Evaluation of Physiological and Yield Parameters for Improving Water Use Efficiency and Drought Tolerance in (*Phaseolus vulgaris* L.)

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Abstract: The common bean (*Phaseolus vulgaris* L.) is considered the most important food legume for humans globally. However, drought is the most constraining factors to *P. vulgaris* production globally. This study was conducted to identify the variation in water use efficiency (WUE), and drought tolerance in common beans under cool temperatures, with the aim of targeting beans for a mild winter growing season in southern Australia. Four genotypes of common bean were compared with a control species, narrow leaf lupin (*Lupinus angustifolius* cv. Mandelup). The results showed that narrow leaf lupin had the lowest WUE, total dry matter (TDM), root to shoot ratio (R/S), reproductive dry matter (RDM) and relative growth rate (RGR) during pre-anthesis to early post-anthesis growth, and ranked lowest and third during the early post-anthesis to physiological maturity growth. In addition, *P. vulgaris* genotype Arwon had the highest TDM, R/S, RGR and WUE during pre-anthesis to early post-anthesis growth, and Spearfelt and Arwon were the bean genotypes with the highest values during the early post-anthesis to physiological maturity growth. This study demonstrated genetic variation in WUE under moderate growing temperatures, suggesting this crop could be potential alternative legume for rain-fed and mild winter growing in Western Australia.

Keywords: Common bean, Drought tolerance, Water use efficiency, Germination, Pre-anthesis, Early post-anthesis, physiological maturity growth

1. Introduction

The common bean (*Phaseolus vulgaris* L.) (2n = 2x = 22) is the most important direct food legume for humans globally (Guzmán-Maldonado et al. 2000) comprising a major part of the diet (Gepts and Debouck 1991). *P. vulgaris* seeds are a significant source of protein, calories, vitamins and minerals (House et al. 2002). In addition, it is considered a major product in global trade, and is consumed by large numbers of poor people in Latin America and Africa (Singh 1999). The common bean is a warm-season species originating from Central and South America (Nleya et al. 2005) where it was domesticated more than 7,000 years ago (Gepts and Debouck 1991).

According to data published by the Food and Agriculture Organization (FAO), the total production of dry beans in Australia and the world in 2011 were 65,247 and 23,250,253 tons, respectively. Grain legume crops in Western and Eastern Australia have been adopted as an important component of field crop rotations in the country (Brinsmead et al. 1991, Marsh et al. 2000). In Western Australia, concerted efforts have been made to increase the number of crops that can be used in rotation with cereals according to the type of soil and climate in the Western Australian wheat belt (Robertson et al. 2010).

Legume crops differ in their temperature tolerances, many are best adapted to tropical and subtropical climates, or growing in the warm seasons in temperate regions. For instance, *P. vulgaris* is more sensitive to high temperatures than soybean (*Glycine max* L.) and cowpea (*Vigna unguiculata* L.) (Pita and Munns 1987). Low temperatures can adversely affect beans. The lowest temperature the common bean can germinate at is 12°C, but some varieties can germinate at temperatures lower than 8°C (Nleya et al. 2005). However, Kotowski (1926) stated that bean seed germination at temperatures lower than 15°C is poor. In general, *P. vulgaris* grows well at temperatures ranging from 17.5°C to 25°C (Gepts 1998).

The wheatbelt of Western Australia as an agricultural region has a Mediterranean climate with dry hot summers and mild winters (Doole et al. 2009), it comprises about 14 million ha, 'in a broad band east of Perth, from Geraldton in the northwest to Esperance in the southeast' (Hobbs 1993). The average annual rainfall in the wheatbelt is below 400mm (Anderson et al. 1995) and the mean minimum and maximum temperatures are 13.6°C and 25.9°C, respectively (Australian Government Bureau of Meteorology 2012). Narrow-leaved lupin (*Lupinus angustifolius* L.), a cool-season species, grows well in the WA wheatbelt, and is the most common grain legume planted in rotation with cereals (Edwards 2009). Moreover, beans have been proposed as an alternative crop for mild winter growing seasons in Western Australia, and potentially for other
Adopting technologies that increase the proportion of water capacity to produce biomass (Webber et al. 2006). WUE in common bean has a strong association with specific plant characteristics and soil type. There are two approaches to increase crop WUE: 1- Adapting technologies that increase the proportion of water that is transpired by the crop and 2- Increasing the crop’s capacity to produce biomass (Webber et al. 2006).

