluated in a temperature-controlled greenhouse under soil waterlogging and normal conditions. At the germination stage, we identified a candidate gene region for emergence (% of control) after four days of waterlogging controlled by a limited number of genes. Similarly, the candidate gene regions of the other measured traits were controlled by a limited number of genes. Significantly, the GWAS identified a pleiotropic candidate gene associated with three traits (shoot, root and total dry mass). The identified genomic regions and associated markers will be valuable for isolating waterlogging tolerance genes to improve mungbean breeding.

5.5.1 Genetic diversity of mini core genotypes

The mini core panel clustered into three subpopulations towards the extremities of the PCA plot based on genetic differences (Figures 5.1 and 5.2). Studies on the same mini core collection of mungbean reported different population structures for different traits of interest including: (i) four subpopulations for seed coat lustre (Breria et al., 2019), (ii) four subpopulations for hypocotyl colour and maturity under abnormally hot weather with temperatures ranging from 24.4 to 39.9°C (day), with the lowest 0.3°C (night), and different photoperiods (Sokolkova et al., 2019); (iii) three subpopulations for seed weight (Akhtar et al., 2021). The reason for these differences might be the different traits measured. The phylogenetic tree constructed by the neighbour-joining method indicated no significant relationship between subpopulation and geographical origin in the mini core genotypes, especially the South Asian genotypes in all three subpopulations (Figures 5.1 and 5.2), which could be due to limitations in the accuracy of ancestry inference within and among regions (Royal et al., 2010), or incomplete sampling (ASHG, 2008), or shared ancestry (Li et al., 2008). Gayacharan et al. (2020) recently reported no significant relationship between genetic diversity and the geographical origin of 1,232 mungbean genotypes for traits such as seed weight, seed length and seed breadth.

The average distance of LD decay across the entire genome was estimated as ~190,784 bp at r^2=0.1, suggesting that DArT marker coverage was adequate for GWAS analysis for the traits of interest in the current study. A wide range of LD decay has been reported in the mungbean mini core, including r^2=0.25 with ~350 kb (Breria et al., 2019) and r^2=0.29 with ~105 kb (Sokolkova et al., 2019). Comparing these studies with the current study is difficult due to various factors, including the different traits measured and the SNP filtering used. Noble et al. (2018) reported ~100 kb of LD decay for seed coat lustre in cultivated mungbean and ~60 kb in wild mungbean. In soybean, for example, different LD decays for waterlogging tolerance traits occurred in different populations, with ~1.60 Mb in a panel of 347 soybean breeding population lines (Yu et al., 2019) and ~400 kb in a panel of worldwide soybean germplasm collections (Sharmin et al., 2021). Mungbean is a self-pollinated species and is therefore expected to have smaller genome-wide recombination rate and higher LD than cross-pollinated species (Flint-Garcia et al., 2003). Nonetheless, the current GWAS analysis identified significant QTL for all traits measured, except SPAD unit_2, demonstrating genetic diversity in the mini core collection of mungbean.

5.5.2 QTL for waterlogging tolerance at the germination

At the germination stage, emergence (% of control) after four days of transient soil waterlogging was associated with a candidate gene for regulating zinc finger protein 8 (*ZFP8*). In *Arabidopsis thaliana, ZFP8* had decreased sensitivity to inhibited germination by abscisic acid (ABA) (Gan et al., 2007), and the ZFP subfamily of zinc finger factors regulated light and ABA responses during germination and early seedling development (Joseph et al., 2014). The ZFP family plays a critical role in abiotic stress tolerance. For example, B-box type zinc finger, *AtCOL4* plays a key role in ABA and salt stress response (Min et al., 2015). Other ZFP families, B-box (VvZFPL) and C2H2, showed increased tolerance to multiple stresses such as cold, drought and salt in transgenic Arabidopsis plants (Kobayashi et al., 2012; Liu et al., 2022). Recently, a B-box type zinc finger protein, MdBBX10, revealed that *GmSFT* might be the candidate gene regulating seed-flooding tolerance in soybean (Yu et al., 2019). In addition, a CCCH-type zinc finger protein was induced by hypoxia stress under submergence, suggesting its potential role in flooding tolerance (Pandey and Kim, 2012). However, further functional evidence and validation are required to confirm its involvement in governing waterlogging tolerance in soybean and mungbean.

5.5.3 QTL for waterlogging tolerance at the seedling stage

The adverse effect of waterlogging is the lack of oxygen in the root zone, which damages root tissues reducing normal growth and water and nutrient uptake (Valliyodan et al., 2017). Adventitious root formation is a significant trait in many waterlogging- and flood-tolerant plant species (Jackson and Armstrong, 1999), including soybean (Valliyodan et al., 2014; Kim et al., 2015), common bean (Soltani et al., 2018), cotton (Zhang et al., 2021) and barley (Borrego-Benjumea et al., 2021). The main function of adventitious roots is to support water and nutrient uptake during waterlogging (Colmer and Greenway, 2011), contributing to plant survival under stress. In this study, FGGY carbohydrate kinase domain-containing protein was associated with adventitious root formation under waterlogging stress. Interestingly, this finding suggests a link between carbohydrate metabolism and adventitious root development during waterlogging, in addition to the well-known shift in metabolism from aerobic to anaerobic pathways and the availability of soluble sugars during low oxygen stress (Bailey-Serres and Voesenek, 2010). In Arabidopsis, the FGGY carbohydrate kinase family are involved in carbohydrate kinase activity, phosphotransferase activity, and the alcohol group as an acceptor in the carbohydrate metabolic process (www.arabidopsis.org). In sorghum seedlings, the FGGY carbohydrate kinase family shares the same phylogenetic nodes with plant-growth-promoting rhizobacteria (PGPR), including several phytobeneficial and desired traits such as increased production or tolerance during biotic or abiotic stress (Etesami et al., 2018). Further investigations of the biological function(s) in plants of FGGY are needed (Singh et al., 2017) and, as highlighted here, should include the potential link to adventitious rooting during waterlogging stress in mungbean.

This study showed the HHO5 transcription factor (TF) as a pleiotropic gene associated with the three traits—shoot, root and total dry mass. HHO5 TF regulates N absorption, essential for normal plant growth and development (Varala et al., 2018). Nitrate supply can influence lateral root growth and counteract the effect of glutamate on primary root growth (Vidal et al., 2010). Likewise, HHO5 TF can interact with ULT1 (protein ULTRAPETALA 1) and inhibit the expression of the central transcription factor WUSCHEL (WUS), controlling the growth rate of shoot apical meristems in plants (Li et al., 2021). An understanding of the role and functioning of HHO TFs may be key to understanding the relationship between N and P signalling pathways and shoot and root development in plants; however, further research is needed to explore the role of these genes in plant growth, development and abiotic stress

responses (Li et al., 2021). In addition to HHO5, uncharacterised protein LOC106780119 was associated with shoot growth in the present study.

For chlorophyll content, SPAD unit_1 (SPAD chlorophyll content on the first trifoliate leave after 8 days of waterlogging, 23 DAS) was controlled by 3-oxoacyl-[acyl-carrier-protein] synthase, mitochondrial. GO annotations related to this gene expressed as transferase activity and 3-oxoacyl-[acyl-carrier-protein] synthase activity. There are three isoforms of ketoacyl-[acp] synthase, namely *KASI*, *KASII*, and *KASIII*. In Arabidopsis, a T-DNA insertion mutant, *KASI* showed multiple morphological defects, including chlorotic and curly leaves, reduced fertility, and semi-dwarfism, demonstrating pleiotropic effects of FA synthesis on plant growth (Wu and Xue, 2010). In tobacco, *KASI* genes played a key role in regulating fatty acid synthesis and mediating plant growth and development (Yang et al., 2016). In spinach (*Spinacia oleracea*; *So KAS III*), overexpression of *KASIII* reduced the rate of lipid synthesis (Dehesh et al., 2001). In plants, lipids are essential for cell and organelle integrity, acting as a hydrophobic barrier for membranes and a signal molecule for regulating cell metabolism (Ohlrogge and Browse, 1995; Li-Beisson et al., 2013). Li and Yu (2018) reported two lipids, MGDG (monogalactosyldiacylglycerol) and DGDG (digalactosyldiacylglycerol), are important for facilitating the photosynthetic light reaction in the thylakoid membrane and for plant survival under abiotic stresses such as phosphate starvation and freezing. The current study observed chlorotic, curly leaves and semi-dwarf plants in the waterlogging treatment. However, further studies are needed to confirm what type of ketoacyl-[acp] synthase is involved in the tolerance of mungbean to waterlogging stress, especially in the chlorophyll content and photosynthesis pathway.

The seed weight of mini core genotypes was associated with many genes (Akhtar et al., 2021). In the current study, there were three SNPs significant SNPs for marker-trait associations: BPI/LBP (BPI, bactericidal/permeability-increasing protein; LPS, lipopolysaccharide-binding protein), probable alpha, alpha-trehalose-phosphate synthase, and pentatricopeptide repeat-containing protein At5g59600. In Arabidopsis, BPI/LBP is known as At1g04970, playing an important role in the lipopolysaccharide and LPS-induced immune response of plants (Iizasa et al., 2016). Trehalose-6-phosphate synthase (TPS) is responsible for drought tolerance in maize (*Zea mays*) seedlings (Acosta-Pérez et al., 2020). Pentatricopeptide repeat-containing protein (i.e. At5g59600/ PCMP-E1 in *Arabidopsis thaliana*) plays an important role in chloroplast development and cold stress tolerance in *Oryza sativa* (Liu et al., 2018).

In summary, zinc finger protein (*ZFP8*) was identified as a candidate gene for emergence after 4 days of waterlogging. Promising candidate genes for the waterlogging response at the seedling stage were FGGY carbohydrate kinase domain-containing protein for adventitious root formation, transcription factor HHO5 for shoot, root and total plant dry mass, 3-oxoacyl-[acyl-carrier-protein] synthase, mitochondrial for leaf chlorophyll (SPAD content), and LBP/BPI (*At1g04970*), trehalose-6-phosphate synthase (TPS) and PCMP-E1/*At5g59600* for seed weight. These findings strengthen our understanding of the genetic mechanisms underlying transient waterlogging tolerance in mungbean and provide candidates for future marker-assisted breeding or gene cloning to develop waterlogging-tolerant mungbean lines. However, further studies are needed to validate the associated candidate genes and develop markers based on the associated SNPs.

5.6 CONCLUSION

Waterlogging is a major abiotic stress in plants. Understanding the genetic basis of waterlogging tolerance is crucial for improving plant survival and production under stress. Breeding for waterlogging tolerance involves managing complex quantitative traits (Hamachi et al., 1990). The robust screening methodologies developed for each growth stage helped identify the specific loci underlying traits, demonstrating significant marker-trait associations for waterlogging tolerance in mungbean. A gene pyramiding and marker-assisted backcrossing strategy to introgress favourable alleles into commercially cultivated varieties could be one route to developing advanced waterlogging-tolerant cultivars.

CHAPTER 6 GENERAL DISCUSSION

Mungbean is an important leguminous crop cultivated by many smallholder farmers in South and Southeast Asia and on a smaller scale in Africa and Oceania (Nair and Schreinemachers, 2020). In Asia, the crop is widely grown in upland and lowland ecosystems. Waterlogging resulting from excess soil water immediately after rice harvesting in rice–legume cropping systems prevents the succeeding mungbean crop from achieving its yield potential (Zaman et al., 2018; Douglas et al., 2020). Waterlogging stress has arisen due to the high frequency of unseasonal rainfall caused by global climate change. The literature review (Chapter 2) revealed that waterlogging is a primary limiting factor for crop production. Mungbean is sensitive to waterlogging (Nair et al., 2012), mainly during early growth (Tickoo et al., 2006; Douglas et al., 2020). However, there is limited information on the genetic basis of waterlogging tolerance in mungbean. The novel genetic and phenotypic diversity of wild and domesticated mungbean has not been explored extensively to identify valuable loci and germplasm for waterlogging tolerance. Understanding mungbean's genetic and phenotypic variation in waterlogging tolerance will also help contribute to the broader knowledge of waterlogging tolerance in legumes.

The research undertaken for this thesis explored the phenotypic and genetic diversity of waterlogging tolerance in a mini-core collection of mungbean germplasm and expanded our understanding of the genetic mechanisms controlling waterlogging in this species, particularly at the germination and seedling stages. Several research questions were investigated:

- Can the seeds of two *Vigna* species germinate under waterlogged conditions?
- Does extending the waterlogging duration decrease the germination rate?
- What are the tolerance mechanisms of mungbean to waterlogging stress at the germination and seedling stages?
- How long can mungbean be waterlogged at the seedling stage to recover upon drainage?
- Is there variation in waterlogging tolerance in mungbean at early growth stages?
- What is the genetic basis of waterlogging tolerance in mungbean?

Here, the General Discussion addresses the collective implications of the three experimental chapters within this thesis for understanding waterlogging tolerance at the germination and seedling stages, details the study's limitations and outlines priorities for future studies.

6.1 EXPANDING OUR KNOWLEDGE ON WATERLOGGING TOLERANCE IN MUNGBEAN

Mungbean is an orphan crop—that is, a crop with comparatively low global production but extreme regional importance. Farmers, particularly in Asian countries, face production losses due to soil waterlogging. However, there is limited data on mungbean production and economic losses, with the adverse effects of soil waterlogging on plant growth and biomass only described for a few genotypes in the literature (Chapter 2). Before commencing this thesis, no study had characterised phenotypic and genotypic variation in waterlogging tolerance in the representative mungbean diversity panel or identified significant marker-trait associations for waterlogging tolerance traits. The results of this thesis contribute to addressing knowledge gaps on the mechanisms and genetic control of waterlogging tolerance in mungbean.

A significant contribution of this thesis includes the development of robust screening methodologies to identify phenotypic variation present in the mini core population (Experiments A and B, Chapter 3). The waterlogging tolerance of two green-testa mungbean cultivars—Jade-AU (large seeded) and Celera II-AU (small seeded)—and a black-testa blackgram cultivar (*Vigna mungo*; Onyx-AU) as a benchmark crop was evaluated at the germination and seedling stages. The experiments included different waterlogging durations in a sandy clay loam under temperature-controlled glasshouse conditions (with the temperature inside the glasshouse ranging from 21±4°C (night) to 32±3°C (day) based on the recommended optimum temperature for mungbean (Poehlman, 1978) and thermal time calculations in the APSIM model (Carberry, 2007; Chauhan et al., 2010). Waterlogging durations were 0, 1, 2, 3, 4, 5, 6, 7 and 8 days during the germination stage and 0, 2, 4, 8 and 16 days during the seedling stage, each followed by 7 days of recovery.

Soil redox potential was measured throughout the experiments using platinum electrodes (Pt) and an Ag–AgCl reference electrode with a handheld Digital Multimeter (Fluke 114, Everett, Washington, USA) to understand the effect of waterlogging on soil aeration. Soil redox potential indicates the chemical changes during waterlogging (Pezeshki and DeLaune, 1998). Waterlogging stress significantly decreased soil redox potential as the waterlogging duration increased, returning to its drained control condition after two days of recovery at both the germination and seedling stages. The gradual increase in soil redox potential at the seedling stage occurred alongside adventitious root formation that helped relieve the stress (Experiment B, Chapter 3).

Waterlogging stress affected the rhizobia nodules at the seedling stage (Table 3.4, Chapter 3), inhibiting their function at depth in waterlogged soil as their colour changed from pink to white. All waterlogged plants produced root nodules near the soil surface, as did control plants, and these nodules were always pink and survived up to 16 days of waterlogging; presumably, they remained functional into the recovery stage. Similar nodulation was observed in a tropical forage legume, *American jointvetch* (*Aeschynomene americana* L.), which maintained nitrogenase activity and net assimilation rate for growth under waterlogged conditions (Tobisa et al., 2014). Borella et al. (2014) reported that alcohol dehydrogenase, pyruvate decarboxylase and lactate dehydrogenase activated in hypoxic roots and hypoxic nodules of soybean. Legumes also can change the oxygen diffusion pathway to nodules (Roberts et al., 2010) and develop aerenchyma that connects to the outer cortex of nodules, presumably enabling root nodule function in waterlogged soils (Shimamura et al., 2003; Thomas et al., 2005). In this thesis, waterlogged seedlings survived by producing adventitious roots and rhizobia nodules near the crown; thus, rhizobium application in mungbean cultivation might help mitigate the effect of waterlogging. More research is needed on the physiological pathways and molecular mechanisms of nodules under waterlogging stress in mungbean.

