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Article

Evaluation of Water Use Efficiency in Mungbean using the Inverted-Bottle Pot System

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ABSTRACT

Mungbean (*Vigna radiata* (L.) Wilczek) is a short-duration legume crop that is valuable for crop rotation. However, its yield potential is often limited by the water availability. Improving water use efficiency (WUE) in mungbean could increase mungbean production in water-limiting areas. Identifying genetic variability in mungbean for WUE is the first step to improving WUE and requires a fast and reliable screening method. This study evaluated twelve mungbean genotypes for WUE using Hunter's inverted water bottle pot system (IBP) on two potting media (a potting mix of composting pine bark [UQ23] and Gatton vertosol soil). Morphology, agronomy, and physiology traits were measured and recorded, including six WUE traits. These WUE traits comprised a combination of two types of WUE (above-ground dry matter [WUEbio] and seed weight [WUEyield]) and three water consumption (total, before flowering, and after flowering [post]). Despite the difference in magnitude where plants in UQ23 used more water than in soil, the pattern of weekly water consumption was similar between these two potting media. The difference in water consumption among genotypes was observed after 46 DAS, and the peak water consumption occurred around flowering time (around 60 DAS). The variability due to the genotype-by-media interaction was very small (<10%) for most traits. The total and after-flowering WUE had large genotypic variance (>50%), similar heritability (0.6 for WUEbio and 0.8 for WUEyield), and strongly correlated ($r > 0.95, p < 0.001$). The ranking of genotypes based on total WUE and after-flowering WUE was reasonably similar across the two potting media. However, the ranking based on WUEyield could differ from the ones based on WUEbio. Top genotypes in WUEyield (e.g., Berken) were only ranked in the middle in WUEbio, while top genotypes in WUEbio (e.g., King) were ranked lower in WUEyield. These results indicated that the size of the plants does not always correspond to seed weight. Therefore, WUEyield would be a better target trait than WUEbio to improve yield in mungbean. This study demonstrated

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the use of the IBP system to detect genotypic variability in WUE among mungbean genotypes under non-limiting water conditions for screening after-flowering WUE in mungbean.

KEYWORDS: water use efficiency (WUE); mungbean; inverted-bottle pot system; crop breeding; screening

ABBREVIATIONS

WUE, water use efficiency; IBP, inverted water bottle pot; DAS, days after sowing

INTRODUCTION

Global climate change and increasing competition for freshwater make it harder for agriculture to get enough water [1]. Agricultural production needs to increase productivity and reduce water consumption [2,3]. This can be partly achieved by short-season crops that use water more efficiently. Mungbean (*Vigna radiata* (L.) Wilczek) is an important food legume crop that can be targeted for improvement in water use efficiency [4]. Mungbean is recognised for its dual benefits, serving as a cost-effective, high-protein option while carrying significant ecological and financial advantages [5]. Incorporating mungbean into crop rotations has been reported to benefit the environment by enhancing soil fertility through nitrogen fixation in a symbiotic relationship [6,7]. Therefore, it supports more sustainable agricultural production by boosting the yield of subsequent crops [8]. In a rice-wheat cropping system in India, planting mungbean and using residues for green fertilisation not only significantly increased the yield of the rice-wheat cropping system 0.5–1.3 t/ha/y compared to systems without a summer crop but also greatly improved the crop's absorption of nitrogen sources [9]. Because in such systems, the yield of mungbean is often constrained by limited water availability, improving water use efficiency (WUE) is a critical research priority. WUE, defined as the ratio of biomass produced to the amount of water used, is one of the key traits for enhancing agricultural productivity under limited water supply [10]. Improving WUE in mungbean not only supports the sustainability of crop production but also addresses broader environmental concerns by reducing the water footprint of agriculture [11].

However, the complexity of WUE studies makes it difficult to screen many genotypes. Environmental factors such as temperature, humidity, soil moisture, and light intensity can significantly influence plant water uptake and transpiration rates, making it challenging to assess WUE accurately under varying conditions [12]. Plants also exhibit dynamic responses to changing environmental conditions, adjusting their water use strategies through stomatal regulation and root morphology changes [12]. These physiological adaptations complicate consistently ranking

genotypes for water use efficiency (WUE), as the rankings may vary throughout the plant's growth cycle and in response to different environmental conditions. The challenge lies in accurately measuring a plant's water use at any given time and ensuring that these genotype rankings remain reliable across diverse environments. This variability underscores the need for robust methodologies that can reliably compare WUE among genotypes under fluctuating conditions, facilitating the selection of genotypes that consistently perform well in water efficiency [13].

Several methods have been developed to measure water use at the whole plant level, each with advantages and limitations. One commonly used technique is the lysimeter, which directly measures water fluxes in and out of a defined soil volume containing the plant root system [14]. Lysimeters provide valuable insights into plant water uptake dynamics and can help quantify WUE under controlled conditions [15]. Continuous weighing platforms allow for non-invasive monitoring of changes in weight over time, facilitating the calculation of whole-plant water use [12]. Hydrological models, such as the FAO Penman-Monteith equation or the SWAP (Soil-Water-Atmosphere-Plant) model, integrate data on plant physiology, soil properties, and climate to estimate whole-plant water use based on environmental factors [16]. Tracer techniques involving isotopically labelled water enable the tracing of water movement within plants, offering insights into whole-plant water use dynamics [17]. The stem heat balance method also allows for estimating whole-plant water use by measuring heat fluxes within the plant stem [18]. These methods collectively provide researchers with various tools to assess water use and its response to environmental conditions. However, these experimental materials are relatively complex and costly. Therefore, we need a simpler and more economical method to compare WUE of mungbean.

An inverted water-bottle pot (IBP) system, proposed by Hunter [19], provides an economical approach to measuring water use. This system was initially designed for zinc deficiency studies in soybeans but may also be used to measure individual plant water consumption. This system and modifications have proved to be a low-cost and easily scalable research tool for screening many genotypes [19,20].

Many species have grown satisfactorily in this closed-pot bottom watering system. They include: soybean (*Glycine max*), sunflower (*Helianthus annuus*), sorghum (*Sorghum bicolor*), cotton (*Gossypium hirsutum*), wheat (*Triticum aestivum*), citrus (*Citrus* sp.), coffee (*Coffea arabica*), and marigold (*Tagetes patula*) [21], guar (*Cyamopsis tetragonoloba*) [22], banana (*Musa* sp.) [23], capicum (*Capsicum annuum*), sweet corn (*Zea mays*) and tomato (*Lycopersicon esculentum*) [20], and lesser yam (*Dioscorea esculenta*) [24]. However, this system has yet to be used for mungbean, which is sensitive to waterlogging [25]. Thus, this study aims to use Hunter's system to evaluate the WUE for several mungbean genotypes and to optimise it for WUE screening.