Drought is the most constraining factor to *P. vulgaris* production globally (Teran and Singh, 2002). Grajales et al. (2008) stated that 60% of bean production occurs in regions suffering from water deficiency. Significantly, as the common bean has low tolerance to drought (Souza et al. 2003), brief periods of water deficiency have negative effects on its quality and yield (Konsens et al. 1991). Bean production worldwide has been affected by drought. Africa loses about 300,000 tons of beans annually because of drought (Wortmann et al. 1988). The intensity, type, and duration of the stress are the three ways drought can impose its effects on common bean (Munoz-Perea et al. 2006).

Water use efficiency (WUE) is defined as the biomass increase per unit of water transpired (De Costa and Ariyawansha 1996). Increasing WUE is a way to increase agricultural production in arid and semi-arid regions (Webber et al. 2006). WUE in common bean has a strong association with specific plant characteristics and soil type. There are two approaches to increase crop WUE: 1- Adapting technologies that increase the proportion of water that is transpired by the crop and 2- Increasing the crop’s capacity to produce biomass (Webber et al. 2006).

The development of common bean genotypes tolerant to drought is a practical and economical approach to reduce the negative effects of drought on crop production (Xiong et al. 2006). Many studies have reported that drought tolerance in crops is a complex physiological process (Araus et al. 2002). The WUE and drought tolerance of beans and winter-adapted legumes has not been compared before. Hence, the primary objective of this study was to determine the pre-anthesis to early post-anthesis WUE and the early post-anthesis to physiological maturity drought tolerance under cool temperatures of the common bean in comparison to narrow leaf lupin—*Lupinus angustifolius* L., an adapted cool-season grain legume—and to test the hypotheses that, there is variation in WUE between *P. vulgaris* genotypes and the control species, *Lupinus angustifolius* L. under cool conditions and there is variation among *P. vulgaris* cultivars with respect to post-anthesis drought tolerance.

## 2. Materials and Methods

### Plant materials and growth conditions

The study was carried out from July to November 2012 in the glasshouse at the University of Western Australia, Perth, Australia. Four genotypes (bush type) of common bean (*Phaseolus vulgaris* L.) – Spearfelt and Arwn (Australia), Kariba ‘green bean type’ and Dongara (South Africa) – were compared with a control species, narrow leaf lupin (*Lupinus angustifolius* L. cv. Mandalup) (WA, Australia), and tested under well watered ‘100% field capacity’ (T1) and drought ‘30% field capacity’ (T2), during early post-anthesis to physiological maturity growth stages under cool temperatures.

The following figure shows that three phases of plant growth, vegetative (up to 46 DAS), flowering (46.6-56.8 DAS) and reproductive growth (52-107 DAS), with times of three harvest times, first harvest (H1), second harvest (H2) (two weeks after flowering) and third harvest (H3) (at physiological maturity) (Fig. 2). It shows the time of application of the two different treatments, 100% field capacity pre-anthesis to early post-anthesis and T1 and T2 early post-anthesis to physiological maturity.

### 3. Experimental Design

A completely randomised factorial design (5 genotypes × 2 water treatments) was used in this study with pots as the experimental unit. Three seeds were sown in each pot at 15-20°C. The experiment included three stages of harvest (H1 36 DAS, H2 63-74 DAS depending on the flowering time and H3 at physiological maturity 96-107 DAS) and two treatments T1 and T2. H1 comprised three replications, H2 and H3 comprised five replications. Methods were similar to those used by Armstrong et al. (1994).

#### Germination and vegetative growth conditions

In the laboratory, germination was tested and 180 seeds for each genotype were germinated and distributed into 18 Petri dishes with ten seeds per dish. Seeds were soaked in water for six hours. Then excess water was removed, ensuring that the filter paper within each Petri dish remained wet. To prevent seed-borne disease infection, fungicide P-Pickel T® was applied after two days. The seeds were maintained in the Petri dishes in the laboratory at 21°C for four days (Siminovitch and Cloutier 1982) prior to sowing in pots.

In the glasshouse, 95 plastic pots (height of 27cm and diameter 24cm) were prepared (90 pots for sowing and five blank pots to measure soil evaporation) with a mixture of blue metal (1.93 kg), Gin Gin loam (10.5 kg; pH 6.5) and 5.0 g of paper (three × paper towels). After germination, six seedlings of each genotype were transferred to each pot. One week later, they were thinned to three healthy plants per pot.

The glasshouse was maintained in the temperature range of 13.2-23.8°C (monthly max. and min. are shown in Fig. 1). During early vegetative growth, pots were irrigated equally every second day. Starting three weeks after sowing, each pot was fertilised once a week with 1.0 g/L of Poly-Feed® complete liquid fertiliser.