In brief, the two *Vigna* species (mungbean and blackgram) had delayed emergence in response to shorter periods of transient waterlogging but failed to germinate under longer periods at the germination stage (Experiment A, Chapter 3). In the seedlings waterlogged 15 days after sowing (Experiment B, Chapter 3), adventitious root formation and crown nodulation varied between genotypes, and 16 days of waterlogging substantially reduced growth but did not cause plant death.

The results of the screening methodology in Chapter 3 have significant implications for understanding mungbean waterlogging tolerance. Hence, they were used to screen the mungbean mini core collection genotypes to identify any phenotypic variation and understand the genetic control of waterlogging tolerance. The statistical analysis (Experiment A, Chapter 3) determined that 4 days of waterlogging at the germination stage was suitable for identifying phenotypic variation among the mungbean population.

Phenotypic screening of 292 mini core genotypes exposed to 4 days of waterlogging, using the same protocols from Chapter 3, was undertaken in a temperature-controlled glasshouse (Chapter 4). Mungbean seeds do not emerge under hypoxia due to insufficient oxygen. The seedlings emerged after 2 days of draining waterlogged pots. A wide range of genotypic variation in seedling emergence was observed among the mini core collection genotypes (Table

4.6, Chapter 4), and the waterlogging tolerance was related to maintaining seed viability under hypoxia and subsequent emergence on the release of hypoxia. This finding agrees with studies on common bean and soybean, illustrating the failure to emerge under waterlogged conditions because the low oxygen levels restrict respiration (Morinaga, 1926; Cardwell, 1984; Hou and Thseng, 1992; Tian et al., 2005; Rajashekar and Baek, 2014). In contrast, tolerant genotypes of grass pea, field pea, lentil and faba bean emerged after 8 days of waterlogging (Wiraguna et al., 2021). Hence, mungbean is highly sensitive to waterlogging compared to these four legumes.

Being a sensitive crop, 4 days of transient waterlogging reduced mungbean emergence by 48% on average relative to drained control (Table 4.6, Chapter 4). Highly tolerant genotypes had 90% emergence after 2–3 days of stress release, whereas susceptible genotypes failed to emerge, similar to barley germplasm screened for waterlogging tolerance (Takeda and Fukuyama, 1987). Shoot dry mass (r^2=0.56), root dry mass (r^2=0.48) and total dry mass (r^2=0.56) significantly correlated with emergence, indicating that these traits are not expressed independently (Chapter 4). The Kolmogorov–Smirnov normality test estimated that emergence (%), shoot dry mass, root dry mass and total dry mass are controlled by dominant and dominant-based duplicate epistasis. The highest broad-sense heritability (81%) was estimated to occur for emergence (Table 4.6, Chapter 4).

Furthermore, the molecular markers for DArT sequencing mini core collection genotypes helped identify the specific genomic region facilitating adaptation in waterlogging-tolerant lines. The GWAS analysis detected significant marker-trait associations and zinc finger protein 8 (*ZFP8*) as a candidate gene or causative allele for waterlogging tolerance at the germination stage (Chapter 5). The *ZFP8* gene had decreased sensitivity to inhibited germination by abscisic acid (ABA) in *Arabidopsis thaliana* (Gan et al., 2007). The ZFP subfamily of zinc finger factors regulated light and ABA responses during germination and early seedling development in *Arabidopsis* thaliana (Joseph et al., 2014), and the zinc finger family plays a key role in multiple abiotic stresses such as cold, drought, salt and hypoxia in soybean (Kobayashi et al., 2012; Pandey and Kim, 2012; Min et al., 2015, Yu et al., 2019, Liu et al., 2022). The research findings described in Chapter 5 will help the genetic improvement programs of mungbean for waterlogging tolerance.

At the seedling stage, the phenotypic screening methodology developed in Experiment B of Chapter 3 revealed that 8 days of waterlogging is suitable for assessing phenotypic variation of the soybean mini core population (Chapter 4). Furthermore, adventitious root formation in waterlogged plants helped restore the soil redox potential under waterlogging. Mungbean

produced adventitious roots to acclimate to soil O_2 deprivation and the associated energy crisis and thus play a major role in stress tolerance (Chapter 3), as reported for other legume species, including common bean (Soltani et al., 2017) and soybean (Ye et al., 2018).

In the current study, the genetic variation estimated 56% of the broad-sense heritability for adventitious root formation with the trait following dominant-based complementary epistatic gene action (Chapter 4). Furthermore, the GWAS identified specific loci underlying the trait, revealing an association between FGGY carbohydrate kinase domain-containing protein and adventitious root formation (Chapter 5). Hence, carbohydrate metabolism might be linked to adventitious root formation in mungbean. Interestingly, a proteome analysis of soybean roots under waterlogging stress at an early vegetative stage revealed that soybean copes with waterlogging by managing carbohydrate consumption and regulating programmed cell death (Alam et al., 2010).

Generally, the greatest adventitious root formation under waterlogged conditions results in the quickest recovery of root activity after releasing the waterlogging stress in soybean (Morita et al., 2004) and the highest root and shoot weights (Soltani et al., 2017). Comparatively, in the mungbean mini core genotypes, adventitious root formation significantly correlated with root dry mass (r^2=0.77), shoot dry mass (r^2=0.52) and total dry mass (r^2=0.97). The highest broad-sense heritability occurred for shoot dry mass (81%), root dry mass (78%) and total dry mass (79%). The Kolmogorov–Smirnov normality test estimated that adventitious root formation, shoot dry mass, root dry mass and total dry mass followed dominant-based complementary epistatic gene action (Fisher, 1932; Robson, 1956; Chapter 4) and *HHO5* TF is a causative pleiotropic gene related to the three traits. Varala et al. (2018) reported that *HHO5* TF regulates N absorption, essential for the growth of shoot apical meristems (Li et al., 2021) and lateral root growth by counteracting primary root growth (Vidal et al., 2010). In addition, in the present study, uncharacterised protein LOC106780119 was associated with shoot growth.

Many genes govern the seed weight of soybean mini core genotypes (Akhtar et al., 2021). Similarly, the current GWAS analysis revealed many genes associated with seed weight, involving trehalose-6-phosphate synthase (TPS), LBP/BPI (At1g04970) and PCMP-E1 (At5g59600). Trehalose-6-phosphate synthase is responsible for drought tolerance in maize (*Zea mays*) seedlings (Acosta-Pérez et al., 2020). In Arabidopsis, At1g04970 plays an important role in the immune response of plants (Iizasa et al., 2016), whereas At5g59600 is involved in cold stress tolerance in *Oryza sativa* (Liu et al., 2018). Further research is needed to understand the crosstalk between abiotic stress response mechanisms in plants.

6.2 COMMON METHODOLOGICAL LIMITATIONS OF THE STUDY

This is the first waterlogging stress tolerance study using a diverse panel of mungbean germplasm at the germination and seedling stages. The estimation of heritability combined with identifying sources of waterlogging tolerance (Chapter 4) demonstrated the possibility of selecting the traits measured and accelerating mungbean breeding for climate-resilient cultivars. However, the phenotypic and genetic variation assessment was based on screenings conducted in a temperature-controlled glasshouse environment. Therefore, a subset of phenotypic screening mini core genotypes should be further evaluated under waterlogged field conditions to assess the correlation between field and temperature-controlled glasshouse data and identify the most extreme genotypes. Soltani et al. (2018) reported an association between waterlogging tolerance and pathogenic *Pythium* spp. resistance in common bean. However, the present study did not examine pathogen susceptibility of the mini core genotypes during the waterlogged and recovery stage, rather effort towards controlling seed-borne and seedling root pathogens was by sterilising the seed surface with 1% commercial bleach (active ingredient 40 mg L^{-1} NaOCl) and applying P-Pickel T liquid fungicide. Under field conditions, waterlogging may be confounded by additional environmental factors (Setter and Waters, 2003).

The second limitation of this research involves the identification of putative causative alleles in the GWAS analyses (Chapter 5), based on published DArT sequencing data that were filtered such that only markers with known physical positions on pseudochromosomes were used. DArT sequencing molecular markers facilitate the identification of associated loci that influence traits of interest and account for a greater proportion of additive genetic effects than phenotype alone (Schafleitner, 2020). However, the mungbean reference genome (draft) sequencing covers 80% of the total estimated genome size and has 7.2% gaps in the total scaffold, with only 239 of 2,748 scaffolds mapped to pseudochromosomes (Kang et al., 2014). Using this draft reference genome, mungbean breeders conduct quantitative trait locus (QTL) mapping or map-based cloning and transfer QTL information from soybean through translational genomics (Kim et al., 2015). Nonetheless, the complete and filtered marker sets do not capture all structural variations at this stage, and ongoing whole-genome resequencing might help identify significant genomic variants in mungbean (Schafleitner, 2020). Hence, the marker-trait associations should be re-analysed with an updated reference genome assembly. Given the genetic basis for waterlogging tolerance in mungbean and the novelty of these findings, further work is needed to identify plausible candidate genes for waterlogging tolerance in mungbean.

6.3 PRACTICAL APPLICATION FOR WATERLOGGING TOLERANCE OF MUNGBEAN AND GERMPLASM COLLECTION

An important motivation for studying waterlogging tolerance in mungbean was laying the foundations for developing climate-resilient cultivars that tolerate waterlogging, thus mitigating the adverse effects of waterlogging stress on crops and economic losses for growers. This is the first report to assess phenotypic variation and study the genetic control of waterlogging tolerance traits and their possible signalling mechanisms using GWAS analyses in mungbean mini core collection genotypes. Several tolerant genotypes were identified within the mini core collection that could be used as donor parents in breeding studies.

Different mungbean genotypes were identified at different growth stages for waterlogging tolerance: VI001284, VI003658, VI003720, VI001533, VI001974, VI002173_1, VI002999, VI003490, VI003699, VI004010 and VI005066 had 80–90% emergence under waterlogging relative to their drained controls after removing the stress at the germination stage, while VI001124, VI002523, VI002469, VI002739, VI003954, VI004024, VI004691, VI004710, VI004743, VI005030 and VI014178 were highly tolerant to waterlogging at the seedling stage. This finding validates the hypothesis that different tolerance mechanisms exist at different growth stages, as found for barley, oats and wheat (Setter and Waters, 2003). Further studies should be undertaken to validate these results, particularly in field environments, and explore the genetic variation of waterlogging tolerance at other growth stages, such as flowering or the reproductive stage. In addition, genetic and phenotypic variation in waterlogging tolerance were not associated with geographical origin, which could be due to the unequal sampling of countries, limitations in the accuracy of ancestry inference within and among regions (Royal et al., 2010), incomplete sampling (ASHG, 2008) and shared ancestry (Li et al., 2008). Therefore, I recommend to collect more genotypes for these various regions to help explore mungbean evolution based on geographic region and tolerance to abiotic stresses prevalent in regions.

The waterlogging-tolerant genotypes and elite mungbean breeding lines identified in this thesis could be used to develop a multi-parent advanced generation inter-cross (MAGIC) population(s). MAGIC populations combine the advantages of natural and synthetic populations with extensive recombination of genetic variation during population development to generate novel allelic combinations (Bandillo et al., 2013; Scott et al., 2020). These populations can be evaluated in multilocation field trials under waterlogged conditions to phenotype yield and yield component traits. In addition, RILs from MAGIC populations have been adopted readily as new varieties in rice, chickpea, wheat and cotton (Scott et al., 2020).

As an example, the rice Bio-MAGIC population developed at IRRI demonstrates the 'pyramiding' of several beneficial alleles for multiple genes for three diseases (blast, bacterial blight and brown plant hopper) without backcrossing (Leung et al., 2015). Moreover, Descalsota et al. (2018) and Zaw et al. (2019) identified rice MAGIC RILs with beneficial allelic combinations across grain yield, grain zinc content, flowering time, plant height and amylose content using resequencing data. In cotton, Thyssen et al. (2019) identified MAGIC RILs that pyramid eight alleles with positive effects on four different fibre quality measurements. A similar scenario could be applied to mungbean for abiotic stress tolerance, such as waterlogging tolerance.

6.4 FINAL REMARKS

Waterlogging stress is becoming an increasingly frequent and problematic issue for mungbean production due to climate change. However, a lack of knowledge surrounding the physiological mechanisms and genetic basis for waterlogging tolerance in this species has restricted crop improvement. Therefore, phenotypic and genetic diversity for waterlogging tolerance in the mungbean mini core collection were characterised under controlled conditions for the first time. An efficient screening methodology was established to discriminate between susceptible and tolerant germplasm and identified key traits (such as adventitious roots) that best facilitate adaptation or acclimation to waterlogging. In addition, several genotypes with waterlogging tolerance at different growth stages were identified, which potentially can be used as parents for pyramiding tolerance alleles and breeding robust cultivars that remain productive in the event of transient waterlogging during the germination and/or seedling growth stages. Finally, several genetic markers significantly associated with waterlogging tolerance were identified using GWAS. These associations provide the first insights into the molecular genetic control of waterlogging tolerance in mungbean and provide a means for adopting MAS to increase breeding efficiency. The research undertaken in this thesis provides an excellent foundation for understanding waterlogging tolerance in mungbean, which must be further built upon and validated under field conditions.