MATERIALS AND METHODS

Inverted-Bottle Pot System (IBP) Setup

The IBP system (Figure 1, Supplementary Figure S1) was modified based on the method described by Hunter [19]. Initially, a plastic sheet was positioned at the base of a 4L ANOVApot®, followed by a moistened capillary mat to facilitate consistent moisture distribution (Figure 1A). A plastic mesh was inserted into the central hole to touch the marble in the water bottle so the water could flow out steadily. A central PVC pipe measuring 225 mm in height and 89 mm in outer diameter was placed at the centre of the pot to separate the water bottle from the potting media. A 1 L bottle, capped and modified with a central 15 mm hole containing a 16 mm marble (Figure 1B), was filled with water and inverted within the central pipe to maintain a continuous water supply. To minimise water evaporation from the potting media, the pots were covered with a silver reflective insulation sheet, which featured a central hole for the pipe and a smaller hole for plant growth. Although evaporation from the central pipe is possible, this setup is standardised across all pots to ensure uniformity in the experimental conditions. Additionally, the pots were wrapped with silver reflective insulation to protect the plants from sunlight-induced heat stress.

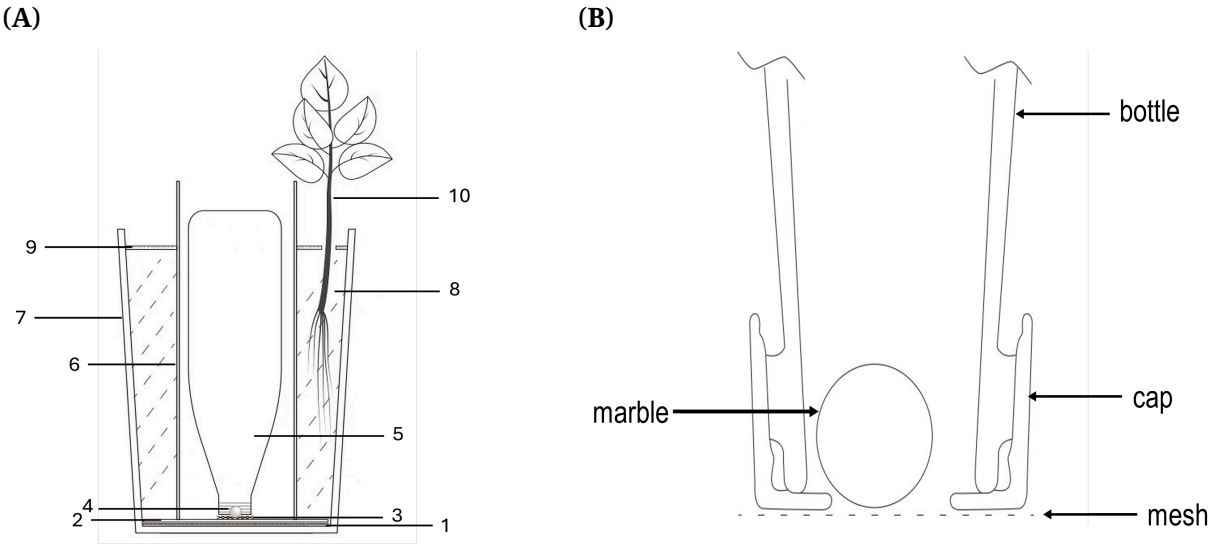

Figure 1. Construction of inverted bottle system (IBP) (**A**) The complete system: (1) Plastic sheet; (2) Capillary mat; (3) Plastic grid (10 mm × 10 mm); (4) marble (16 mm); (5) 1L bottle with cap (40 mm diameter) with a central hole (15mm); (6) Central pipe (225 mm × 89 mm outside diameter (OD)); (7) 4 L ANOVApot®; (8) Potting media; (9) A cover using silver light duty (LD) reflective insulation with a central and plant hole; (10) A plant. (**B**) The detail of the cap and marble valve in the open position, with the bottle reservoir resting on nylon mesh.

Experimental Design

An experiment was conducted between August and November 2023 in an evapotranspiration glasshouse at The University of Queensland St

Lucia campus in Brisbane (27.4913°S 153.0033°E). This experiment evaluated the WUE of 12 mungbean genotypes (Table 1). Opal-AU, Crystal, Jade-AU, Celera II-AU, Satin II, and Berken are popular mungbean varieties in Australia and have been used in other studies (e.g., [25–29]). Green Dragon is the latest addition to Australian mungbean varieties. King, Barimung 5, Chaeraejeong 8, and Yellow mungbean are varieties from the Australia Grain Genebank collection, and Accession 54 (Acc54) is the wild mungbean (*V. radiata* ssp. *sublobata*). Acc54 has wild-type traits like prostrate habit, twinning form and small hard seeds, with a short-growth cycle, high yield and high harvest index [30]. Acc54 was included in this set as it has been successfully crossed to Crystal and Jade and may be of value in improving mungbean varieties. This set of genotypes was selected to provide coverage of the key traits (Table 1).

Table 1. The twelve mungbean genotypes, country of origin, pedigree (if known), and key features. The term in bracket is the abbreviation used in some of the figures.

Genotype	Country	Pedigree	Key Features
Jade-AU (Jade)	Australia	3511-9/VC2768A	• Medium maturation • High yield potential • Large and shiny seeds • Moderate susceptibility to Powdery Mildew and Tan Spot
Crystal (Cry)	Australia	White Gold/ VC1628A/Emerald	• Medium maturation • Large and shiny seeds • Consistent performance across all regions • Moderate susceptibility to Powdery Mildew and Halo Blight • Moderate resistance to Tan Spot
Berken (Ber)	Australia	Selection from Jumbo Mung and Oklahoma; no documented pedigree history	• Medium-early maturation • Low yield potential • Large and shiny seeds • Susceptibility to Powdery Mildew, Halo Blight and Tan Spot
Opal-AU (Opal)	Australia	M07032/AGG321818 MUNG	• Medium maturation • High yield potential (12% higher yielding than Jade-AU) • Large and shiny seeds • Moderate resistance to Powdery Mildew and Halo Blight • Moderate susceptibility to Tan Spot
Satin II (Sa)	Australia	White Gold/Deha	• Medium maturation • Large and dull seeds • Moderate susceptibility to Powdery Mildew, Halo Blight and Tan Spot

Table 1. *Cont*.

Genotype	Country	Pedigree	Key Features
Celera II-AU (Cel)	Australia	M773/OAEM58-62	• Early maturation • Small and shiny seeds • Moderate resistance to Halo Blight • Moderate susceptibility to Powdery Mildew and Tan Spot
Green Dragon (Green)	Australia	Unknown	• High yield potential • Large and shiny seeds • Resistance to Powdery Mildew, Halo Blight and Tan Spot • Indeterminate
King	Australia	EG-MG-7	• High yield potential • Large seeds • Resistance to Powdery Mildew in Australia • Susceptible to Powdery Mildew and Cercospora Leaf Spot in Thailand
Yellow Mungbean (Yel)	Unknown	Unknown	• Yellow seeds
Chaeraejong 8 (Cha)	Korea, South	Unknown	• Dark green seeds • Ovate terminal leaflets • Low susceptibility to shattering
Barimung 5 (Bar)	Bangladesh	From the NM-92 line	• High yield • Photo-insensitive mungbean variety • Tolerant to Mungbean Yellow Mosaic Virus (MYMV)
Accession 54 (Acc54)	Madagascar	Wild accession (*V. radiata* ssp. *sublobata*)	• Small seeds • Short duration • High yield • High harvest index • Susceptibility to shattering

The two potting media were a composted pine bark (70%) + coco peat (30%) fertilised with 2 g/L Osmocote and 1g/L Dolomite (potting mix-UQ23) and Grey Vertosol soil (soil) from the University of Queensland's Gatton research field. A soil test was conducted (Supplementary Table S1), and no additional supplement was added. Each pot was filled with 1400 g media for the potting mix treatment. While for the soil treatment, each pot was filled with 2500 g air-dried soil.