Gravimetric water use was recorded during the pre-anthesis to early post-anthesis, and early post-anthesis to
physiological maturity periods to determine the water use efficiency (WUE) for each genotype. In the early post-anthesis to physiological maturity period, fertilisation was increased to 2.0 g/L/week because of increased biomass. In addition, 0.4 kg of Alkathene® plastic beads (approximately 2cm thick layer) were added to the soil surface of pots (from pre-anthesis to early post-anthesis period) to prevent water evaporation. Soil water content and soil evaporation from the five blank pots were also measured. The experiment was sprayed prophylactically with insecticides and fungicides (tau-fluvalinate, fipronil, Mancozeb, primicarb, carbaryl and abamectin) to manage pests and diseases.

Harvest

Three harvests of plant shoots and roots were undertaken. H1 was undertaken 36 DAS to calculate the first biomass. At H1, shoots and roots were separated, oven-dried for two days at 70°C, and then weighed. Water use was monitored starting after H1 by weighing pots and replacing water to the target weight.

H2 was two weeks after flowering when WUE began to be calculated for 100% field capacity, so the water used by the plants for this harvest was measured from the pre-anthesis to early post-anthesis period. To calculate WUE at 100% field capacity, the pots were well watered before sunset (6 pm) then weighed early the next day and the weight of each pot recorded as the field capacity weight for each pot individually.

Each pot during the pre-anthesis to early post-anthesis period was put on an individual close-fitting tray to prevent loss of water through the holes in the bottom of pots. Each pot was weighed every 2-3 days after the initial weighing then the amount of water equal to that lost from each pot was added. Finally, 63-74 DAS, the plants were harvested depending on the date of appearance of the first open flowers for each genotype. After separating leaves, stems, pods and roots at harvest, samples were processed as at H1.

The early post-anthesis to physiological maturity period water use was examined for T1 and T2. H3, 96-107 DAS, was undertaken at physiological maturity. The target amount of water in T1 pots was achieved using the same method as that used in H2, while T2 pots required a different process to obtain the target amount of water for each genotype as follows:

First, a sample of Gin Gin loam was weighed and oven-dried at 70°C for four days, then reweighed and the dry weight of the sample recorded. Thus, the mass of the Gin Gin loam after weighing it both moist and dry was determined using the following equation:

\[
\text{Weight of moist soil sample} = \text{Weight of field capacity soil in pot only} - (\text{weight of empty pot} + \text{weight of Gin Gin loam} + \text{tray weight} + \text{Alkathene® weight} + \text{weight of paper})
\]

\[
\text{Moisture in T2 Gin Gin loam} = 0.3\times \text{Field capacity percent moisture content of gin gin loam soil}
\]

\[
\text{Weight of T2 soil} = (1 + \frac{\text{Moisture in T2 Gin Gin loam}}{100}) \times \text{Mass of Gin Gin loam}
\]

\[
\text{Target amount of water under T2} = \text{Weight of T2 soil} + \text{Weight of empty pot} + \text{Weight of paper} + \text{Weight of blue metal} + \text{Tray weight} + \text{Alkathene® weight}
\]

All the pots under T1 and T2 conditions were weighed every two to three days then the amount of water equal to the water lost per pot was added, as in H2. Every addition of water during H2 and H3 was recorded and the WUE value for each genotype during these two periods determined. The calculation of WUE required total dry matter (TDM) as follow:

\[
\text{WUE} = \frac{\text{Total water used for growth period (pot)}}{\text{Average blank pot water used}}
\]

As well as, reproductive dry matter (RDM) and relative growth rate (RGR) were calculated using the following equations:

\[
\text{RDM} = \text{Reproductive dry weight (whole of immature pods) per plant}
\]

\[
\text{RGR (Pre-anthesis)} = \frac{\text{TDMH2-TDMH1}}{\text{days up to H2}}
\]

\[
\text{RGR (Post-anthesis at T1)} = \frac{\text{TDMH3} - \text{TDMH2}}{\text{days from H2 to H3}}
\]

\[
\text{RGR (Post-anthesis at T2)} = \frac{\text{TDMH3} - \text{TDMH2}}{\text{days from H2 to H3}}
\]

Statistical analysis

The data were analysed by analysis of variance (ANOVA) using R Project software and Microsoft Excel. Germination and early vegetative data were determined using two-factors ANOVA (genotype and replications). The late vegetative – reproductive growth data were determined using three-factors ANOVA (genotype, replications and water treatment (T1 and T2)). ANOVA and Microsoft Excel were also used to analyse the study data to obtain WUE, TDM, RDM, RGR and the root to shoot ratio for both H2 and H3. Correlations were calculated by GenStat 12th Edition | VSN International.
4. Results

Stage of growth: Vegetative

ANOVA indicated that the difference between the four *P. vulgaris* genotypes and lupin (control) in total dry matter/plant (TDM) at 36 DAS was not statistically significant (Table 1).