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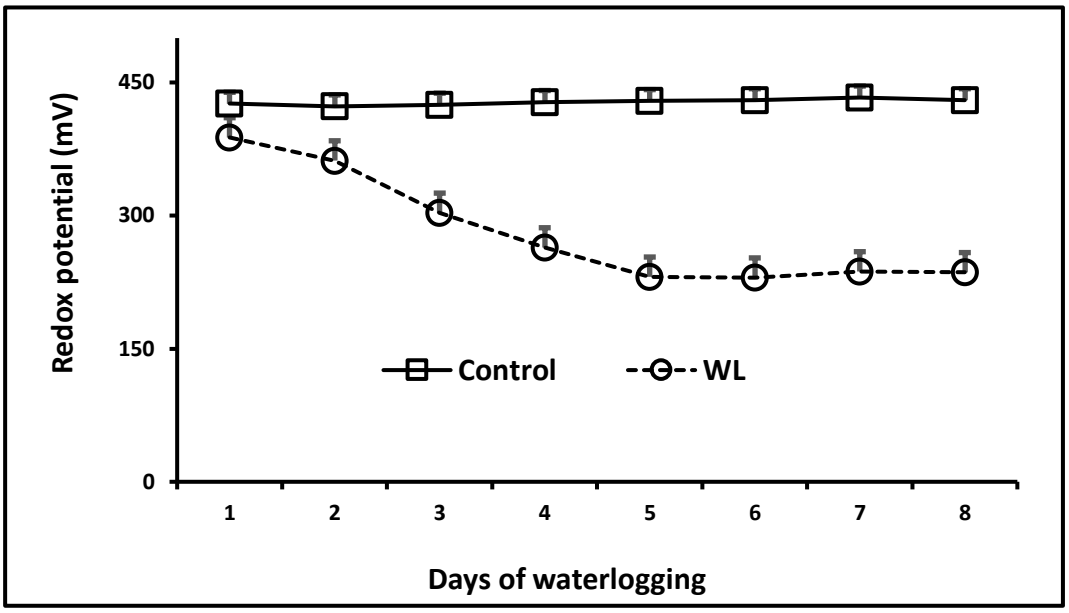
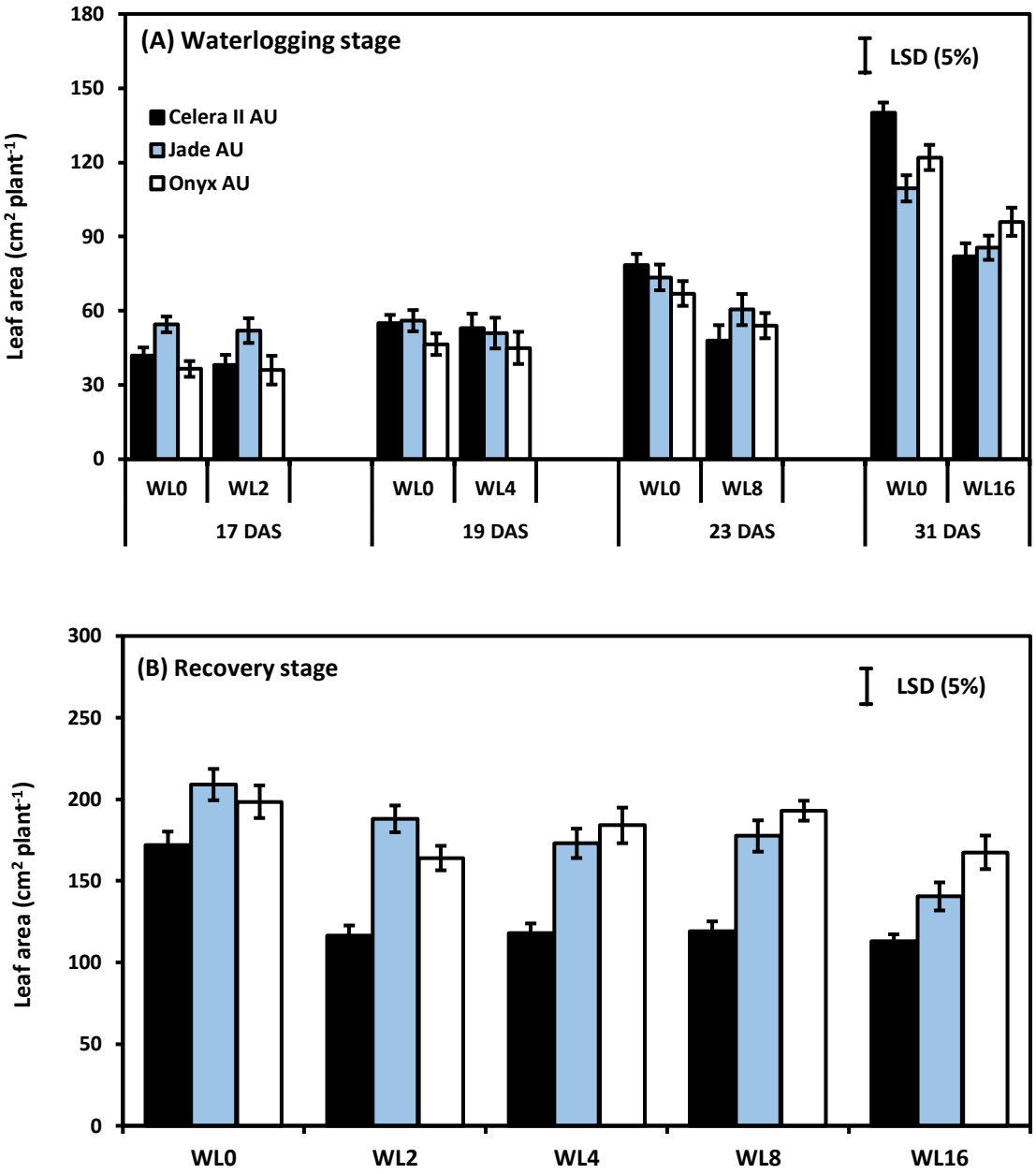
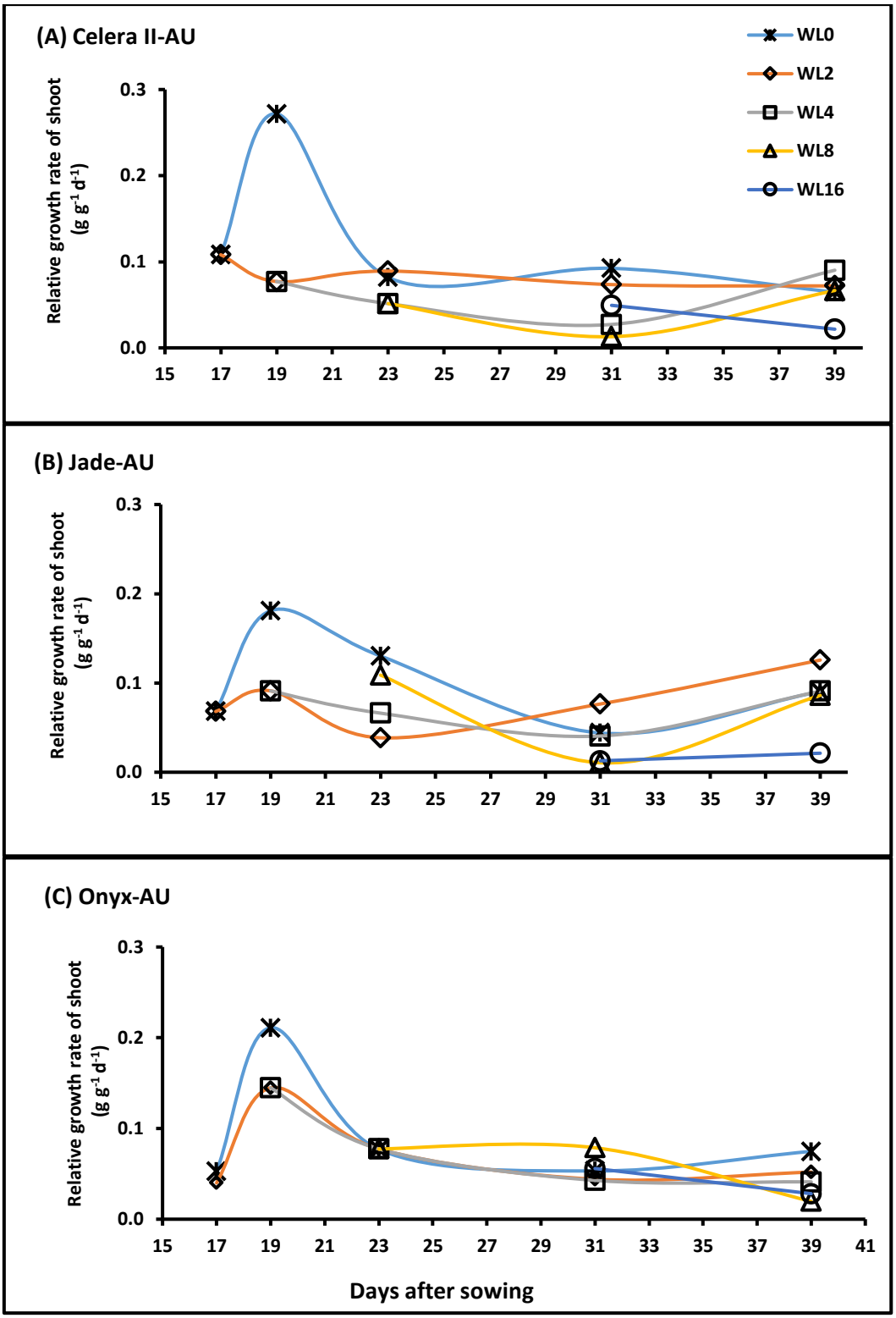
A.1. SUPPLEMENTARY FIGURES



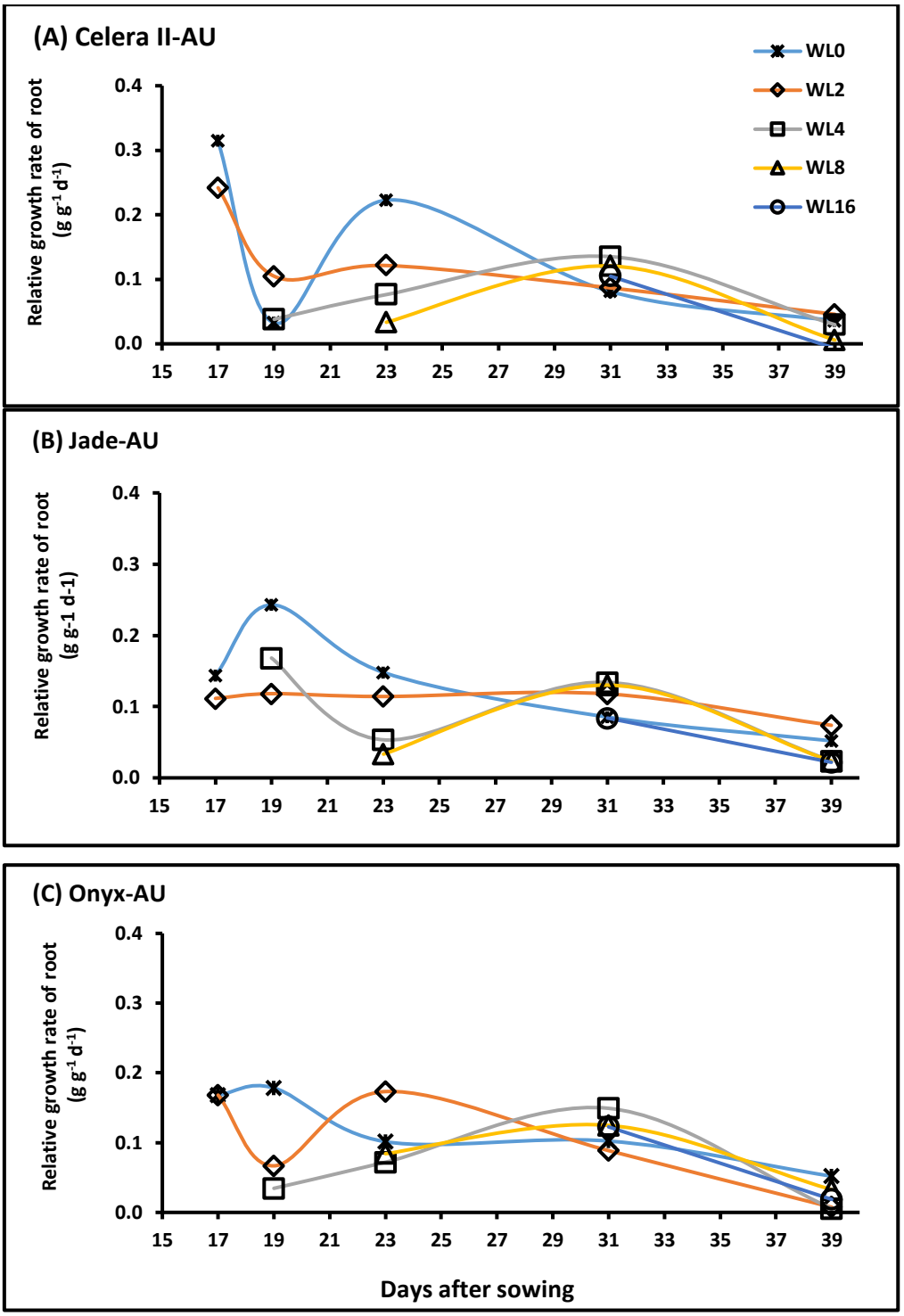
SUPPLEMENTARY FIGURE 3.1 EFFECT OF WATERLOGGING ON SOIL REDOX POTENTIAL AT THE GERMINATION STAGE IN WATERLOGGED (WL) TREATMENTS RELATIVE TO THE DRAINED CONTROL. ERROR BARS REPRESENT STANDARD ERRORS OF THE MEAN.



SUPPLEMENTARY FIGURE 3.2 COMPARISON OF TOTAL LEAF AREA BETWEEN DRAINED CONTROL AND WATERLOGGED PLANTS FOR THREE GENOTYPES AFTER **(A)** DIFFERENT WATERLOGGING DURATIONS AND **(B)** RECOVERY. WL0, DRAINED CONTROL; WL2, WATERLOGGING FOR 2 DAYS, RECOVERY FOR 22 DAYS; WL4, WATERLOGGING FOR 4 DAYS, RECOVERY FOR 20 DAYS; WL8, WATERLOGGING FOR 8 DAYS, RECOVERY FOR 16 DAYS; WL16, WATERLOGGING FOR 16 DAYS, RECOVERY FOR 8 DAYS. BARS ARE MEANS ±SE OF FOUR REPLICATES. LEAST SIGNIFICANT DIFFERENCES (LSD) AT P=0.05 FOR THE GENOTYPE.



SUPPLEMENTARY FIGURE 3.3 SHOOT RELATIVE GROWTH RATE UNDER DIFFERENT WATERLOGGING DURATIONS AND SUBSEQUENT RECOVERY FOR **(A)** CELERA II-AU, **(B)** JADE-AU, **(C)** ONYX-AU. WL0, DRAINED CONTROL; WL2, WATERLOGGING FOR 2 DAYS, RECOVERY FOR 22 DAYS; WL4, WATERLOGGING FOR 4 DAYS, RECOVERY FOR 20 DAYS; WL8, WATERLOGGING FOR 8 DAYS, RECOVERY FOR 16 DAYS; WL16, WATERLOGGING FOR 16 DAYS, RECOVERY FOR 8 DAYS. RGR IS ESTIMATED FROM THE MEAN OF FOUR REPLICATE POTS.



SUPPLEMENTARY FIGURE 3.4 ROOT RELATIVE GROWTH RATE UNDER DIFFERENT WATERLOGGING DURATIONS AND SUBSEQUENT RECOVERY FOR **(A)** CELERA II-AU, **(B)** JADE-AU, **(C)** ONYX-AU. WL0, DRAINED CONTROL; WL2, WATERLOGGING FOR 2 DAYS, RECOVERY FOR 22 DAYS; WL4, WATERLOGGING FOR 4 DAYS, RECOVERY FOR 20 DAYS; WL8, WATERLOGGING FOR 8 DAYS, RECOVERY FOR 16 DAYS; WL16, WATERLOGGING FOR 16 DAYS, RECOVERY FOR 8 DAYS. RGR IS ESTIMATED FROM THE MEAN OF FOUR REPLICATE POTS.

A.2. ARTICLE PUBLISHED IN FRONTIERS IN PLANT SCIENCE, 2021

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Response of Mungbean (cvs. Celera II-AU and Jade-AU) and Blackgram (cv. Onyx-AU) to Transient Waterlogging

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Mungbean [*Vigna radiata* (L.) Wilczek] and blackgram [*Vigna mungo* (L.) Hepper] are important crops for smallholder farmers in tropical and subtropical regions. Production of both crops is affected by unexpected and increasingly frequent extreme precipitation events, which result in transient soil waterlogging. This study aimed to compare the waterlogging tolerance of mungbean and blackgram genotypes under the varying duration of waterlogging stress at germination and seedling stages. We evaluated the responses to different durations of transient waterlogging in a sandy clay loam under temperature-controlled glasshouse conditions. Waterlogging durations were 0, 1, 2, 3, 4, 5, 6, 7, and 8 days during germination and 0, 2, 4, 8, and 16 days during the seedling stage. We used two mungbean genotypes (green testa), Celera II-AU (small-seeded), and Jade-AU (large-seeded), contrasting in seed size and hypocotyl pigmentation, and a blackgram genotype (black testa), Onyx-AU. Waterlogging reduced soil redox potential, delayed or even prevented germination, decreased seedling establishment, and affected shoot and root development. In the seedlings waterlogged (WL) at 15 days after sowing (DAS), adventitious root formation and crown nodulation varied between the genotypes, and 16 days of waterlogging substantially reduced growth but did not result in plant death. Plants in soil with waterlogging for 8–16 days followed by drainage and sampling at 39 DAS had reduced shoot and root dry mass by 60–65% in mungbean and 40% in blackgram compared with continuously drained controls, due at least in part to fewer lateral roots. Soil plant analysis development (SPAD) chlorophyll content was also reduced. Onyx-AU, a blackgram genotype, was more tolerant to transient waterlogging than Jade-AU and Celera II-AU in both growth stages. Of the two mungbean genotypes, Celera II-AU had a greater seedling establishment than Jade-AU post waterlogging imposed at sowing. In contrast, Jade-AU had more plant biomass and greater recovery growth than Celera II-AU after waterlogging and recovery during the seedling stage. Both species were delayed in emergence in response to the shorter periods of transient waterlogging at germination, and with the longer waterlogging germination and emergence failed, whereas at the seedling stage both showed adaptation by the formation of adventitious roots.

Keywords: mungbean, blackgram, waterlogging, adventitious roots, germination

A.3. SUPPLEMENTARY TABLES

SUPPLEMENTARY TABLE 3.1 THE ANALYSIS OF SOIL SAMPLES. BEFORE ANALYSIS, THE SOIL SAMPLES WERE RANDOMLY COLLECTED AND DRIED IN THE 60°C SOIL DRYING ROOM FOR 5 DAYS AND SIEVED WITH 2 MM SIEVING MACHINE. THE DATA REPRESENT THE MEANS OF FOUR REPLICATIONS.