The experiment was arranged as a resolvable row-column design with 8 rows and 15 columns across five replications. Water was added to the bottle before planting to initiate the initial water table. Seeds were treated with the fungicide Thiram and inoculated with Nodule N^{TM} Legume Inoculant for mungbean (Strain CB1015, 0.1 g inoculant per 4 g seeds).

Three seeds were planted in each pot and then gradually thinned to a single plant 18 days after sowing (DAS). Weekly water consumption was measured by calculating the difference in the pot weight before and after filling the water bottle. Water bottles were filled up to four times weekly to ensure they were never empty.

Morphology, agronomy, and physiological traits were recorded from this experiment (Table 1). The statistical analysis included 16 main traits (Table 2A), while others were intermediary traits (Table 2B). The main traits (Table 2A) were selected as they were traits of interest for water use efficiency and included in the statistics analysis. These main traits include six WUE traits, which are a combination of the two types of WUE, i.e., based on total above-ground dry matter and seed weight, and three estimates of water consumption, i.e., pre-flowering, after-flowering, and total water consumption (Table 2A).

Table 2. This list of traits includes abbreviations used (in brackets, if any), units, and descriptions.

Trait	Unit	Description
(A) The main traits included in the statistical analysis		
Plant height (Height)	cm	Measured with a ruler at harvest from the base of the media line to the tip of the tallest stem.
Leaf area (LeafArea)	cm^2	Measured total leaf area (collected all leaves) using LI-3100C Area Meter at harvest.
Above-ground dry matter (ShootDW)	g	The leaves, stems, and pods were harvested and kept in separate brown paper bags. Leaves and stems were dried at 65 ℃ and pods at 35 ℃ for over 48 hours, then weighed using a digital electric scale. Above-ground dry matter was calculated as the dry weight of leaves, stems and pods.
Seed weight (SeedWt)	g	The weight of dried seed from each plant was taken using a digital electric weighing scale.
Harvest Index (HarvestIndex)	-	A ratio of seed weight and above-ground dry matter.
Total water consumption (WUtotal)	g	Weekly water consumption from 18 days after sowing (DAS) to harvest time.
Water consumption before flowering (WUpre)	g	Cumulative weekly water consumption from 18 DAS to the week of the first flower.
Water consumption after flowering (WUpost)	g	Cumulative weekly water consumption the week of the first flower to harvest time.
Stomatal conductance before flowering (SC44)	$mmol/m^2/s$	It was measured in the morning on day 44 DAS by the Li-COR 600 Porometer. Measure the newest fully expanded leaf in the plant.
Stomatal conductance after flowering (SC53)	$mmol/m^2/s$	It was measured in the morning on day 53 DAS the Li-COR 600 Porometer. Measure the newest fully expanded leaf in the plant.

Table 2. *Cont*.

Trait	Unit	Description
(A) The main traits included in the statistical analysis		
WUE-yield (WUEyield_total)	g/kg	A ratio of seed weight and total water consumption.
WUE-yield before flowering (WUEyield_pre)	g/kg	A ratio of seed weight and total water consumption before flowering.
WUE-yield after flowering (WUEyield_post)	g/kg	A ratio of seed weight and total water consumption after flowering.
WUE-biomass (WUEbio_total)	g/kg	A ratio of above-ground dry matter and total water consumption.
WUE-biomass before flowering (WUEbio_pre)	g/kg	A ratio of above-ground dry matter and total water consumption before flowering.
WUE-biomass after flowering (WUEbio_post)	g/kg	A ratio of above-ground dry matter and total water consumption after flowering.
(B) Additional traits not included in the statistical analysis		
Branch number	-	The number of primary branches counted at harvest.
Leaf number	-	The number of full trifoliate leaves counted at harvest.
Days of flower	DAS	Number of days from sowing to the first flower buds.
Days of pod	DAS	Number of days from sowing to the first pod.
Days of maturity	DAS	Number of days when 80% of pods are black.
Leaf fresh weight	g	Fresh weight of leaves taken on harvest day.
Stem fresh weight	g	Fresh weight of stem taken on harvest day.
Pod fresh weight	g	Fresh weight of pods taken on harvest day.
Pod number	-	The number of total pods (black and green pods) per plant counted at harvest maturity.
Pod length	cm	Length from the point of pod attachment to the tip of the pod taken after harvest. Ten pods were randomly selected from each plant and averaged.
Seed number	-	The number of total seeds taken using a SeedCount SC6000R.
1000-seed weight	g	The weight of 1000-seed is estimated using a SeedCount SC6000R.

Statistical Analysis

The data was analysed using the mixed model implemented in ASREML-R [31]. The analysis was done for each trait (Table 2) using the following model:

$$y_{i(mn)jk} = \mu + B_i + R_m|B_i + C_n|B_i + P_j + G_k + (PG)_{jk} + \varepsilon_{i(mn)jk} \quad (1)$$

where $y_{i(mn)jk}$ is the observation for row m ($m = 1, \ldots, 8$) and column n ($n = 1, .., 3$) in replication i ($i = 1, \ldots, 5$) for genotype k ($k = 1, \ldots, 12$), and potting media j ($j = 1, 2$); μ is the grand mean; B_i is the fixed effect of replication i; $R_m|B_i$ is the fixed effect of row m within replication i; $C_n|B_i$ is the fixed effect of the column n within replication i; P_j is the random effect of potting media j; G_k is the random effect of genotype i, and $\varepsilon_{i(mn)jk}$ is the random effect of residual. Heritability for each trait is calculated using the following formula:

$$H^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \hat{\sigma}_{gm}^2 + \hat{\sigma}_\varepsilon^2} \quad (2)$$

where $\hat{\sigma}_g^2$ is the estimated genotypic variance, $\hat{\sigma}_{gm}^2$ is the estimated variance for genotype-by-potting media interaction and $\hat{\sigma}_\varepsilon^2$ is the estimated residual variance.

Predicted values (BLUP, best linear unbiased prediction) were calculated for each treatment (i.e., genotype × potting media combination) for each trait, resulting in a two-way, two-mode table of treatment-by-trait. This table was used to calculate Pearson's correlation matrix among traits and summarise using pattern analysis.