Name	Unit	Mukinbudin
Colour		BRGR
Gravel	%	0
Texture		3
Ammonium nitrogen	mg kg^{-1}	1.7
Nitrate nitrogen	mg kg^{-1}	20
Phosphorus Colwell	mg kg^{-1}	15
Potassium Colwell	mg kg^{-1}	554
Sulphur	mg kg^{-1}	25
Organic carbon	%	0.3
Conductivity	dS m^{-1}	0.5
pH ($CaCl_2$)		7.8
pH (H_2O)		8.5
DTPA copper	mg kg^{-1}	2.1
DTPA iron	mg kg^{-1}	9.9
DTPA manganese	mg kg^{-1}	16.0
DTPA zinc	mg kg^{-1}	0.7
Exchangeable aluminium	meq 100 g^{-1}	0.1
Exchangeable calcium	meq 100 g^{-1}	8.6
Exchangeable magnesium	meq 100 g^{-1}	3.2
Exchangeable potassium	meq 100 g^{-1}	1.2
Exchangeable sodium	meq 100 g^{-1}	2.2
Boron hot $CaCl_2$	mg kg^{-1}	2.7

SUPPLEMENTARY TABLE 4.1 SCREENING OF MUNGBEAN MINI CORE GENOTYPES FOR WATERLOGGING TOLERANCE AT THE GERMINATION STAGE. THE TREATMENT WAS WATERLOGGED FOR 4 DAYS (WL 4 DAYS) AND COMPARED WITH THEIR RESPECTIVE DRAINED CONTROL. THE WATERLOGGED POTS RECOVERED FOR 7 DAYS IMMEDIATELY AFTER THE RELEASE OF WATERLOGGING. HARVESTING OF WATERLOGGED AND CONTROL POTS OCCURRED ON THE SAME DAY. DATA REPRESENT THE MEAN VALUE FOR THREE REPLICATIONS.

Sr. No.	Accession Num	VI_num	Origin	Germination stage							
				Emergence (%)		Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)	
				Control	WL	Control	WL	Control	WL	Control	WL
1	AGG325466	VI000020AY	SEA	100	53	0.40	0.29	0.21	0.13	0.61	0.43
2	AGG325467	VI000099AG	SA	100	75	0.53	0.42	0.41	0.06	0.94	0.49
3	AGG325468	VI000105BG	SA	97	20	1.13	0.09	0.48	0.05	1.61	0.15
4	AGG325469	VI000164BG	SWA	100	32	0.26	0.06	0.14	0.03	0.40	0.10
5	AGG325470	VI000170B-BR	SWA	100	39	0.97	0.06	0.37	0.04	1.34	0.11
6	AGG325471	VI000175BY	SA	100	27	0.77	0.16	0.32	0.07	1.09	0.25
7	AGG325472	VI000188A-BLM	SWA	100	29	0.69	0.08	0.25	0.05	0.94	0.15
8	AGG325473	VI00203B-BR	SWA	94	30	1.09	0.05	0.38	0.02	1.47	0.08
9	AGG325474	VI000212A-BLM	NA	100	56	0.53	0.14	0.28	0.08	0.81	0.23
10	AGG325475	VI000232AG	SWA	92	36	1.23	0.15	0.46	0.08	1.69	0.24
11	AGG325476	VI000238AG	SWA	96	37	0.71	0.12	0.26	0.07	0.97	0.21
12	AGG325477	VI000253AG	SA	93	46	0.60	0.19	0.24	0.09	0.84	0.29
13	AGG325478	VI000316AG	SWA	96	70	0.64	0.09	0.20	0.05	0.84	0.16
14	AGG325479	VI000317BG	SWA	99	62	0.53	0.07	0.20	0.05	0.72	0.13
15	AGG325480	VI000319AG	SWA	94	31	0.73	0.04	0.23	0.03	0.96	0.08
16	AGG325481	VI000380AG	SEA	98	49	0.98	0.13	0.32	0.03	1.31	0.17
17	AGG325482	VI000461BG	SEA	94	18	0.76	0.04	0.25	0.02	1.01	0.07
18	AGG325483	VI000470AG	SWA	100	56	0.38	0.11	0.26	0.04	0.63	0.16
19	AGG325484	VI000532BG	SA	94	49	0.62	0.18	0.29	0.07	0.90	0.27
20	AGG325485	VI000537BG	SA	95	66	0.49	0.19	0.20	0.06	0.68	0.26
21	AGG325486	VI000542BY	SA	100	31	0.71	0.21	0.20	0.05	0.91	0.27
22	AGG325487	VI000551AG	SA	100	70	0.36	0.08	0.34	0.04	0.70	0.13
23	AGG325488	VI000554AG	SA	100	49	0.47	0.10	0.21	0.05	0.68	0.17
24	AGG325489	VI000559AG	SA	100	56	0.37	0.04	0.19	0.04	0.56	0.10
25	AGG325490	VI000578AG	SA	99	66	0.51	0.13	0.61	0.06	1.12	0.20
26	AGG325491	VI000589B-BR	SA	98	0	1.21	0.00	0.68	0.00	1.89	0.00
27	AGG325492	VI000616BG	SAM	95	35	0.68	0.10	0.31	0.06	0.99	0.16
28	AGG325493	VI000618AG	SA	95	4	0.78	0.01	0.47	0.02	1.26	0.04
29	AGG325494	VI000625B-BR	SA	94	30	0.51	0.13	0.32	0.08	0.83	0.22
30	AGG325495	VI000680AG	NA	94	47	0.60	0.26	0.24	0.13	0.84	0.40
31	AGG325496	VI000723AG	SWA	96	53	0.60	0.08	0.18	0.05	0.79	0.14
32	AGG325497	VI000732AG	SA	94	34	0.55	0.09	0.35	0.05	0.90	0.15
33	AGG325498	VI000735BG	SA	100	30	0.43	0.03	0.21	0.02	0.64	0.06
34	AGG325499	VI000736AG	SA	100	62	0.64	0.25	0.27	0.14	0.91	0.39
35	AGG325500	VI000749AG	SA	97	60	0.70	0.18	0.17	0.05	0.87	0.25
36	AGG325501	VI000764AG	SA	99	21	0.55	0.04	0.25	0.02	0.79	0.07
37	AGG325502	VI000766BG	SA	95	54	0.61	0.16	0.17	0.13	0.78	0.30
38	AGG325503	VI000805BG	SA	92	51	1.20	0.28	0.54	0.14	1.74	0.43
39	AGG325504	VI000815BG	SA	94	74	0.89	0.26	0.28	0.15	1.17	0.42
40	AGG325505	VI000818BG	SA	94	29	1.12	0.08	0.48	0.06	1.60	0.15
41	AGG325506	VI000852AG	SA	94	44	0.85	0.35	0.38	0.15	1.23	0.51
42	AGG325507	VI000938AG	SA	94	72	0.84	0.43	0.23	0.17	1.07	0.61
43	AGG325508	VI000942AG	SA	92	48	1.11	0.21	0.34	0.04	1.45	0.27
44	AGG325509	VI000953AG	SA	100	50	1.49	0.25	0.77	0.12	2.26	0.39
45	AGG325510	VI000981BG	SEA	95	75	0.95	0.50	0.30	0.15	1.25	0.66
46	AGG325511	VI001023BG	SA	94	31	1.05	0.09	0.37	0.06	1.41	0.16
47	AGG325512	VI001066BG	OP	97	46	0.31	0.19	0.21	0.02	0.52	0.23
48	AGG325513	VI001096AG	OP	97	56	1.33	0.37	0.45	0.13	1.77	0.51
49	AGG325514	VI001124AG	OP	97	65	1.67	0.36	0.55	0.16	2.22	0.53
50	AGG325515	VI001126BG	OP	95	15	0.81	0.05	0.50	0.02	1.31	0.08
51	AGG325516	VI001162AG	OP	92	64	0.61	0.08	0.24	0.03	0.85	0.12

Sr. No.	Accession Num	VI_num	Origin	Germination stage							
				Emergence (%)		Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)	
				Control	WL	Control	WL	Control	WL	Control	WL
52	AGG325517	VI001191BG	SEA	97	71	0.93	0.26	0.36	0.08	1.29	0.35
53	AGG325518	VI001211AG	SEA	92	32	0.96	0.10	0.27	0.05	1.24	0.16
54	AGG325519	VI001221AG	SEA	95	49	1.23	0.15	0.56	0.07	1.78	0.23
55	AGG325520	VI001244AG	SEA	96	16	1.41	0.09	0.39	0.04	1.80	0.14
56	AGG325521	VI001268BG	SA	94	49	0.70	0.13	0.32	0.07	1.02	0.20
57	AGG325522	VI001282AG	SA	95	61	0.61	0.09	0.19	0.05	0.80	0.14
58	AGG325523	VI001284AG	SA	100	90	0.79	0.17	0.25	0.07	1.05	0.25
59	AGG325524	VI001339AG	SEA	92	55	1.14	0.38	0.45	0.07	1.59	0.46
60	AGG325525	VI001385AG	SA	93	41	0.61	0.15	0.20	0.08	0.80	0.25
61	AGG325526	VI001400AG	SA	92	49	0.98	0.13	0.23	0.16	1.22	0.30
62	AGG325527	VI001403BR	SA	97	65	0.67	0.29	0.26	0.14	0.94	0.45
63	AGG325528	VI001406BG	SWA	96	0	0.39	0.00	0.14	0.00	0.53	0.00
64	AGG325529	VI001408BG	SA	92	45	0.46	0.11	0.14	0.03	0.60	0.15
65	AGG325530	VI001411AG	SA	100	76	0.75	0.23	0.25	0.08	1.00	0.32
66	AGG325531	VI001412AG	SA	95	56	0.72	0.19	0.24	0.08	0.96	0.28
67	AGG325532	VI001419BG	SA	93	42	0.72	0.19	0.20	0.06	0.93	0.27
68	AGG325533	VI001435AG	NA	92	48	0.68	0.19	0.19	0.07	0.87	0.26
69	AGG325534	VI001448A-BLM	SA	100	51	0.41	0.02	0.12	0.02	0.53	0.05
70	AGG325535	VI001471AG	SA	93	67	1.33	0.30	0.41	0.12	1.73	0.43
71	AGG325536	VI001482BG	SA	94	33	0.84	0.21	0.23	0.14	1.07	0.36
72	AGG325537	VI001490AG	SWA	100	15	0.80	0.02	0.23	0.02	1.03	0.05
73	AGG325538	VI001509AG	SWA	100	45	0.76	0.13	0.21	0.08	0.97	0.21
74	AGG325539	VI001514AG	SA	100	56	0.92	0.15	0.30	0.05	1.22	0.21
75	AGG325540	VI001520A-BLM	SA	100	51	0.47	0.15	0.15	0.10	0.62	0.26
76	AGG325541	VI001533BG	SA	100	82	0.39	0.14	0.15	0.07	0.54	0.23
77	AGG325542	VI001535BG	SA	100	18	0.88	0.06	0.39	0.01	1.26	0.08
78	AGG325543	VI001539AG	SA	95	29	0.60	0.06	0.17	0.03	0.76	0.10
79	AGG325544	VI001548AG	SA	97	53	0.70	0.19	0.22	0.08	0.92	0.28
80	AGG325545	VI001556BG	SA	100	39	1.16	0.09	0.15	0.06	1.31	0.15
81	AGG325546	VI001557BG	NA	100	51	0.29	0.14	0.12	0.08	0.41	0.24
82	AGG325547	VI001562AG	SA	100	75	0.32	0.13	0.18	0.06	0.50	0.20
83	AGG325548	VI001576BG	SA	95	73	0.44	0.13	0.19	0.09	0.63	0.23
84	AGG325549	VI001579BG	SA	95	45	0.77	0.11	0.30	0.05	1.07	0.17
85	AGG325550	VI001605BG	SA	97	42	1.01	0.18	0.50	0.07	1.51	0.26
86	AGG325551	VI001612AG	UK	100	53	0.38	0.13	0.13	0.04	0.51	0.18
87	AGG325552	VI001628AG	SA	100	30	1.06	0.10	0.87	0.05	1.93	0.16
88	AGG325553	VI001651BG	SA	100	40	0.47	0.08	0.29	0.05	0.76	0.14
89	AGG325554	VI001652BG	SA	100	31	0.77	0.09	0.25	0.04	1.02	0.14
90	AGG325555	VI001654BG	SA	98	62	1.52	0.14	0.61	0.08	2.14	0.23
91	AGG325556	VI001678BG	SA	94	70	1.05	0.20	0.41	0.09	1.47	0.29
92	AGG325557	VI001692AG	SA	96	32	0.70	0.05	0.19	0.02	0.89	0.08
93	AGG325558	VI001698BG	SA	100	65	1.01	0.22	0.33	0.07	1.34	0.29
94	AGG325559	VI001728AG	SA	100	52	0.53	0.11	0.19	0.06	0.72	0.19
95	AGG325560	VI001733BG	SA	96	35	0.99	0.13	0.37	0.05	1.37	0.18
96	AGG325561	VI001743BG	SA	94	38	0.78	0.22	0.34	0.14	1.12	0.37
97	AGG325562	VI001756BG	SA	99	23	0.97	0.09	0.26	0.04	1.22	0.14
98	AGG325563	VI001762A-GM	SA	94	50	1.00	0.15	0.39	0.08	1.39	0.24
99	AGG325564	VI001806AG	SWA	94	49	0.97	0.21	0.35	0.08	1.32	0.30
100	AGG325565	VI001806BG	SWA	99	9	0.96	0.03	0.28	0.03	1.24	0.06
101	AGG325566	VI001820BG	EUR	100	25	0.96	0.09	0.37	0.05	1.33	0.14
102	AGG325567	VI001859BG	SEA	100	57	1.41	0.35	0.49	0.18	1.90	0.54
103	AGG325568	VI001974BG	EA	100	86	1.08	0.39	0.34	0.18	1.42	0.59
104	AGG325569	VI001993BG	EA	93	0	0.73	0.00	0.21	0.00	0.94	0.00
105	AGG325570	VI002009BG	SA	96	65	1.17	0.14	0.81	0.06	1.98	0.21
106	AGG325571	VI002012BG	SA	100	22	0.58	0.38	0.25	0.09	0.83	0.48
107	AGG325572	VI002051BG	SA	94	53	0.58	0.28	0.22	0.16	0.79	0.46
108	AGG325573	VI002063BG	NA	100	63	0.96	0.38	0.25	0.16	1.21	0.55

Sr. No.	Accession Num	VI_num	Origin	Germination stage							
				Emergence (%)		Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)	
				Control	WL	Control	WL	Control	WL	Control	WL
109	AGG325574	VI002173AG	SA	91	33	0.72	0.16	0.25	0.06	0.97	0.22
110	AGG325575	VI002173BG	SA	93	82	1.03	0.45	0.35	0.21	1.37	0.66
111	AGG325576	VI002176AG	SA	95	55	1.08	0.12	0.35	0.09	1.43	0.23
112	AGG325577	VI002176BG	SA	93	10	0.50	0.02	0.21	0.02	0.71	0.05
113	AGG325578	VI002190BG	SA	95	80	0.76	0.31	0.49	0.15	1.25	0.47
114	AGG325579	VI002195AG	SEA	92	60	1.20	0.38	0.51	0.12	1.70	0.51
115	AGG325580	VI002197BG	EA	100	16	1.01	0.04	0.22	0.02	1.23	0.07
116	AGG325581	VI002206AG	SEA	92	51	0.92	0.21	0.23	0.10	1.15	0.32
117	AGG325582	VI002239AG	SWA	96	61	0.70	0.07	0.26	0.06	0.95	0.14
118	AGG325584	VI002284BG	SWA	94	11	0.56	0.10	0.19	0.02	0.76	0.14
119	AGG325585	VI002402BG	SEA	96	53	0.76	0.07	0.32	0.06	1.09	0.14
120	AGG325586	VI002432AG	SEA	100	39	0.88	0.33	0.26	0.09	1.14	0.43
121	AGG325587	VI002437 BG	EA	100	49	1.12	0.11	0.59	0.05	1.71	0.17
122	AGG325588	VI002456AG	EA	100	53	1.07	0.36	0.29	0.21	1.36	0.58
123	AGG325589	VI002469AG	SEA	95	70	1.20	0.18	0.29	0.06	1.49	0.25
124	AGG325590	VI002487AG	SWA	100	71	0.72	0.19	0.27	0.05	0.98	0.25
125	AGG325591	VI002523AG	SEA	98	21	0.52	0.08	0.23	0.03	0.76	0.12
126	AGG325593	VI002532AG	SA	98	57	0.70	0.34	0.28	0.11	0.98	0.46
127	AGG325594	VI002537 AG	SWA	92	33	0.99	0.09	0.24	0.04	1.23	0.13
128	AGG325595	VI002569BG	AFR	100	53	0.89	0.28	0.20	0.11	1.09	0.41
129	AGG325596	VI002587AG	OP	98	32	1.18	0.16	0.51	0.38	1.69	0.55
130	AGG325597	VI002611AG	SEA	100	47	1.14	0.15	0.46	0.10	1.60	0.26
131	AGG325598	VI002646AG	SEA	98	67	1.12	0.65	0.39	0.30	1.50	0.96
132	AGG325599	VI002647AG	SEA	100	17	1.11	0.11	0.24	0.04	1.36	0.16
133	AGG325600	VI002672AG	SEA	100	63	1.44	0.27	0.46	0.09	1.90	0.37
134	AGG325601	VI002739AG	SWA	100	49	0.51	0.37	0.17	0.12	0.69	0.05
135	AGG325602	VI002802A-BR	SWA	100	19	1.43	0.08	0.81	0.04	2.23	0.13
136	AGG325603	VI002859BG	SWA	100	46	0.76	0.16	0.32	0.07	1.08	0.24
137	AGG325604	VI002860AG	SWA	100	43	1.32	0.15	0.28	0.09	1.59	0.25
138	AGG325605	VI002872BG	SWA	100	35	0.89	0.17	0.39	0.08	1.28	0.26
139	AGG325606	VI002877BG	SWA	100	30	0.46	0.06	0.13	0.02	0.59	0.09
140	AGG325607	VI002894B-BR	SWA	94	14	1.13	0.05	0.35	0.02	1.49	0.08
141	AGG325608	VI002926AG	SA	100	64	0.52	0.14	0.20	0.08	0.72	0.22
142	AGG325609	VI002934AG	SA	100	33	0.88	0.05	0.21	0.03	1.09	0.09
143	AGG325610	VI002986AG	SA	95	28	0.29	0.09	0.15	0.03	0.44	0.14
144	AGG325611	VI002993BG	SA	95	15	0.35	0.04	0.29	0.04	0.65	0.09
145	AGG325612	VI002999AG	SA	96	96	0.42	0.27	0.10	0.08	0.52	0.36
146	AGG325613	VI003019A-BLM	UK	95	28	0.63	0.18	0.26	0.09	0.89	0.28
147	AGG325614	VI003019ABG	UK	92	21	0.62	0.04	0.25	0.03	0.86	0.08
148	AGG325615	VI003034BG	SA	94	23	0.79	0.01	0.17	0.01	0.96	0.03
149	AGG325616	VI003035AG	SA	96	28	0.70	0.04	0.22	0.02	0.92	0.05
150	AGG325617	VI003057BG	SA	100	60	0.33	0.29	0.39	0.10	0.72	0.40
151	AGG325618	VI003062BG	SA	100	45	0.94	0.15	0.22	0.07	1.17	0.23
152	AGG325619	VI003068A-BR	SA	100	51	0.69	0.11	0.15	0.04	0.85	0.13
153	AGG325620	VI003070AG	SA	95	66	0.79	0.28	0.17	0.11	0.96	0.37
154	AGG325621	VI003083BG	SA	95	70	1.33	0.37	0.36	0.13	1.69	0.48
155	AGG325622	VI003114AG	SA	100	72	1.17	0.31	0.22	0.14	1.38	0.43
156	AGG325623	VI003135B-BL	SA	97	42	0.66	0.13	0.19	0.07	0.85	0.18
157	AGG325624	VI003159AG	SA	100	42	0.71	0.20	0.22	0.07	0.93	0.26
158	AGG325625	VI003172BG	SA	100	55	0.75	0.10	0.21	0.02	0.96	0.13
159	AGG325626	VI003181B-GM	SA	100	66	1.20	0.61	0.32	0.17	1.52	0.78
160	AGG325627	VI003183AG	SA	100	51	0.94	0.55	0.28	0.18	1.22	0.74
161	AGG325628	VI003187BG	SA	97	25	1.43	0.29	0.30	0.14	1.73	0.41
162	AGG325629	VI003212B-BLM	SA	100	40	1.01	0.12	0.20	0.03	1.21	0.13
163	AGG325630	VI003220AG	SA	100	74	1.36	0.41	0.32	0.17	1.68	0.57
164	AGG325631	VI003232AG	SA	98	79	0.63	0.47	0.15	0.11	0.78	0.57
165	AGG325632	VI003235AG	SA	100	60	1.15	0.19	0.31	0.15	1.46	0.33

Sr. No.	Accession Num	VI_num	Origin	Germination stage							
				Emergence (%)		Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)	
				Control	WL	Control	WL	Control	WL	Control	WL
166	AGG325633	VI003242AG	SA	100	44	1.39	0.28	0.41	0.08	1.81	0.35
167	AGG325634	VI003251A-BL	SA	100	54	0.96	0.24	0.26	0.07	1.23	0.30
168	AGG325635	VI003251A-BLM	SA	97	57	0.71	0.49	0.29	0.20	1.00	0.70
169	AGG325636	VI003252BG	SA	97	75	0.22	0.14	0.07	0.04	0.29	0.18
170	AGG325637	VI003255AG	SA	97	57	0.58	0.37	0.21	0.13	0.80	0.49
171	AGG325638	VI003276BG	SA	97	55	0.58	0.29	0.17	0.11	0.75	0.38
172	AGG325639	VI003329AG	SA	100	37	1.11	0.04	0.24	0.18	1.34	0.22
173	AGG325640	VI003332AG	SA	96	78	0.76	0.37	0.26	0.17	1.02	0.53
174	AGG325641	VI003337BG	SA	92	10	0.87	0.05	0.24	0.02	1.11	0.07
175	AGG325642	VI003364AG	SA	100	70	1.49	0.35	0.32	0.11	1.81	0.45
176	AGG325643	VI003379BG	SA	96	76	0.60	0.44	0.31	0.24	0.91	0.65
177	AGG325644	VI003382BG	SA	92	62	0.84	0.36	0.24	0.06	1.07	0.42
178	AGG325645	VI003407AG	SA	96	80	0.48	0.09	0.16	0.03	0.63	0.11
179	AGG325646	VI003413BG	SA	100	15	0.70	0.08	0.19	0.02	0.88	0.09
180	AGG325647	VI003440AG	SA	95	54	0.75	0.58	0.23	0.19	0.98	0.77
181	AGG325648	VI003455AG	SA	96	40	0.42	0.13	0.11	0.05	0.52	0.17
182	AGG325649	VI003456AG	UK	100	55	1.08	0.19	0.27	0.07	1.35	0.24
183	AGG325650	VI003456BG	SA	97	78	1.01	0.27	0.23	0.11	1.24	0.36
184	AGG325651	VI003470BG	SA	97	9	1.18	0.11	0.29	0.04	1.46	0.15
185	AGG325652	VI003480BG	SA	97	46	0.45	0.07	0.20	0.03	0.65	0.08
186	AGG325653	VI003490AG	SA	100	86	0.53	0.10	0.23	0.03	0.76	0.12
187	AGG325654	VI003493BG	SA	97	71	0.70	0.13	0.31	0.06	1.01	0.19
188	AGG325655	VI003514BG	SA	98	39	0.25	0.03	0.08	0.01	0.33	0.02
189	AGG325656	VI003517BG	SA	97	40	0.89	0.17	0.31	0.08	1.20	0.24
190	AGG325657	VI003534AG	SA	100	27	1.05	0.10	0.31	0.05	1.36	0.15
191	AGG325658	VI003534BG	SA	98	49	0.74	0.32	0.21	0.05	0.94	0.36
192	AGG325659	VI003548AG	SA	93	35	0.82	0.12	0.24	0.02	1.06	0.12
193	AGG325660	VI003554AG	SA	97	74	0.57	0.20	0.20	0.09	0.78	0.28
194	AGG325662	VI003563A-BR	SA	100	51	1.05	0.16	0.32	0.12	1.37	0.28
195	AGG325663	VI003557AG	SA	97	60	0.44	0.07	0.13	0.06	0.57	0.13
196	AGG325664	VI003602AG	SA	96	32	0.93	0.10	0.27	0.16	1.20	0.36
197	AGG325665	VI003642AG	SA	98	61	1.33	0.29	0.40	0.10	1.73	0.41
198	AGG325666	VI003648BG	SA	95	57	1.17	0.15	0.27	0.04	1.44	0.16
199	AGG325667	VI003658BG	SA	100	92	0.93	0.35	0.27	0.10	1.20	0.44
200	AGG325668	VI003664AG	SA	100	66	0.97	0.59	0.29	0.20	1.26	0.78
201	AGG325669	VI003678BG	SA	100	62	0.96	0.21	0.32	0.04	1.28	0.23
202	AGG325670	VI003685AG	SA	100	80	1.33	0.38	0.40	0.13	1.73	0.49
203	AGG325671	VI003699BG	SA	97	85	0.12	0.05	0.15	0.02	0.27	0.07
204	AGG325672	VI003720BG	SA	100	85	0.82	0.14	0.21	0.06	1.03	0.18
205	AGG325673	VI003726BG	SA	92	47	1.17	0.27	0.47	0.14	1.64	0.41
206	AGG325674	VI003733BG	SA	100	61	0.73	0.05	0.19	0.02	0.92	0.05
207	AGG325675	VI003734B-BR	SA	100	52	1.14	0.26	0.28	0.08	1.41	0.33
208	AGG325676	VI003734B-DG	SA	100	55	1.03	0.34	0.28	0.09	1.31	0.42
209	AGG325677	VI003744AG	SA	100	13	1.16	0.10	0.30	0.03	1.47	0.13
210	AGG325678	VI003755BG	SA	100	62	1.08	0.30	0.27	0.15	1.35	0.44
211	AGG325679	VI003760BG	SA	98	42	0.33	0.17	0.11	0.08	0.44	0.24
212	AGG325680	VI003785BG	SA	96	80	0.70	0.35	0.27	0.08	0.97	0.42
213	AGG325681	VI003795AG	SA	95	37	1.39	0.20	0.33	0.09	1.72	0.27
214	AGG325682	VI003801BG	SA	95	44	1.44	0.38	0.35	0.18	1.79	0.54
215	AGG325683	VI003882A-BLM	SWA	99	15	0.80	0.10	0.26	0.06	1.06	0.16
216	AGG325684	VI003886 B-BR	SA	100	70	1.03	0.38	0.31	0.15	1.34	0.67
217	AGG325685	VI003886 BY	SA	100	55	1.68	0.30	0.37	0.12	2.05	0.40
218	AGG325686	VI003893AG	SA	100	70	1.09	0.51	0.30	0.22	1.39	0.72
219	AGG325687	VI003894 B-BLM	SA	92	35	0.98	0.28	0.22	0.11	1.20	0.39
220	AGG325688	VI003907AG	SWA	95	50	0.97	0.41	0.31	0.13	1.28	0.54
221	AGG325689	VI003914AG	SA	95	44	0.95	0.26	0.26	0.11	1.21	0.35
222	AGG325690	VI003925 B-BLM	SA	92	20	0.53	0.07	0.25	0.03	0.77	0.08

Sr. No.	Accession Num	VI_num	Origin	Germination stage							
				Emergence (%)		Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)	
				Control	WL	Control	WL	Control	WL	Control	WL
223	AGG325691	VI003927AG	SA	100	60	0.57	0.19	0.13	0.06	0.70	0.24
224	AGG325692	VI003929A-BL	SA	92	32	0.42	0.14	0.20	0.14	0.62	0.27
225	AGG325693	VI003942AG	SWA	98	70	0.92	0.42	0.27	0.17	1.18	0.58
226	AGG325694	VI003944 B-BR	SWA	95	10	0.73	0.11	0.21	0.04	0.95	0.13
227	AGG325695	VI003947 B-BR	SA	95	64	0.93	0.23	0.23	0.09	1.15	0.31
228	AGG325696	VI003948 B-BR	SA	93	5	0.60	0.06	0.27	0.02	0.87	0.09
229	AGG325697	VI003951AG	SA	97	58	1.12	0.55	0.31	0.17	1.43	0.71
230	AGG325698	VI003954BG	SA	97	12	1.05	0.09	0.33	0.02	1.39	0.09
231	AGG325699	VI003957AG	SA	99	77	1.02	0.57	0.30	0.18	1.33	0.76
232	AGG325700	VI003958 B-BLM	SA	100	19	0.84	0.04	0.23	0.01	1.07	0.03
233	AGG325701	VI003959 BG	SA	92	76	0.77	0.51	0.27	0.25	1.04	0.96
234	AGG325702	VI004006 A-GM	SA	92	34	1.07	0.18	0.24	0.10	1.31	0.27
235	AGG325703	VI004010 AG	SA	95	84	1.09	0.58	0.25	0.16	1.34	0.74
236	AGG325704	VI004024 AG	OP	97	67	1.57	0.23	0.30	0.07	1.88	0.28
237	AGG325705	VI004044 BG	SA	96	61	0.73	0.23	0.22	0.07	0.95	0.28
238	AGG325706	VI004045 A-DGM	SA	94	20	1.30	0.10	0.32	0.05	1.63	0.16
239	AGG325707	VI004048 A-DGM	SA	94	63	0.88	0.69	0.20	0.19	1.08	0.86
240	AGG325708	VI004069BG	SA	94	50	0.94	0.12	0.27	0.06	1.22	0.16
241	AGG325709	VI004096 AG	SA	97	45	1.02	0.08	0.22	0.02	1.24	0.08
242	AGG325710	VI004096 BG	SA	97	62	0.51	0.09	0.10	0.05	0.61	0.13
243	AGG325711	VI004129 A-BLM	UK	96	70	0.63	0.45	0.27	0.19	0.90	0.62
244	AGG325712	VI004138 BG	SA	97	32	1.15	0.18	0.28	0.06	1.42	0.24
245	AGG325713	VI004145 B-BLM	SWA	99	50	0.52	0.17	0.15	0.07	0.67	0.23
246	AGG325714	VI004184 AG	EUR	98	32	0.90	0.20	0.22	0.08	1.13	0.26
247	AGG325715	VI004243 B-BR	SWA	97	30	0.76	0.15	0.20	0.07	0.96	0.20
248	AGG325716	VI004244 B-BR	SA	98	55	0.83	0.26	0.23	0.13	1.07	0.38
249	AGG325717	VI004297 AG	SWA	93	55	0.93	0.55	0.32	0.17	1.25	0.73
250	AGG325718	VI004302 AG	SWA	95	65	0.88	0.42	0.28	0.20	1.16	0.62
251	AGG325719	VI004307 AG	SWA	94	45	0.82	0.26	0.28	0.13	1.10	0.40
252	AGG325720	VI004312 AG	SA	97	25	1.12	0.06	0.30	0.02	1.42	0.07
253	AGG325721	VI004347 B-BLM	SA	99	37	1.35	0.19	0.36	0.06	1.71	0.25
254	AGG325722	VI004351 AG	SA	98	70	0.72	0.18	0.23	0.09	0.94	0.26
255	AGG325723	VI004423 AG	SWA	94	37	0.83	0.25	0.24	0.08	1.06	0.33
256	AGG325724	VI004432 B-BR	SWA	95	42	1.10	0.32	0.34	0.15	1.44	0.47
257	AGG325725	VI004480 AG	SWA	96	30	1.33	0.12	0.35	0.07	1.69	0.17
258	AGG325726	VI004639 AG	SWA	92	35	0.30	0.21	0.06	0.04	0.36	0.24
259	AGG325727	VI004666 AG	SWA	94	40	1.07	0.14	0.29	0.07	1.37	0.22
260	AGG325728	VI004691 AG	SWA	95	68	1.03	0.33	0.33	0.13	1.36	0.45
261	AGG325729	VI004694 BG	SWA	96	65	1.12	0.36	0.39	0.15	1.51	0.51
262	AGG325730	VI004710 AG	SWA	98	50	1.12	0.43	0.29	0.17	1.41	0.59
263	AGG325731	VI004734 AG	SWA	93	22	1.32	0.21	0.29	0.10	1.61	0.31
264	AGG325732	VI004743 AG	SA	95	78	0.43	0.07	0.16	0.02	0.58	0.07
265	AGG325733	VI004789	SA	95	59	1.42	0.41	0.40	0.18	1.82	0.58
266	AGG325734	VI004810 BG	SA	97	47	1.03	0.43	0.26	0.09	1.29	0.50
267	AGG325735	VI004811 BG	SA	98	62	0.69	0.16	0.23	0.04	0.91	0.20
268	AGG325736	VI004822 BG	SA	97	47	0.82	0.22	0.27	0.08	1.09	0.29
269	AGG325737	VI004838 AG	SA	98	52	0.85	0.12	0.25	0.05	1.10	0.16
270	AGG325738	VI004842 AG	SA	97	67	0.82	0.36	0.21	0.09	1.03	0.43
271	AGG325739	VI004853 BG	SA	98	52	0.70	0.25	0.18	0.08	0.88	0.32
272	AGG325740	VI004871 BG	SA	96	64	0.91	0.43	0.22	0.19	1.13	0.62
273	AGG325741	VI004877 AG	SA	96	69	0.68	0.27	0.21	0.08	0.89	0.34
274	AGG325742	VI004915 BG	SA	98	80	1.54	0.24	0.27	0.07	1.81	0.31
275	AGG325743	VI004931 AG	SWA	96	30	0.96	0.28	0.27	0.10	1.23	0.38
276	AGG325744	VI004933 AG	SWA	96	56	0.82	0.21	0.21	0.07	1.03	0.28
277	AGG325745	VI004934 AG	SWA	95	75	0.72	0.32	0.24	0.16	0.95	0.47
278	AGG325746	VI004937 AG	SWA	97	60	1.05	0.14	0.24	0.18	1.28	0.31
279	AGG325747	VI004942 BG	SWA	95	42	1.15	0.19	0.27	0.09	1.42	0.28