Pattern analysis, i.e., a joint use of clustering and ordination techniques [32], was applied to this two-way table after the column was standardised. Hierarchical cluster analysis using the average Squared Euclidean Distance (SED) as the dissimilarity measure and Ward's method as the clustering strategy [33]. Clustering was performed for both treatments and traits. Ordination was performed using Singular Value Decomposition. The cluster analysis results were displayed using an optimised dendrogram [34], and the ordination results were displayed using biplots [35].

RESULTS

Weekly Water Consumption

The pattern of weekly water consumption was similar between the two potting media (Figure 2), although differing in magnitude. Water consumption increased after the initiation of flowering (53–60 DAS) and decreased at maturity (Figure 2). The weekly water consumption was higher in the potting mix UQ23 than in the soil. The water content in the soil was initially higher than the potting mix UQ23 (742 g and 523 g, respectively). As mungbean is averse to waterlogging, the higher initial water content in the soil impended the initial plant growth, resulting in smaller plants than those in the potting mix; hence, they used less water

(Figure 2). While the overall pattern was the same between the two media, there were different genotype responses (Figure 2), especially from 46–53 DAS onwards.

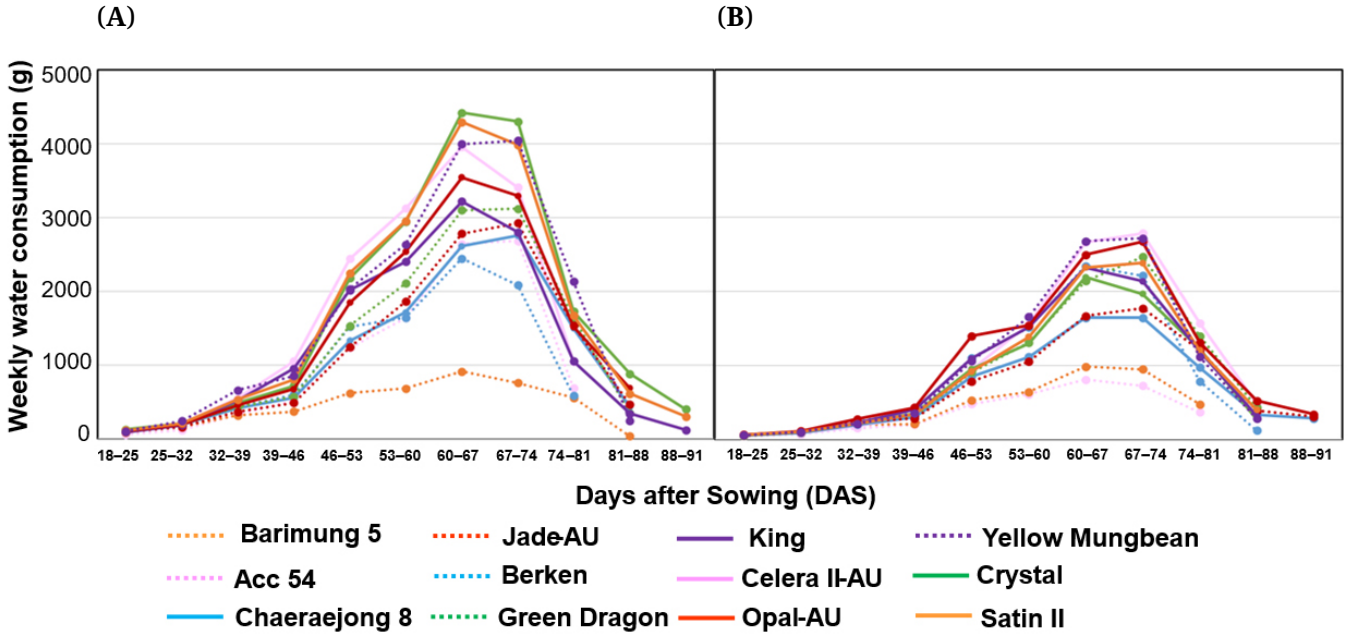

Figure 2. Weekly water consumption of the twelve mungbean genotypes (**A**) in potting mix UQ23 (70% composted pine bark + 30% coco peat with 2 g/L Osmocote and 1 g/L Dolomite) and (**B**) in Gatton Vertosol soil.

There was also variation in maturity among these genotypes, with the soil tend to have longer maturity time (Figure 2). The delay in flowering and maturity could be due to the low night temperature (<17 ℃) in the first few weeks after planting. This low night temperature slowed the initial growth, but normal growth resumed when the average night temperature rose above 17 ℃.

Cumulative water consumption increased linearly from 46–53 DAS to 81–88 DAS (Figure 3). UQ23 showed a higher water consumption rate (i.e., larger slope) than soil. This was expected as plants in UQ23 tend to be bigger than the ones in the soil. Except for Berken and Barimung 5, there were larger differences in water consumption between soil and UQ23 after the week of 60–67 DAS, where most genotypes started to flower.

While the linear lines for soil and UQ23 were not always parallel, the lack of crossover (Figure 3) indicates no interaction between the two potting media during this period. The slopes were varied among genotypes, indicating differences in the water consumption rate among the genotypes (Figure 3). The slope variation among genotypes was observed in both potting media, but more pronounced UQ23. Crystal and Satin II had the highest water consumption rate, while Barimung 5 and Berken had the lowest (Figure 3).

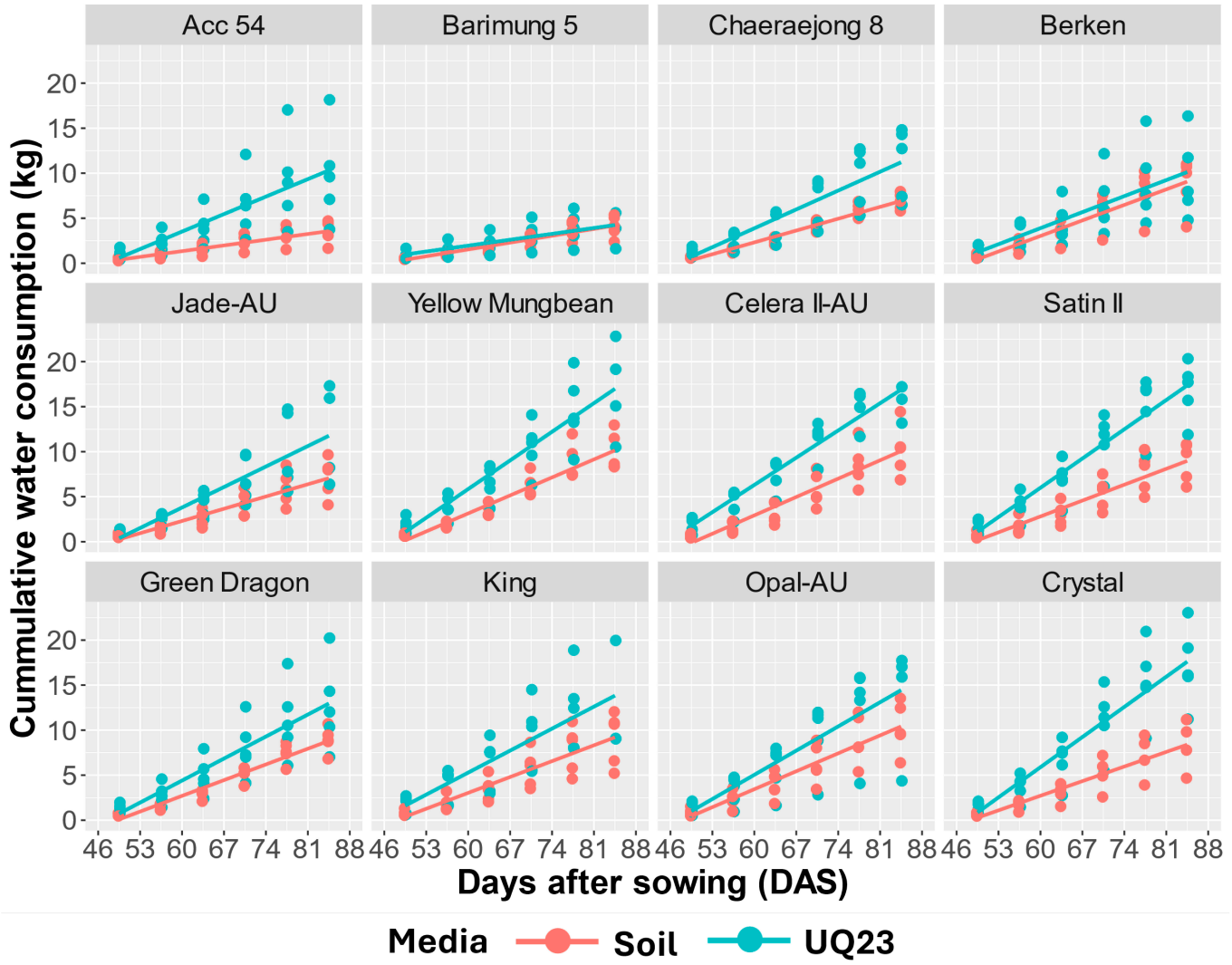

Figure 3. Cumulative water consumption (kg) of the twelve mungbean genotypes calculated from weekly water consumption from 46–53 DAS to 81–88 days after flowering (DAS) in Gatton vertosol soil (Soil, red), and potting mix of 70% composted pine bark + 30% coco peat with 2 g/L Osmocote and 1 g/L Dolomite (UQ23, blue). Cumulative water consumption was calculated from the weekly water consumption and plotted on the mid-week. Each point is a replication, and the lines indicate the linear trend.

Variance Components

Variance components were obtained by fitting the mixed model analysis for each of the 16 traits (Supplementary Table S2). For comparisons, the variance components for each trait were presented as a ratio to the total variance (Figure 4A). The total variance was calculated as the sum of the variances due to potting media, genotypes, the interaction, and the residual. For most traits, the variance due to the interaction between genotypes and media was the smallest source of variation. For all WUE traits, the genotypic variance was larger (25%–75%) than that due to potting media (<25%) (Figure 4A). For water use (WU) and stomatal conductance (SC44 and SC53), the variance due to the potting media was larger than the genotypic variance (Figure 4A).

For both types of WUE traits (i.e., WUEbio and WUEyield), the heritability of the WUE total and after flowering was higher than that of WUE before flowering (Figure 4B). The low heritability for both WUE before flowering is mainly due to a large residual variance (Figure 4A). In addition to larger residual variance, the genotypic variance for WUE before flowering is also smaller than after flowering, especially (Figure 4A). Large residual and small genotype variances for WUE before flowering could be due to the lack of differentiation in water consumption among genotypes before 46–53 DAS (Figures 2 and 3).

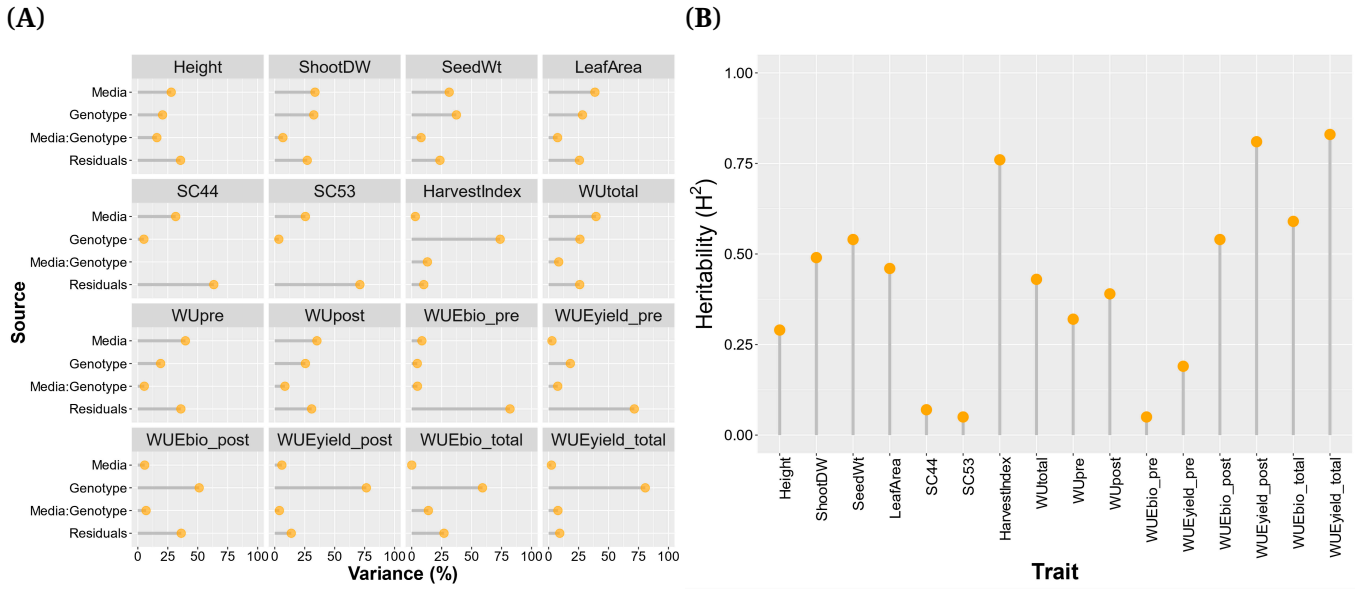

Figure 4. Lollipop plot for the (**A**) percent variance components and (**B**) heritability obtained for each of the 16 traits (Table 2A). The percentage is calculated as the ratio of each component to the total (the sum of variances due to potting media, genotype, potting media-by-genotype interaction, and residual). The heritability is calculated as the ratio of the variance due to genotype and the phenotypic variance (the sum of variance due to the genotype, potting media-by-genotype interaction, and residual). Refer to Table 2A for the details of the traits.