Sr. No.	Accession Num	VI_num	Origin	Germination stage							
				Emergence (%)		Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)	
				Control	WL	Control	WL	Control	WL	Control	WL
280	AGG325748	VI004954BG	SWA	97	45	0.86	0.04	0.35	0.02	1.21	0.05
281	AGG325749	VI004956 AG	SWA	93	5	0.95	0.03	0.22	0.01	1.18	0.02
282	AGG325750	VI004957 AG	SWA	94	75	0.98	0.35	0.24	0.14	1.22	0.48
283	AGG325751	VI004958 BG	SWA	95	63	1.26	0.42	0.31	0.14	1.57	0.56
284	AGG325752	VI004965 BG	SWA	98	62	1.22	0.50	0.32	0.16	1.53	0.65
285	AGG325753	VI004968 AG	SWA	97	79	0.55	0.37	0.19	0.14	0.75	0.49
286	AGG325754	VI004969 AG	SWA	98	74	1.11	0.29	0.30	0.11	1.41	0.38
287	AGG325755	VI004973 B-BLM	SA	97	19	0.41	0.04	0.18	0.02	0.59	0.06
288	AGG325756	VI005022 BG	SA	96	15	0.87	0.04	0.25	0.01	1.12	0.03
289	AGG325758	VI005030 BY	MA	94	42	0.86	0.23	0.21	0.10	1.06	0.32
290	AGG325759	VI005066 A-GM	UK	96	50	1.23	0.35	0.40	0.12	1.64	0.46
291	AGG325760	VI005041 AG	SA	98	86	1.69	0.79	0.57	0.30	2.26	1.08
292	AGG325761	VI014178 BG	AFR	96	17	0.99	0.10	0.21	0.04	1.20	0.12

SUPPLEMENTARY TABLE 4.2 SCREENING OF MUNGBEAN MINI CORE GENOTYPES FOR WATERLOGGING TOLERANCE AT THE SEEDLING STAGE. THE TREATMENT WAS WATERLOGGED FOR WL 8 DAYS AT THE SEEDLING STAGE AND COMPARED WITH THEIR RESPECTIVE DRAINED CONTROL. THE WATERLOGGED POTS RECOVERED FOR 7 DAYS IMMEDIATELY AFTER THE RELEASE OF WATERLOGGING. HARVESTING OF WATERLOGGED AND CONTROL POTS OCCURRED ON THE SAME DAY. DATA REPRESENT THE MEAN VALUE FOR THREE REPLICATIONS.

Sr. No.	Accession Number	VI_number	Origin	Seedling stage													
				Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)		No. adventitious roots	SPAD_1		SPAD_2		SPAD_3		100-seed weight (g)
				Control	WL	Control	WL	Control	WL		Control	WL	Control	WL	Control	WL	
1	AGG325466	VI000020AY	SEA	1.44	0.73	0.41	0.28	1.85	1.01	17	37	26	49	30	31	28	6.7
2	AGG325467	VI000099AG	SA	0.44	0.32	0.25	0.11	0.69	0.43	12	40	34	43	33	26	25	3.2
3	AGG325468	VI000105BG	SA	0.71	0.42	0.39	0.15	1.10	0.57	14	41	33	43	29	33	23	3.1
4	AGG325469	VI000164BG	SWA	0.68	0.52	0.35	0.18	1.03	0.70	12	38	32	45	33	35	29	5.3
5	AGG325470	VI000170B-BR	SWA	0.40	0.23	0.13	0.07	0.52	0.31	6	40	31	45	35	41	23	3.1
6	AGG325471	VI000175BY	SA	0.81	0.38	0.29	0.15	1.10	0.53	10	35	22	39	24	31	17	2.9
7	AGG325472	VI000188A-BLM	SWA	0.80	0.66	0.30	0.21	1.10	0.87	12	35	32	43	33	35	24	3.3
8	AGG325473	VI00203B-BR	SWA	0.51	0.36	0.25	0.12	0.76	0.48	10	46	34	52	36	39	27	3.7
9	AGG325474	VI000212A-BLM	NA	0.56	0.30	0.23	0.06	0.79	0.36	3	39	31	43	30	33	26	3.3
10	AGG325475	VI000232AG	SWA	0.72	0.45	0.34	0.14	1.06	0.59	11	39	30	44	30	30	20	2.6
11	AGG325476	VI000238AG	SWA	0.39	0.24	0.22	0.08	0.61	0.32	6	40	30	51	27	39	27	2.9
12	AGG325477	VI000253AG	SA	0.89	0.41	0.43	0.14	1.31	0.55	15	38	32	40	31	30	23	3.0
13	AGG325478	VI000316AG	SWA	0.88	0.37	0.26	0.10	1.14	0.46	6	40	29	47	29	37	22	3.6
14	AGG325479	VI000317BG	SWA	1.16	0.30	0.28	0.02	1.44	0.32	0	43	29	40	36	35	28	2.9
15	AGG325480	VI000319AG	SWA	0.94	0.51	0.32	0.12	1.27	0.63	12	42	34	43	28	27	25	3.8
16	AGG325481	VI000380AG	SEA	1.52	0.96	0.57	0.31	2.08	1.27	16	39	30	52	32	36	21	3.7
17	AGG325482	VI000461BG	SEA	0.94	0.58	0.32	0.18	1.27	0.76	7	37	31	42	34	38	21	3.2
18	AGG325483	VI000470AG	SWA	1.60	0.85	0.50	0.24	2.10	1.08	15	41	27	51	36	38	24	4.2
19	AGG325484	VI000532BG	SA	0.54	0.14	0.25	0.05	0.79	0.19	3	40	29	41	32	28	21	3.3
20	AGG325485	VI000537BG	SA	0.65	0.41	0.20	0.11	0.85	0.51	14	38	31	45	29	33	24	3.6
21	AGG325486	VI000542BY	SA	0.56	0.25	0.31	0.08	0.87	0.33	4	39	30	49	30	35	23	3.7
22	AGG325487	VI000551AG	SA	0.57	0.31	0.24	0.07	0.81	0.38	4	31	28	47	31	33	23	3.2
23	AGG325488	VI000554AG	SA	0.60	0.19	0.18	0.09	0.78	0.28	3	38	30	46	32	33	19	3.5
24	AGG325489	VI000559AG	SA	0.42	0.27	0.23	0.12	0.65	0.39	12	41	31	42	32	31	25	3.8
25	AGG325490	VI000578AG	SA	0.67	0.45	0.41	0.13	1.08	0.58	16	36	30	52	35	30	27	4.1
26	AGG325491	VI000589B-BR	SA	1.13	0.47	0.45	0.15	1.58	0.62	18	38	31	54	29	45	25	3.2
27	AGG325492	VI000616BG	SAM	0.82	0.36	0.22	0.12	1.04	0.48	15	37	31	50	28	43	22	2.7
28	AGG325493	VI000618AG	SA	0.63	0.28	0.32	0.11	0.95	0.39	7	39	28	45	28	30	22	2.7
29	AGG325494	VI000625B-BR	SA	0.61	0.25	0.18	0.09	0.79	0.34	10	38	27	53	32	38	25	3.3
30	AGG325495	VI000680AG	NA	1.30	0.43	0.53	0.10	1.83	0.53	6	35	29	44	29	36	24	3.4

Sr. No.	Accession Number	VI_number	Origin	Seedling stage													
				Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)		No. adventitious roots	SPAD_1		SPAD_2		SPAD_3		100-seed weight (g)
				Control	WL	Control	WL	Control	WL		Control	WL	Control	WL	Control	WL	
31	AGG325496	VI000723AG	SWA	0.55	0.32	0.32	0.10	0.87	0.42	10	43	38	49	32	35	21	3.3
32	AGG325497	VI000732AG	SA	0.66	0.46	0.21	0.10	0.87	0.57	14	39	32	54	32	41	23	3.5
33	AGG325498	VI000735BG	SA	1.11	0.39	0.37	0.10	1.48	0.48	8	36	26	44	30	36	23	2.6
34	AGG325499	VI000736AG	SA	0.95	0.46	0.26	0.09	1.21	0.54	9	42	31	50	32	43	21	3.4
35	AGG325500	VI000749AG	SA	0.59	0.38	0.27	0.14	0.86	0.52	11	41	32	51	36	34	24	3.8
36	AGG325501	VI000764AG	SA	0.67	0.24	0.25	0.07	0.92	0.31	16	39	30	47	30	33	25	5.2
37	AGG325502	VI000766BG	SA	0.86	0.49	0.36	0.13	1.21	0.62	10	39	31	44	30	39	27	3.4
38	AGG325503	VI000805BG	SA	1.23	0.60	0.36	0.16	1.59	0.77	13	41	25	46	29	37	21	3.3
39	AGG325504	VI000815BG	SA	0.65	0.35	0.24	0.10	0.89	0.45	6	43	27	54	34	39	29	4.3
40	AGG325505	VI000818BG	SA	0.75	0.46	0.30	0.17	1.04	0.63	15	40	27	39	32	26	23	5.7
41	AGG325506	VI000852AG	SA	1.07	0.74	0.47	0.23	1.53	0.97	18	43	32	42	32	35	28	3.6
42	AGG325507	VI000938AG	SA	0.66	0.22	0.20	0.06	0.86	0.28	6	40	27	45	34	34	26	3.4
43	AGG325508	VI000942AG	SA	1.53	0.69	0.35	0.21	1.87	0.90	16	41	34	54	30	39	24	2.7
44	AGG325509	VI000953AG	SA	1.27	0.51	0.32	0.16	1.59	0.67	8	44	29	50	29	34	23	3.5
45	AGG325510	VI000981BG	SEA	1.08	0.38	0.51	0.17	1.59	0.55	14	37	29	40	30	31	24	3.0
46	AGG325511	VI001023BG	SA	0.88	0.41	0.34	0.15	1.23	0.56	7	38	24	42	31	36	22	3.2
47	AGG325512	VI001066BG	OP	0.80	0.32	0.43	0.21	1.24	0.53	14	45	31	50	31	35	26	3.2
48	AGG325513	VI001096AG	OP	1.28	0.62	0.36	0.21	1.64	0.83	15	41	36	52	38	38	21	3.4
49	AGG325514	VI001124AG	OP	1.28	0.96	0.52	0.32	1.80	1.28	22	47	37	47	36	37	27	2.9
50	AGG325515	VI001126BG	OP	0.73	0.34	0.25	0.13	0.97	0.47	11	37	28	43	30	36	26	3.1
51	AGG325516	VI001162AG	OP	0.49	0.25	0.20	0.11	0.69	0.36	11	42	32	46	31	38	25	3.0
52	AGG325517	VI001191BG	SEA	0.87	0.55	0.35	0.17	1.22	0.72	16	37	28	44	29	34	19	3.7
53	AGG325518	VI001211AG	SEA	1.03	0.18	0.32	0.07	1.35	0.25	11	39	27	48	25	30	19	3.2
54	AGG325519	VI001221AG	SEA	0.99	0.51	0.42	0.14	1.42	0.65	9	41	28	51	27	41	22	6.1
55	AGG325520	VI001244AG	SEA	1.32	0.65	0.44	0.17	1.76	0.83	15	43	29	49	29	37	23	4.1
56	AGG325521	VI001268BG	SA	0.86	0.40	0.28	0.07	1.14	0.47	11	38	31	46	31	33	25	2.9
57	AGG325522	VI001282AG	SA	0.54	0.28	0.23	0.08	0.77	0.36	7	35	25	49	29	40	26	3.1
58	AGG325523	VI001284AG	SA	0.76	0.36	0.20	0.12	0.96	0.48	13	40	30	47	33	38	22	5.1
59	AGG325524	VI001339AG	SEA	0.80	0.50	0.39	0.18	1.20	0.68	12	39	33	54	35	43	29	3.4
60	AGG325525	VI001385AG	SA	0.46	0.22	0.21	0.10	0.67	0.32	8	36	25	45	31	37	25	3.4
61	AGG325526	VI001400AG	SA	0.60	0.36	0.22	0.11	0.82	0.46	11	36	30	44	30	24	22	3.6
62	AGG325527	VI001403BR	SA	0.86	0.29	0.35	0.11	1.21	0.39	9	44	34	48	32	38	27	3.1
63	AGG325528	VI001406BG	SWA	0.83	0.47	0.36	0.14	1.20	0.61	8	44	33	40	31	38	22	3.8
64	AGG325529	VI001408BG	SA	0.76	0.39	0.23	0.08	0.99	0.47	7	40	29	46	28	32	20	4.1
65	AGG325530	VI001411AG	SA	0.56	0.27	0.24	0.06	0.80	0.33	9	37	30	43	30	33	22	3.2