Pattern Analysis

The WUE after flowering (WUEbio_post and WUEyield_post) was highly positively correlated ($r > 0.95, p < 0.001$) with the WUE total for both types of WUE (WUEbio_total and WUEyield_total) across the two potting media (Figure 5A). The WUE total based on seed weight (WUEyield_post) was also highly correlated with the harvest index ($r = 0.96, p < 0.001$). The two measures of stomatal conductance were moderately correlated with each other ($r = 0.6, p < 0.001$) and negatively correlated ($-0.03 \leq r \leq -0.7$) with the most traits (Figure 5A). Genotypes with lower stomatal conductance typically exhibit higher water use, hence higher biomass [32]. Similar correlation patterns among traits were observed within each media (Supplementary Figure S2).

Based on the dendrogram, the traits were divided into three groups: (1) agronomy and water used, (2) WUE and harvest index, and (3) the two

stomatal conductance (Figure 5B). There was a positive correlation between group (1) and (2), a negative correlation between group (1) and (3) and a weak correlation between group (2) and (3) (Figure 5C). A similar grouping of traits was observed in soil (Supplementary Figure S2A) but not in potting mix (Supplementary Figure S2B). In the potting mix, SC53 was grouped with WUE before flowering, and SC44 was grouped with the agronomic and water use traits (Supplementary Figure S2B).

The dendrogram for the genotypes showed that the grouping was mainly based on the type of potting media (Figure 5B). At five-group levels, Acc 54 in soil (AccSo) was not grouped with other treatments, while Acc 54 in potting mix (Acc23) was groped with Barimung 5 on the same media (Bar23, Figure 5A). Other genotypes were mainly grouped based on the potting media, except for Berken, where both the soil (BerSo) and potting mix (Ber23) were in the same group and Jade-AU in the potting mix (Jade23) that grouped with Opal in the soil (OpalSo) and other genotypes in soil, but not with Jade-AU in soil (JadeSo, Figure 5B).

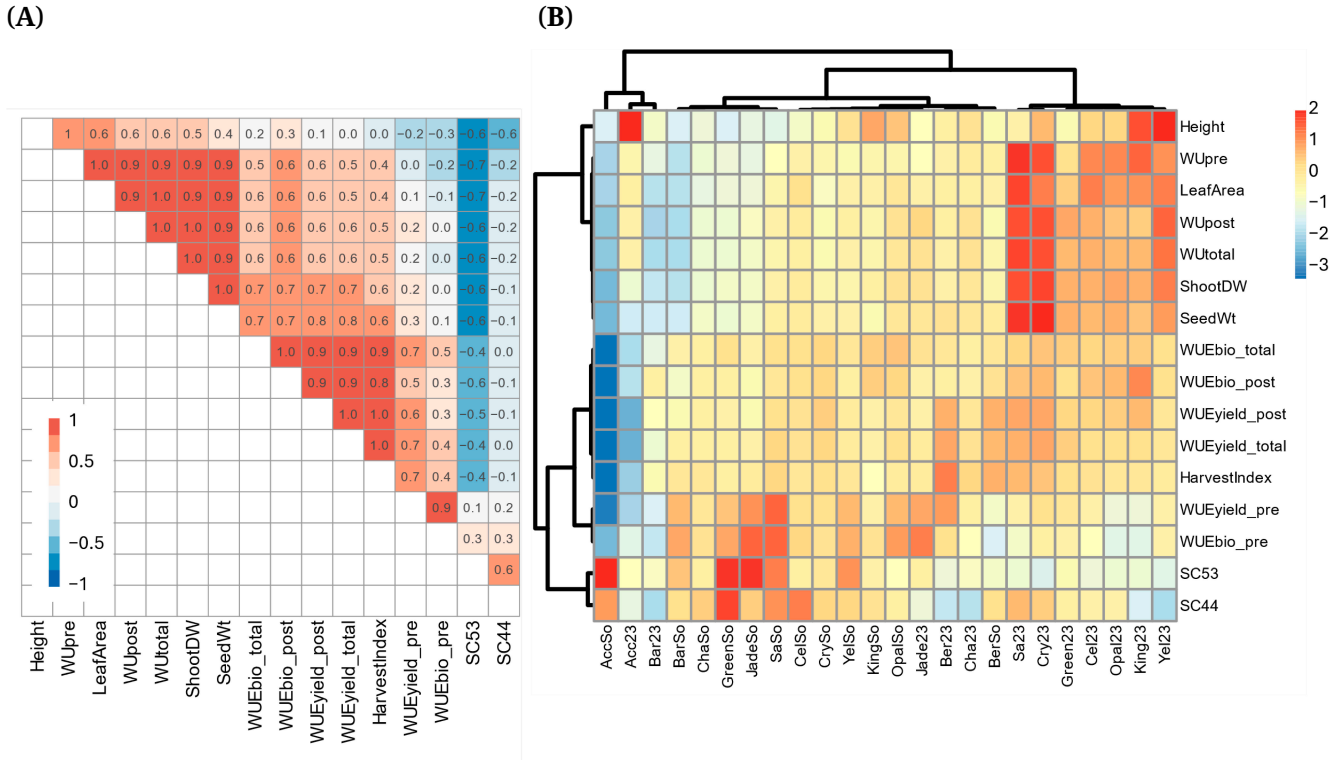

Figure 5. (**A**) Heatmap for the correlation matrix among the 16 traits (Table 2A). (**B**) Heatmap for the column standardised predicted values of treatments (i.e., the combination of genotypes and potting media)-by-traits table. The traits and treatments are ordered based on their optimised dendrogram (right and bottom). Each dendrogram used average squared Euclidean distance as dissimilarity measures and Wards' method as the clustering strategy. The treatment name combines genotype abbreviation (Table 1) and media (So = Gatton Vertosol Soil; 23 = potting mix UQ23). (**C**) Biplots for the first three components from the ordination based on singular value decomposition. The vectors indicate traits, and the points indicate treatments.

(C)