Sr. No.	Accession Number	VI_number	Origin	Seedling stage													
				Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)		No. adventitious roots	SPAD_1		SPAD_2		SPAD_3		100-seed weight (g)
				Control	WL	Control	WL	Control	WL		Control	WL	Control	WL	Control	WL	
66	AGG325531	VI001412AG	SA	0.41	0.24	0.22	0.07	0.62	0.30	5	36	22	46	29	41	25	4.2
67	AGG325532	VI001419BG	SA	0.46	0.30	0.23	0.09	0.69	0.39	7	39	25	35	26	29	20	3.1
68	AGG325533	VI001435AG	NA	0.86	0.33	0.41	0.10	1.27	0.43	7	41	36	50	31	41	29	3.2
69	AGG325534	VI001448A-BLM	SA	0.40	0.17	0.13	0.05	0.53	0.22	7	45	30	39	30	34	26	3.0
70	AGG325535	VI001471AG	SA	0.67	0.30	0.22	0.09	0.89	0.39	7	41	35	52	39	40	28	4.8
71	AGG325536	VI001482BG	SA	0.67	0.41	0.31	0.13	0.98	0.54	8	43	36	43	34	42	29	3.1
72	AGG325537	VI001490AG	SWA	0.94	0.55	0.29	0.14	1.23	0.69	12	42	35	48	35	24	24	3.2
73	AGG325538	VI001509AG	SWA	0.96	0.46	0.41	0.16	1.37	0.62	10	37	35	39	34	40	24	3.3
74	AGG325539	VI001514AG	SA	0.57	0.28	0.27	0.09	0.83	0.36	9	42	30	48	33	36	25	3.2
75	AGG325540	VI001520A-BLM	SA	0.58	0.23	0.35	0.06	0.93	0.29	9	38	24	50	31	38	25	3.2
76	AGG325541	VI001533BG	SA	0.42	0.21	0.16	0.07	0.57	0.28	10	37	24	42	31	41	26	3.0
77	AGG325542	VI001535BG	SA	0.69	0.47	0.35	0.17	1.04	0.64	18	42	28	37	26	44	24	3.7
78	AGG325543	VI001539AG	SA	0.76	0.31	0.20	0.10	0.96	0.42	12	38	31	44	32	37	24	3.0
79	AGG325544	VI001548AG	SA	0.41	0.17	0.19	0.06	0.60	0.23	10	40	30	39	30	24	24	3.5
80	AGG325545	VI001556BG	SA	0.51	0.35	0.25	0.08	0.77	0.43	7	43	30	45	33	34	24	3.4
81	AGG325546	VI001557BG	NA	0.63	0.48	0.21	0.12	0.84	0.60	12	40	26	44	30	39	21	3.6
82	AGG325547	VI001562AG	SA	0.77	0.36	0.22	0.12	0.99	0.48	16	37	33	46	31	39	28	5.8
83	AGG325548	VI001576BG	SA	0.58	0.53	0.25	0.14	0.82	0.67	13	34	30	42	30	39	24	3.4
84	AGG325549	VI001579BG	SA	1.05	0.39	0.44	0.14	1.49	0.53	10	39	29	47	27	33	23	3.3
85	AGG325550	VI001605BG	SA	1.07	0.46	0.35	0.13	1.43	0.59	10	39	30	44	31	37	23	2.6
86	AGG325551	VI001612AG	UK	0.81	0.33	0.34	0.11	1.14	0.44	19	38	29	49	32	39	28	3.7
87	AGG325552	VI001628AG	SA	0.64	0.30	0.20	0.08	0.84	0.38	14	41	29	44	32	39	24	3.3
88	AGG325553	VI001651BG	SA	0.64	0.34	0.39	0.10	1.03	0.44	13	36	31	48	30	43	22	3.0
89	AGG325554	VI001652BG	SA	0.72	0.46	0.26	0.13	0.98	0.59	13	43	35	44	29	33	24	3.7
90	AGG325555	VI001654BG	SA	0.76	0.46	0.29	0.15	1.05	0.61	15	40	32	42	33	36	25	5.1
91	AGG325556	VI001678BG	SA	1.11	0.57	0.35	0.19	1.46	0.76	17	36	26	45	31	36	27	3.8
92	AGG325557	VI001692AG	SA	0.58	0.23	0.23	0.09	0.81	0.32	6	39	27	41	32	41	25	6.5
93	AGG325558	VI001698BG	SA	0.69	0.25	0.29	0.10	0.98	0.36	9	45	30	52	34	50	25	2.9
94	AGG325559	VI001728AG	SA	0.89	0.63	0.24	0.15	1.12	0.78	13	41	26	44	31	37	24	5.1
95	AGG325560	VI001733BG	SA	0.70	0.63	0.27	0.18	0.97	0.81	25	37	27	44	25	28	18	3.6
96	AGG325561	VI001743BG	SA	0.63	0.41	0.29	0.14	0.92	0.55	10	38	29	34	28	36	21	2.7
97	AGG325562	VI001756BG	SA	0.96	0.35	0.45	0.12	1.41	0.46	12	42	28	53	31	36	21	3.4
98	AGG325563	VI001762A-GM	SA	0.79	0.49	0.26	0.13	1.04	0.61	11	39	31	48	29	39	22	5.4
99	AGG325564	VI001806AG	SWA	0.55	0.38	0.28	0.16	0.83	0.54	14	36	27	45	29	35	22	3.8
100	AGG325565	VI001806BG	SWA	0.59	0.33	0.26	0.10	0.85	0.43	5	37	32	45	31	37	24	4.1

Sr. No.	Accession Number	VI_number	Origin	Seedling stage													
				Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)		No. adventitious roots	SPAD_1		SPAD_2		SPAD_3		100-seed weight (g)
				Control	WL	Control	WL	Control	WL		Control	WL	Control	WL	Control	WL	
101	AGG325566	VI001820BG	EUR	0.73	0.33	0.32	0.11	1.05	0.44	8	38	29	48	29	39	24	3.0
102	AGG325567	VI001859BG	SEA	1.08	0.77	0.38	0.18	1.46	0.95	18	37	33	37	30	35	18	3.4
103	AGG325568	VI001974BG	EA	1.02	0.43	0.44	0.16	1.46	0.59	17	42	34	45	28	40	25	3.7
104	AGG325569	VI001993BG	EA	0.83	0.51	0.31	0.20	1.14	0.71	12	41	31	43	34	39	26	3.1
105	AGG325570	VI002009BG	SA	0.76	0.56	0.29	0.18	1.05	0.74	14	34	28	45	33	37	24	3.0
106	AGG325571	VI002012BG	SA	0.76	0.58	0.33	0.22	1.09	0.80	15	37	28	40	29	28	26	4.0
107	AGG325572	VI002051BG	SA	0.95	0.46	0.40	0.29	1.35	0.75	13	36	27	39	28	34	23	3.9
108	AGG325573	VI002063BG	NA	0.93	0.61	0.39	0.27	1.32	0.88	11	33	21	40	25	28	21	4.3
109	AGG325574	VI002173AG	SA	0.99	0.74	0.45	0.28	1.44	1.02	14	39	21	39	27	35	22	3.7
110	AGG325575	VI002173BG	SA	1.00	0.65	0.37	0.17	1.37	0.82	20	37	26	37	24	35	23	3.7
111	AGG325576	VI002176AG	SA	0.77	0.59	0.34	0.19	1.10	0.79	15	38	25	44	28	34	22	3.2
112	AGG325577	VI002176BG	SA	0.92	0.66	0.34	0.26	1.26	0.91	15	36	22	40	31	33	20	3.1
113	AGG325578	VI002190BG	SA	1.28	0.58	0.50	0.26	1.78	0.84	20	35	27	39	32	33	18	2.6
114	AGG325579	VI002195AG	SEA	1.09	0.63	0.51	0.30	1.61	0.93	18	40	22	42	31	32	24	3.1
115	AGG325580	VI002197BG	EA	1.03	0.63	0.39	0.21	1.42	0.84	13	38	23	41	29	35	24	2.6
116	AGG325581	VI002206AG	SEA	1.33	0.60	0.40	0.26	1.73	0.86	15	41	24	47	29	38	23	3.3
117	AGG325582	VI002239AG	SWA	0.98	0.35	0.36	0.20	1.34	0.56	12	37	27	40	26	34	22	3.0
118	AGG325584	VI002284BG	SWA	0.46	0.34	0.30	0.18	0.76	0.51	12	39	34	41	31	34	25	3.2
119	AGG325585	VI002402BG	SEA	0.91	0.43	0.33	0.18	1.25	0.61	12	37	25	42	33	36	22	4.6
120	AGG325586	VI002432AG	SEA	1.18	0.70	0.64	0.32	1.82	1.02	19	41	28	40	28	35	22	3.9
121	AGG325587	VI002437 BG	EA	0.60	0.37	0.28	0.15	0.88	0.52	12	40	25	41	30	37	17	3.8
122	AGG325588	VI002456AG	EA	1.19	0.66	0.46	0.29	1.65	0.95	8	38	29	47	31	34	28	7.1
123	AGG325589	VI002469AG	SEA	1.02	0.85	0.46	0.33	1.48	1.18	15	38	26	41	32	33	25	3.5
124	AGG325590	VI002487AG	SWA	0.61	0.46	0.31	0.16	0.92	0.62	16	41	28	42	32	35	26	3.4
125	AGG325591	VI002523AG	SEA	1.26	0.84	0.48	0.36	1.74	1.21	20	34	27	37	32	34	23	3.8
126	AGG325593	VI002532AG	SA	1.09	0.66	0.68	0.23	1.77	0.89	23	43	30	46	29	38	26	6.0
127	AGG325594	VI002537 AG	SWA	0.85	0.62	0.35	0.19	1.20	0.81	14	38	27	43	31	31	22	3.5
128	AGG325595	VI002569BG	AFR	0.93	0.60	0.38	0.23	1.30	0.82	16	37	27	42	30	33	23	4.6
129	AGG325596	VI002587AG	OP	1.39	0.84	0.53	0.27	1.92	1.11	18	41	30	42	26	34	24	3.2
130	AGG325597	VI002611AG	SEA	0.55	0.44	0.31	0.21	0.86	0.65	11	43	28	46	32	34	27	2.9
131	AGG325598	VI002646AG	SEA	0.99	0.58	0.62	0.22	1.61	0.80	16	37	28	39	27	32	20	4.9
132	AGG325599	VI002647AG	SEA	0.88	0.53	0.44	0.28	1.32	0.82	17	38	29	40	31	35	26	3.6
133	AGG325600	VI002672AG	SEA	0.83	0.66	0.46	0.29	1.28	0.95	20	36	28	39	24	36	20	6.4
134	AGG325601	VI002739AG	SWA	1.20	0.89	0.59	0.34	1.79	1.23	17	42	27	44	28	37	24	4.6
135	AGG325602	VI002802A-BR	SWA	1.37	0.65	0.65	0.27	2.02	0.92	14	39	30	42	31	31	24	2.4

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				Control	WL	Control	WL	Control	WL		Control	WL	Control	WL	Control	WL	
136	AGG325603	VI002859BG	SWA	0.75	0.29	0.29	0.17	1.04	0.47	12	39	24	41	26	34	16	5.3
137	AGG325604	VI002860AG	SWA	0.66	0.35	0.46	0.18	1.12	0.53	8	41	26	42	29	38	25	7.1
138	AGG325605	VI002872BG	SWA	1.24	0.62	0.66	0.26	1.90	0.89	19	38	30	43	32	34	21	5.4
139	AGG325606	VI002877BG	SWA	1.06	0.68	0.38	0.22	1.45	0.90	13	36	27	43	31	36	27	3.9
140	AGG325607	VI002894B-BR	SWA	0.72	0.53	0.31	0.22	1.03	0.76	12	41	25	40	33	38	22	4.2
141	AGG325608	VI002926AG	SA	0.78	0.42	0.34	0.17	1.12	0.59	13	34	31	38	31	33	23	3.5
142	AGG325609	VI002934AG	SA	0.67	0.54	0.35	0.19	1.02	0.72	13	37	28	42	27	35	22	3.7
143	AGG325610	VI002986AG	SA	0.76	0.35	0.32	0.15	1.08	0.50	16	38	27	42	24	32	21	3.2
144	AGG325611	VI002993BG	SA	0.98	0.53	0.36	0.21	1.34	0.74	19	42	27	46	32	33	25	3.4
145	AGG325612	VI002999AG	SA	0.88	0.31	0.26	0.10	1.14	0.41	10	35	24	41	28	33	25	3.7
146	AGG325613	VI003019A-BLM	UK	0.77	0.51	0.35	0.20	1.12	0.71	9	38	26	40	25	26	20	3.4
147	AGG325614	VI003019ABG	UK	0.81	0.48	0.46	0.25	1.27	0.72	15	38	28	43	30	37	23	2.5
148	AGG325615	VI003034BG	SA	0.53	0.34	0.25	0.18	0.79	0.52	9	42	29	41	32	34	28	3.1
149	AGG325616	VI003035AG	SA	0.44	0.38	0.21	0.12	0.66	0.50	13	35	27	40	29	37	24	2.8
150	AGG325617	VI003057BG	SA	0.49	0.25	0.23	0.16	0.73	0.41	9	36	26	39	29	32	24	3.1
151	AGG325618	VI003062BG	SA	0.68	0.39	0.44	0.17	1.11	0.56	9	43	29	41	27	35	22	3.4
152	AGG325619	VI003068A-BR	SA	0.66	0.28	0.32	0.12	0.97	0.41	8	37	26	38	28	32	27	5.0
153	AGG325620	VI003070AG	SA	0.54	0.30	0.23	0.15	0.76	0.45	13	37	27	41	28	33	22	3.1
154	AGG325621	VI003083BG	SA	0.62	0.33	0.30	0.12	0.92	0.45	9	37	31	39	29	33	27	2.6
155	AGG325622	VI003114AG	SA	0.70	0.39	0.23	0.14	0.93	0.52	13	40	33	42	32	33	27	6.5
156	AGG325623	VI003135B-BL	SA	0.35	0.20	0.17	0.07	0.53	0.27	7	35	22	39	30	30	28	7.0
157	AGG325624	VI003159AG	SA	0.56	0.36	0.27	0.16	0.84	0.52	11	39	31	41	31	30	26	3.1
158	AGG325625	VI003172BG	SA	0.57	0.29	0.16	0.12	0.73	0.41	10	40	28	41	28	27	20	3.0
159	AGG325626	VI003181B-GM	SA	0.62	0.47	0.29	0.22	0.91	0.69	12	37	26	40	28	35	21	3.1
160	AGG325627	VI003183AG	SA	0.48	0.29	0.31	0.11	0.79	0.41	10	40	23	42	30	36	20	3.9
161	AGG325628	VI003187BG	SA	0.84	0.54	0.50	0.18	1.34	0.72	14	38	27	40	30	34	26	2.8
162	AGG325629	VI003212B-BLM	SA	0.70	0.36	0.29	0.12	0.99	0.48	8	41	30	42	27	33	28	6.6
163	AGG325630	VI003220AG	SA	0.79	0.58	0.32	0.27	1.12	0.85	14	37	28	41	33	33	28	3.6
164	AGG325631	VI003232AG	SA	0.64	0.44	0.24	0.18	0.88	0.62	9	40	25	41	30	34	23	3.7
165	AGG325632	VI003235AG	SA	0.56	0.36	0.33	0.13	0.89	0.49	10	38	29	40	33	36	26	3.1
166	AGG325633	VI003242AG	SA	0.89	0.52	0.35	0.27	1.24	0.79	14	38	28	39	33	33	27	3.7
167	AGG325634	VI003251A-BL	SA	0.60	0.39	0.25	0.17	0.85	0.56	23	42	26	43	26	34	22	2.9
168	AGG325635	VI003251A-BLM	SA	0.50	0.29	0.21	0.14	0.71	0.42	15	44	27	44	34	35	25	4.2
169	AGG325636	VI003252BG	SA	0.37	0.22	0.13	0.09	0.50	0.32	10	39	30	41	32	33	25	4.1
170	AGG325637	VI003255AG	SA	0.81	0.57	0.37	0.23	1.18	0.81	14	36	27	39	30	34	26	3.4

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				Control	WL	Control	WL	Control	WL		Control	WL	Control	WL	Control	WL	
171	AGG325638	VI003276BG	SA	0.59	0.28	0.22	0.10	0.81	0.38	11	35	27	37	28	34	19	3.1
172	AGG325639	VI003329AG	SA	0.57	0.44	0.27	0.19	0.84	0.63	12	40	30	41	33	35	26	2.1
173	AGG325640	VI003332AG	SA	0.72	0.30	0.32	0.11	1.04	0.41	8	36	30	40	26	34	18	3.8
174	AGG325641	VI003337BG	SA	0.47	0.36	0.19	0.14	0.66	0.49	8	37	28	42	29	33	26	2.9
175	AGG325642	VI003364AG	SA	0.74	0.58	0.36	0.23	1.11	0.82	15	38	23	40	31	37	17	5.2
176	AGG325643	VI003379BG	SA	1.04	0.48	0.43	0.23	1.47	0.71	14	37	26	42	25	35	22	4.8
177	AGG325644	VI003382BG	SA	0.44	0.25	0.26	0.15	0.70	0.40	13	40	26	41	30	34	19	3.4
178	AGG325645	VI003407AG	SA	0.53	0.28	0.31	0.12	0.84	0.40	7	36	25	42	28	31	24	3.5
179	AGG325646	VI003413BG	SA	0.75	0.41	0.33	0.18	1.08	0.59	11	33	26	38	32	31	24	3.6
180	AGG325647	VI003440AG	SA	0.69	0.50	0.21	0.16	0.90	0.65	13	37	26	40	28	37	22	3.7
181	AGG325648	VI003455AG	SA	0.62	0.44	0.31	0.13	0.92	0.57	9	38	26	41	29	34	26	3.4
182	AGG325649	VI003456AG	UK	0.58	0.32	0.30	0.11	0.87	0.43	11	36	25	39	26	36	23	3.0
183	AGG325650	VI003456BG	SA	0.64	0.57	0.41	0.22	1.04	0.79	13	37	24	41	34	34	26	4.2
184	AGG325651	VI003470BG	SA	1.28	0.65	0.58	0.30	1.86	0.95	11	34	26	42	32	33	27	3.0
185	AGG325652	VI003480BG	SA	0.95	0.58	0.49	0.26	1.45	0.84	10	39	36	43	31	31	25	3.2
186	AGG325653	VI003490AG	SA	0.48	0.35	0.25	0.16	0.73	0.51	9	35	29	40	30	35	27	4.1
187	AGG325654	VI003493BG	SA	0.51	0.38	0.24	0.12	0.76	0.50	10	37	29	42	26	32	24	3.2
188	AGG325655	VI003514BG	SA	0.70	0.24	0.40	0.14	1.09	0.38	10	40	22	41	30	35	24	3.6
189	AGG325656	VI003517BG	SA	0.65	0.35	0.46	0.14	1.12	0.49	10	35	25	39	29	32	27	3.2
190	AGG325657	VI003534AG	SA	0.74	0.45	0.26	0.17	1.00	0.62	11	35	31	41	31	37	24	3.1
191	AGG325658	VI003534BG	SA	1.04	0.44	0.44	0.18	1.48	0.62	11	42	27	43	23	35	22	3.7
192	AGG325659	VI003548AG	SA	0.48	0.23	0.26	0.07	0.75	0.30	7	33	26	40	28	32	22	2.7
193	AGG325660	VI003554AG	SA	0.63	0.22	0.33	0.12	0.96	0.34	8	33	26	41	25	36	18	3.3
194	AGG325662	VI003563A-BR	SA	0.65	0.38	0.31	0.12	0.96	0.49	12	37	24	42	28	35	24	2.9
195	AGG325663	VI003557AG	SA	0.49	0.32	0.27	0.16	0.76	0.49	7	40	23	41	29	37	24	3.3
196	AGG325664	VI003602AG	SA	0.57	0.32	0.23	0.11	0.80	0.42	8	38	26	41	25	37	20	2.8
197	AGG325665	VI003642AG	SA	0.53	0.30	0.28	0.14	0.81	0.44	7	36	28	42	28	34	24	5.4
198	AGG325666	VI003648BG	SA	0.66	0.37	0.34	0.15	1.00	0.53	15	37	28	44	28	33	21	3.0
199	AGG325667	VI003658BG	SA	0.72	0.37	0.24	0.13	0.95	0.50	7	40	29	43	28	31	21	3.0
200	AGG325668	VI003664AG	SA	0.61	0.35	0.24	0.14	0.85	0.48	4	34	26	37	28	33	17	4.9
201	AGG325669	VI003678BG	SA	0.66	0.27	0.32	0.15	0.97	0.42	11	39	27	43	28	34	17	3.6
202	AGG325670	VI003685AG	SA	0.57	0.29	0.25	0.13	0.83	0.42	8	37	26	39	26	29	23	3.8
203	AGG325671	VI003699BG	SA	0.22	0.15	0.16	0.09	0.38	0.24	9	41	30	40	33	35	23	4.5
204	AGG325672	VI003720BG	SA	0.59	0.35	0.25	0.12	0.84	0.47	8	37	30	40	32	38	23	4.4
205	AGG325673	VI003726BG	SA	1.25	0.64	0.60	0.26	1.85	0.90	17	42	23	41	34	35	22	3.4

Sr. No.	Accession Number	VI_number	Origin	Seedling stage													
				Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)		No. adventitious roots	SPAD_1		SPAD_2		SPAD_3		100-seed weight (g)
				Control	WL	Control	WL	Control	WL		Control	WL	Control	WL	Control	WL	
206	AGG325674	VI003733BG	SA	1.02	0.49	0.51	0.28	1.53	0.77	19	40	27	42	34	38	22	4.7
207	AGG325675	VI003734B-BR	SA	0.66	0.31	0.47	0.15	1.13	0.46	13	38	17	38	27	32	17	3.1
208	AGG325676	VI003734B-DG	SA	0.96	0.55	0.55	0.32	1.50	0.87	16	40	16	40	34	35	21	3.3
209	AGG325677	VI003744AG	SA	1.08	0.46	0.57	0.20	1.65	0.66	16	38	23	42	30	34	16	4.0
210	AGG325678	VI003755BG	SA	0.67	0.50	0.31	0.23	0.98	0.73	15	40	17	39	31	35	25	5.7
211	AGG325679	VI003760BG	SA	1.11	0.39	0.53	0.22	1.65	0.61	16	40	24	41	32	32	25	3.1
212	AGG325680	VI003785BG	SA	0.50	0.34	0.25	0.14	0.75	0.48	13	40	28	41	29	37	17	3.2
213	AGG325681	VI003795AG	SA	0.72	0.49	0.38	0.26	1.10	0.76	11	41	22	35	27	30	18	3.1
214	AGG325682	VI003801BG	SA	0.50	0.37	0.30	0.20	0.80	0.57	11	32	25	38	35	31	23	3.4
215	AGG325683	VI003882A-BLM	SWA	1.32	0.42	0.69	0.29	2.02	0.71	19	34	21	42	30	32	26	3.0
216	AGG325684	VI003886 B-BR	SA	1.77	0.71	1.07	0.39	2.84	1.10	16	37	28	42	29	37	23	4.0
217	AGG325685	VI003886 BY	SA	0.91	0.54	0.45	0.25	1.35	0.80	12	38	26	41	28	32	24	3.2
218	AGG325686	VI003893AG	SA	1.05	0.65	0.56	0.28	1.60	0.93	19	36	24	37	27	33	19	3.5
219	AGG325687	VI003894 B-BLM	SA	0.66	0.32	0.28	0.21	0.93	0.52	12	38	26	43	23	38	19	3.4
220	AGG325688	VI003907AG	SWA	0.92	0.34	0.43	0.17	1.35	0.51	10	39	20	43	26	30	19	6.8
221	AGG325689	VI003914AG	SA	1.22	0.48	0.48	0.22	1.70	0.70	13	38	24	39	33	37	24	4.3
222	AGG325690	VI003925 B-BLM	SA	0.77	0.46	0.47	0.24	1.24	0.70	11	38	20	41	32	30	21	2.8
223	AGG325691	VI003927AG	SA	0.61	0.47	0.32	0.25	0.93	0.73	16	37	28	38	32	34	25	3.5
224	AGG325692	VI003929A-BL	SA	0.77	0.39	0.45	0.19	1.22	0.58	14	39	23	43	28	33	24	3.6
225	AGG325693	VI003942AG	SWA	0.72	0.40	0.52	0.23	1.24	0.63	13	40	25	42	32	37	24	3.9
226	AGG325694	VI003944 B-BR	SWA	0.97	0.55	0.59	0.31	1.56	0.86	18	34	25	43	28	36	26	3.6
227	AGG325695	VI003947 B-BR	SA	1.18	0.72	0.73	0.46	1.91	1.17	15	38	22	42	29	38	20	3.7
228	AGG325696	VI003948 B-BR	SA	0.80	0.43	0.38	0.26	1.18	0.69	11	38	26	40	29	38	21	3.5
229	AGG325697	VI003951AG	SA	1.09	0.58	0.66	0.29	1.75	0.88	14	38	24	41	31	37	26	4.0
230	AGG325698	VI003954BG	SA	0.72	0.62	0.38	0.35	1.10	0.97	20	38	23	40	28	32	21	3.4
231	AGG325699	VI003957AG	SA	1.18	0.51	0.65	0.33	1.83	0.83	15	42	21	43	30	38	23	3.1
232	AGG325700	VI003958 B-BLM	SA	0.84	0.40	0.48	0.15	1.32	0.55	10	38	27	42	31	37	10	2.5
233	AGG325701	VI003959 BG	SA	0.79	0.37	0.45	0.19	1.23	0.56	19	38	26	45	36	38	29	4.2
234	AGG325702	VI004006 A-GM	SA	0.52	0.41	0.28	0.22	0.79	0.64	16	37	18	39	28	30	19	3.4
235	AGG325703	VI004010 AG	SA	1.10	0.46	0.55	0.25	1.65	0.71	15	37	25	43	34	34	25	2.7
236	AGG325704	VI004024 AG	OP	0.86	0.71	0.47	0.36	1.32	1.07	17	38	20	43	30	34	22	5.4
237	AGG325705	VI004044 BG	SA	1.03	0.47	0.49	0.23	1.52	0.70	12	36	24	39	28	34	24	4.0
238	AGG325706	VI004045 A-DGM	SA	1.08	0.41	0.67	0.21	1.75	0.62	17	37	25	41	34	38	27	4.0
239	AGG325707	VI004048 A-DGM	SA	0.97	0.60	0.53	0.29	1.51	0.89	13	40	22	44	30	39	23	3.7
240	AGG325708	VI004069BG	SA	0.70	0.38	0.27	0.14	0.97	0.52	12	37	29	43	30	36	22	3.6

Sr. No.	Accession Number	VI_number	Origin	Seedling stage													
				Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)		No. adventitious roots	SPAD_1		SPAD_2		SPAD_3		100-seed weight (g)
				Control	WL	Control	WL	Control	WL		Control	WL	Control	WL	Control	WL	
241	AGG325709	VI004096 AG	SA	0.95	0.32	0.49	0.20	1.44	0.52	11	36	24	41	32	37	20	3.4
242	AGG325710	VI004096 BG	SA	0.79	0.35	0.32	0.15	1.12	0.50	13	35	18	42	32	38	27	3.0
243	AGG325711	VI004129 A-BLM	UK	0.71	0.30	0.37	0.14	1.07	0.44	15	38	22	43	30	38	29	3.1
244	AGG325712	VI004138 BG	SA	0.44	0.43	0.32	0.20	0.76	0.63	10	39	25	41	27	35	22	2.8
245	AGG325713	VI004145 B-BLM	SWA	1.02	0.38	0.60	0.17	1.61	0.55	14	39	23	43	26	34	21	3.3
246	AGG325714	VI004184 AG	EUR	0.73	0.39	0.45	0.26	1.18	0.65	14	38	23	42	31	37	21	3.1
247	AGG325715	VI004243 B-BR	SWA	0.58	0.32	0.39	0.18	0.97	0.51	14	35	23	38	28	32	22	3.8
248	AGG325716	VI004244 B-BR	SA	1.11	0.47	0.64	0.26	1.75	0.73	10	37	27	42	29	37	23	3.0
249	AGG325717	VI004297 AG	SWA	1.03	0.52	0.62	0.32	1.65	0.85	9	36	26	42	30	38	19	3.9
250	AGG325718	VI004302 AG	SWA	0.98	0.38	0.54	0.23	1.53	0.60	22	37	25	43	32	39	24	2.9
251	AGG325719	VI004307 AG	SWA	0.91	0.28	0.62	0.19	1.52	0.46	16	36	25	42	31	34	20	4.2
252	AGG325720	VI004312 AG	SA	0.75	0.28	0.53	0.18	1.28	0.46	9	38	25	42	27	35	19	3.2
253	AGG325721	VI004347 B-BLM	SA	1.44	0.48	0.81	0.30	2.25	0.78	14	40	24	40	27	37	18	3.8
254	AGG325722	VI004351 AG	SA	1.24	0.34	0.65	0.15	1.90	0.49	12	38	25	41	30	38	20	3.4
255	AGG325723	VI004423 AG	SWA	0.93	0.38	0.67	0.22	1.60	0.60	14	38	30	42	32	38	26	4.0
256	AGG325724	VI004432 B-BR	SWA	0.67	0.33	0.44	0.21	1.11	0.54	11	36	23	40	27	35	20	3.4
257	AGG325725	VI004480 AG	SWA	0.74	0.40	0.55	0.26	1.29	0.66	11	36	25	41	25	36	22	5.3
258	AGG325726	VI004639 AG	SWA	0.98	0.48	0.80	0.34	1.78	0.82	21	39	26	44	27	33	23	3.3
259	AGG325727	VI004666 AG	SWA	0.73	0.24	0.49	0.15	1.23	0.38	12	37	25	41	28	32	28	5.3
260	AGG325728	VI004691 AG	SWA	0.84	0.48	0.60	0.33	1.44	0.82	15	37	24	39	25	36	28	2.9
261	AGG325729	VI004694 BG	SWA	0.87	0.53	0.62	0.30	1.49	0.83	16	36	28	39	34	34	28	3.2
262	AGG325730	VI004710 AG	SWA	0.61	0.54	0.37	0.32	0.98	0.86	9	39	26	40	29	35	23	3.8
263	AGG325731	VI004734 AG	SWA	0.94	0.50	0.48	0.25	1.41	0.75	15	38	21	42	28	35	24	3.5
264	AGG325732	VI004743 AG	SA	1.08	0.99	0.72	0.51	1.80	1.50	13	36	25	42	32	34	22	2.7
265	AGG325733	VI004789	SA	1.12	0.60	0.59	0.25	1.71	0.85	23	40	22	39	30	34	22	2.6
266	AGG325734	VI004810 BG	SA	1.62	0.81	0.89	0.45	2.51	1.26	19	42	26	45	35	32	24	3.1
267	AGG325735	VI004811 BG	SA	1.01	0.43	0.41	0.21	1.42	0.64	15	36	25	44	33	38	28	3.1
268	AGG325736	VI004822 BG	SA	0.80	0.43	0.43	0.25	1.23	0.68	16	41	28	38	31	36	28	2.9
269	AGG325737	VI004838 AG	SA	0.69	0.28	0.41	0.16	1.10	0.44	11	38	23	43	31	37	23	4.0
270	AGG325738	VI004842 AG	SA	0.60	0.57	0.29	0.27	0.89	0.84	18	33	22	40	29	35	25	7.5
271	AGG325739	VI004853 BG	SA	0.81	0.34	0.55	0.18	1.36	0.53	14	37	26	41	30	36	20	3.4
272	AGG325740	VI004871 BG	SA	0.72	0.45	0.26	0.19	0.98	0.64	12	36	24	44	32	37	23	6.9
273	AGG325741	VI004877 AG	SA	0.67	0.39	0.40	0.19	1.07	0.59	15	35	22	41	35	33	19	3.3
274	AGG325742	VI004915 BG	SA	0.91	0.38	0.39	0.20	1.29	0.57	19	41	26	43	32	36	22	3.8
275	AGG325743	VI004931 AG	SWA	0.99	0.60	0.50	0.25	1.50	0.84	16	38	15	40	29	36	23	4.0

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				Shoot dry mass (g)		Root dry mass (g)		Total dry mass (g)		No. adventitious roots	SPAD_1		SPAD_2		SPAD_3		100-seed weight (g)
				Control	WL	Control	WL	Control	WL		Control	WL	Control	WL	Control	WL	
276	AGG325744	VI004933 AG	SWA	0.92	0.47	0.44	0.19	1.35	0.65	9	38	24	42	33	35	11	3.3
277	AGG325745	VI004934 AG	SWA	0.65	0.45	0.25	0.20	0.90	0.65	15	38	21	42	32	37	20	3.0
278	AGG325746	VI004937 AG	SWA	1.01	0.47	0.45	0.23	1.46	0.70	12	40	20	42	29	30	27	3.7
279	AGG325747	VI004942 BG	SWA	0.75	0.42	0.44	0.19	1.19	0.61	12	38	20	40	27	35	27	3.8
280	AGG325748	VI004954BG	SWA	1.17	0.72	0.62	0.26	1.78	0.99	19	38	27	45	31	31	21	3.4
281	AGG325749	VI004956 AG	SWA	1.33	0.65	0.72	0.22	2.05	0.87	20	40	22	44	22	38	18	3.4
282	AGG325750	VI004957 AG	SWA	0.44	0.38	0.20	0.18	0.64	0.56	14	35	22	41	31	32	25	3.4
283	AGG325751	VI004958 BG	SWA	0.82	0.37	0.46	0.25	1.28	0.62	18	38	29	42	35	39	24	3.4
284	AGG325752	VI004965 BG	SWA	0.66	0.49	0.37	0.25	1.03	0.74	14	37	26	40	32	32	24	2.5
285	AGG325753	VI004968 AG	SWA	1.12	0.49	0.48	0.29	1.60	0.79	18	40	26	42	37	36	27	3.3
286	AGG325754	VI004969 AG	SWA	0.78	0.48	0.35	0.26	1.13	0.74	12	38	16	41	33	32	25	3.3
287	AGG325755	VI004973 B-BLM	SA	0.53	0.43	0.31	0.19	0.83	0.63	16	40	18	44	29	38	24	3.4
288	AGG325756	VI005022 BG	SA	0.76	0.48	0.40	0.27	1.17	0.75	16	40	27	42	34	40	23	3.6
289	AGG325758	VI005030 BY	MA	1.57	0.91	0.86	0.59	2.43	1.50	15	40	23	36	32	31	22	3.2
290	AGG325759	VI005066 A-GM	UK	0.95	0.52	0.56	0.30	1.51	0.82	18	40	24	37	28	34	21	3.3
291	AGG325760	VI005041 AG	SA	1.01	0.57	0.53	0.27	1.54	0.84	16	36	28	37	32	33	25	3.3
292	AGG325761	VI014178 BG	AFR	1.52	0.94	0.83	0.42	2.35	1.36	16	39	25	42	30	37	22	3.8