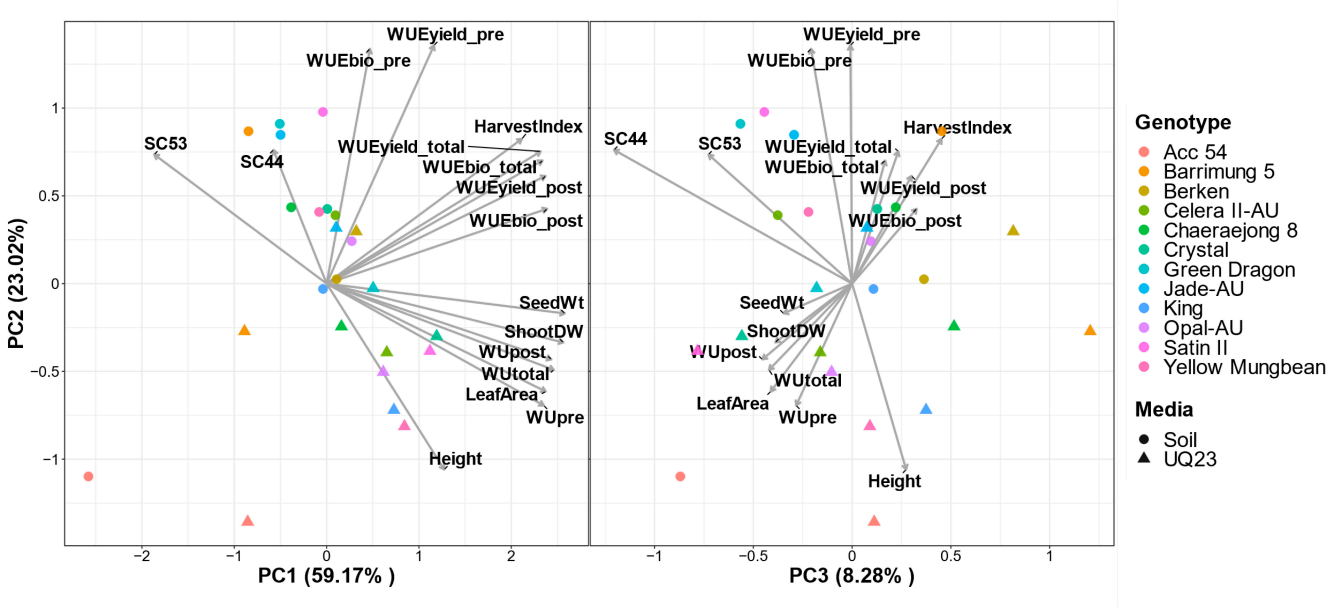

Figure 5. *Cont*.

The genotypes tested in the potting mix had relatively lower stomatal conductance, higher water use, higher seed weight, and higher shoot dry weight than those tested in the soil (Figure 5B,C). The genotypes also showed variability in the traits measured (Figure 5B,C, Supplementary Table S3). Acc 54 has tiny seeds and small leaves and tends to be indeterminate. Satin II and Crystal-AU had the highest seed weight, and Yellow mungbean and King were tall. Barimung 5 had a relatively low value for the agronomic and water use traits, in contrast to Satin II and Crystal-AU in the potting mix. The variation among genotypes in these 16 traits was more detectable in the potting mix than in the soil (Figure 5B, Supplementary Table S3).

Water Use Efficiency

The ranking of genotypes based on WUE for total and after-flowering water consumption were relatively similar (Figure 6A,B for WUEbio; Figure 6C,D for WUEyield). In all cases, the WUE for UQ23 was either the same or higher than for soil, except for Barimung 5 (Figure 6). There was little difference in the agronomic and yield of Barimung 5 between the two potting media but with slightly higher water consumption in UQ23 than in soils, resulting in higher WUE in soil than UQ23 (3.8 g/kg and 3.5 g/kg for WUEbio_total; 1.3 g/kg and 1.1 g/kg for WUEyield_total).

However, there was a change in genotype ranking between WUEbio and WUEyield. For example, Berken was in the top three for WUEyield (Figure 6C) but only in the middle rank for WUEbio (Figure 6A). Whereas King was in the top three for WUEbio but dropped to the 4th position for WUEyield. This result is expected as large biomass does not always translate to high yield. The wild mungbean, Acc 54, had the lowest WUE

and the most different WUE between the two potting media (Figure 6). The WUE for Acc 54 was much higher for UQ23 as this genotype did not grow well in soil.

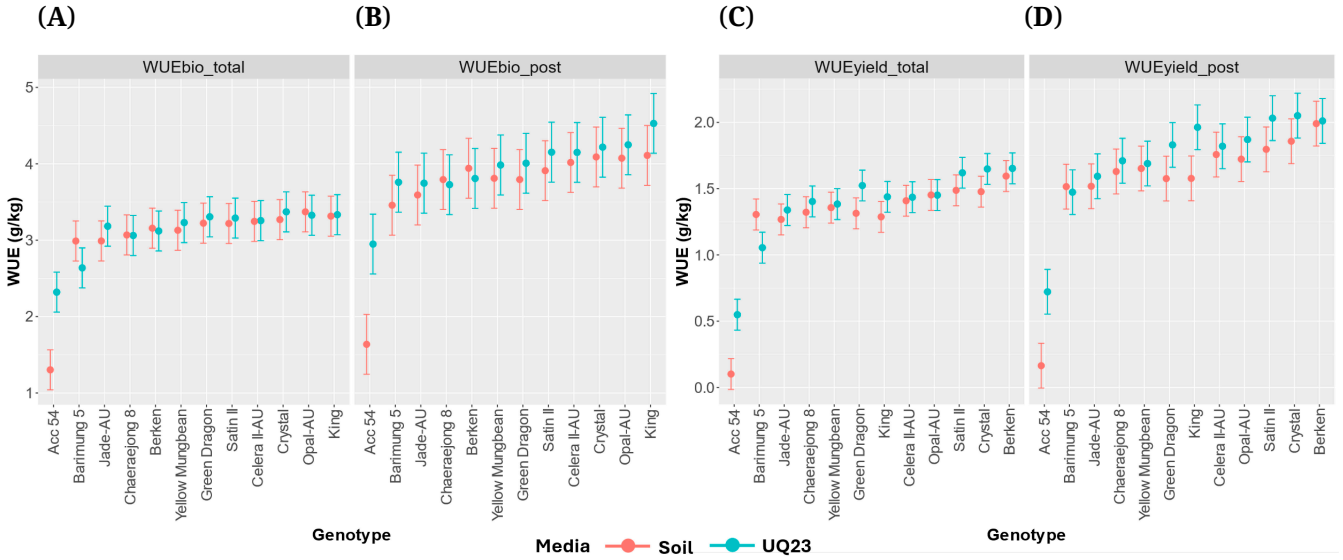

Figure 6. Water use efficiency (WUE, g/kg) for the twelve mungbean genotypes (Table 1) for each potting media (i.e., soil and potting mix [UQ23]). **(A)** WUEbio_post (ratio of above-ground dry matter and the total water consumption). **(B)** WUEbio_post (ratio of above-ground dry matter and the total water consumption after flowering). **(C)** WUEyield_total (ratio of seed weight and the total water consumption). **(D)** WUEyield_total (ratio of seed weight and the total water consumption after flowering). Error bars represent twice the standard error. The genotypes were ordered based on the average WUE across the two-potting media.

DISCUSSION

Genetic variability is important for improving the WUE through breeding [36]. Observable variations in water use among different varieties of the same crop indicate the potential to develop genotypes through selective breeding that are even more efficient in their water usage [37]. Traditional methods often involve extensive resource allocation and may not accurately reflect plant water use at the individual level [38]. The IBP system is cost-effective and scalable. This system detected variability in WUE among the twelve mungbean genotypes evaluated (Figures 2–6). It can potentially screen many mungbean genotypes for WUE under non-limiting water conditions.

In the IBP system, the same genotypes grow better in the potting mix than in soil (Figure 5). This could be due to the higher water saturation in soil that might inhibit mungbean growth. The difference in growth between the two potting media also resulted in the difference in water consumption (Figure 2). However, the pattern of weekly water consumption is similar between the two potting media (Figure 2). The variability due to media and genotype-by-media interaction was smaller than the variability due to genotypes for total WUE (Figure 4A). The ranking of genotypes for WUE is also reasonably consistent across the two

potting media (Figure 6). Based on these results, potting mix UQ23 would be a better option for screening than soil. However, further studies should be conducted to test the suitability of the IBP system for different soil types.

In screening for WUE, the objective is to be able to rank many of the genotypes. This ranking is then used to select either high-WUE parents or a set of contrasting genotypes (high and low WUE) for further studies. As the ranking of genotypes based on total and after-flowering WUE were also reasonably similar (Figure 6), and these traits are highly positively correlated ($r > 0.95, p < 0.001$, Figure 5) with similar heritability (≈ 0.6 for WUEbio and ≈ 0.8 for WUEyield, Figure 4B), after flowering WUE can be used, instead of total WUE, to rank the genotypes. Moreover, the pattern of weekly water consumption (Figure 2) and the trend of cumulative water consumption (Figure 3) indicate that the difference in water consumption among genotypes occurred after 46 DAS, peaked around flowering, and continued until harvest maturity, the is no need to record water consumption before flowering for comparing WUE among genotypes.

Using after-flowering WUE to rank genotypes will save time and labour and potentially enable screening more genotypes. Using less time and money in plant breeding strategies is crucial for enhancing efficiency and reducing the costs of developing new plant varieties. By streamlining breeding processes, breeders can more quickly respond to market demands and environmental challenges. In plant breeding, increasing the efficiency of genotype screenings within the same plant cycle is crucial for meeting the urgent demands of feeding a growing global population and enhancing profitability [39].

However, the ranking of genotypes based on total WUE could differ from the ranking based on seed weight (Figure 6). Therefore, it is important to determine which WUE should be used to rank the genotypes. As yield is the main objective for mungbean breeding, improving WUEyield could be more important than improving WUEbio.

For the WUE study, while the IBP can be used to rank genotypes based on the well-watered WUE (Figure 6), it is important to consider that it might not necessarily translate to a higher yield under water-limited conditions, where the ability to conserve water may become more critical. In wheat, selection in predictable irrigation conditions has led to genetic improvements in yield potential, producing broadly adapted genotypes that perform well in well-watered and water-limiting conditions [40].

Another limitation of this IBP system is the difficulty in recovering the root biomass because they become entangled with the capillary mat and the potting mix particles. Root systems are important for improving WUE and selecting for drought tolerance [41]. Therefore, the WUE based on above-ground dry matter might not provide a full picture of the WUE in mungbean.

However, the IBP system could be used to evaluate a heat-stress response in mungbean without the confounding effect of water stress. Heat and water stress often occur together and are hard to distinguish [42].

The non-water limiting condition in the IBP system eliminates the water stress; hence, a heat stress response can be observed.

CONCLUSIONS

Hunter's IBP system [19] provides a relatively easy system for measuring water consumption to estimate water use efficiency. A modification of this system for better control of water flow and the use of composted pine bark mix (UQ23) potting media enables this system to be used for mungbean despite its sensitivity to waterlogging.

The difference in water consumption among genotypes was observed 46 days after sowing (DAS), with the peak of water consumption around flowering (around 60 DAS). During this period until harvest maturity, there was a linear trend in water consumption, with Crystal having the highest and Barimung 5 having the lowest water consumption rate.

The ranking of genotypes based on WUE calculated using total water consumption and after-flowering water consumption were reasonably similar. There was also a strong positive correlation between total and after-flowering WUE ($r > 0.95, p < 0.001$) and similar heritability between these two WUEs (≈ 0.6 for WUEbio and ≈ 0.8 for WUEyield). Therefore, after-flowering WUE is a good predictor for total WUE and using this trait to screen genotypes would save resources.

However, the ranking of above-ground dry matter WUE (WUEbio) and seed weight WUE (WUEyield) could differ. Berken was in the top three based on WUEyield_post but only ranked 8th for WUEbio_post. In contrast, King was in the top three for WUEbio_post but ranked 4th for WUEyield_post. For mungbean, improving WUEyield will be of more interest in increasing yield production.

This study used the IBP system to rank mungbean genotypes based on their WUE under non-limiting water conditions. Whether these top genotypes can also perform well under water-deficit conditions requires further research.

SUPPLEMENTARY MATERIALS

The following supplementary materials are available online at https://doi.org/10.20900/cbgg20240007. Supplementary Table S1: Test results for the two potting media[1]; Supplementary Table S2: Variance components and their standard error for the 16 traits (Table 2A) from the mixed model analysis; Supplementary Table S3: Predicted value and the standard error of the 16 traits (Table 2A) for the twelve mungbean genotypes for each potting media; Supplementary Figure S1: Construction of the inverted-bottle pot (IBP) system. (**A**) The entire pot set-up based on 4L ANOVApot®. (**B**) Pot with upper water shedding container (65 mm × 116 mm top, 90 mm bottom) removed to reveal the bottom of the inverted bottle (250 mm × 85 mm) lying within the central conduit (89 mm outside diameter (OD) × 225 mm). (**C**) Bottle with cap (40 mm diameter) with a central hole (15 mm) and marble (16 mm) placed beside. (**D**) Internal view

of central conduit revealing capillary mat draped over the central well of 4L ANOVApot® with a section of the plastic grid in place. (**E**) Rainfall plastic deflecting sheet (398 mm diameter) with a central hole (82 mm) and plant hole (30 mm) edge placed 15 mm from the edge of the central hole. Laces threaded through peripheral holes (8 mm) and tightened to secure the deflecting sheet in place. (**F**) Top of central conduit with adhesive tapes to prevent deflecting sheet sliding downwards. (**G**) All components with plant hole conduit (32 mm OD × 17 mm) in position. A plastic sheet beneath the capillary mat is included for added anti-drainage security (unnecessary if the pot is sealed). The wraparound silver reflecting insulation is not included here; Supplementary Figure S2: Heatmap for the correlation matrix among the 16 traits (Table 2A) and heatmap for the column standardised predicted values of genotypes table for (**A**) Gatton vertosol soil and (**B**) potting mix UQ23 (70% composted pine bark + 30% coco peat with 2 g/L Osmocote and 1 g/L Dolomite). The traits and genotypes are ordered based on their optimised dendrogram (right and bottom). Each dendrogram used average squared Euclidean distance as dissimilarity measures and Wards' method as the clustering strategy.

DATA AVAILABILITY

The dataset of this study is available from the authors upon reasonable request.

AUTHOR CONTRIBUTIONS

Conceptualization, YZ, VA, VS, MH and MD; Methodology, MH; Software, YZ and VA; Validation, YZ, VA, VS and MH; Formal Analysis, VA; Investigation, VS; Resources, VA; Data Curation, VA; Writing—Original Draft Preparation, YZ; Writing—Review & Editing, YZ, VA, VS, MD, KB, YC and MH; Visualization, YZ and VA; Supervision, VA; Project Administration, VA; Funding Acquisition, VA.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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