4.1. Stage of growth: Pre-anthesis to early post-anthesis

The time to flower (TF), the time from sowing until the beginning of anthesis, varied from 46.6 to 56.8 DAS among genotypes, where lupin (control) was in the middle (50.4 DAS). The four *P. vulgaris* genotypes were significantly different to the lupin (control) (p<0.001) (Table 1).

The results of Total dry matter (TDM) at two weeks after flowering showed that the four *P. vulgaris* genotypes were significantly different to lupin (control) (p<0.001) (Table 1 and Fig. 3). Among beans, Arwon had the highest TDM (16.1 g/plant) and Kariba (10.7 g/plant) the lowest. Lupin (control) produced the lowest TDM in this harvest stage (7.6 g/plant).

The root/shoot ratio (R/S) was estimated at two weeks after flowering, and varied from 0.19 to 0.43 among genotypes. The four *P. vulgaris* genotypes were significantly different to lupin (control) (p<0.05). Kariba had the highest R/S, followed by Dongara, Arwon and Spearfelt. Lupin (control) had the lowest R/S.

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The root/shoot ratio (R/S) was estimated at two weeks after flowering, and varied from 0.19 to 0.43 among genotypes. The four *P. vulgaris* genotypes were significantly different to lupin (control) (p<0.05). Kariba had the highest root/shoot ratio, followed by Dongara, Arwon and Spearfelt. Lupin (control) had the lowest root/shoot ratio.

The results showed that the *P. vulgaris* genotypes had faster relative growth rate (RGR) than lupin (control) in the period of pre-anthesis to early post-anthesis growth (Table 1). Arwon had the highest RGR (0.9 g/days) among the other *P. vulgaris* genotypes and Kariba (0.7 g/days) the lowest RGR. Finally, lupin (control) had the lowest genotype in RGR among the four *P. vulgaris* genotypes (0.5 g/days).

The four *P. vulgaris* genotypes were significantly different to lupin (control) in reproductive dry matter (RDM)/plant at two weeks after flowering (H2) (p<0.05) (Table 1). Arwon had the highest in RDM (3.4 g/plant) and Kariba had the lowest in RDM (1.3 g/plant) (Fig. 4). Lupin (control) was the fourth genotype in RDM (1.97 g/plant).

For water use efficiency (WUE), the four *P. vulgaris* genotypes did not differ significantly to lupin (control) at pre-anthesis to early post-anthesis period (p=0.73) (p>0.05) (Table 1).

4.2. Stage of growth: Early post-anthesis to physiological maturity

For TDM at physiological maturity, the effects of genotype, water treatments and their interaction (p<0.001) were significant, and there are differences among *P. vulgaris* genotypes and lupin (control) (Table 1 and Fig. 5). Arwon was the highest genotype in TDM (22.6 g/plant) for 100% field capacity (T1) and Spearfelt had the highest TDM (14.3 g/plant) for 30% field capacity (T2). Where Dongara had the lowest TDM among beans for both treatments, T1 and T2, (11.7 and 10.6 g/plant, respectively). Lupin (control) had the lowest TDM for both treatments, T1 and T2, (11.2 and 7.5 g/plant, respectively). Overall, the moisture stress treatment (T2) yielded 5.5 g/plant, which was 42.1% less than the fully-watered treatment (T1). In addition, Spearfelt, Arwon and Kariba genotypes at T2 produced more TDM than lupin (control) at T1. The current study showed a significant correlation only between TDM and RDM (Table 2).

The root/shoot ratio was estimated at physiological maturity, it was significantly different among genotypes (p<0.001) and water treatments (p<0.01), but there was no significant interaction (Table 1). The root/shoot ratio ranged from 0.07 to 0.17 and from 0.07 to 0.21 among genotypes at T1 and T2, respectively. Arwon had the highest root/shoot ratio and Kariba the lowest at T1 and T2 among *P. vulgaris* genotypes. Lupin (control) had the third and lowest root/shoot ratio at T1 and T2, respectively.

The results showed that Spearfelt had the highest RGR at 100% field capacity (T1) (0.2 g/days), and Kariba the highest at 30% field capacity (T2) (0.03 g/days). In addition, Dongara and Arwon had lowest RGR at T1 and T2 (-0.006 and -0.005 g/days, respectively). Lupin (control) had the third in RGR at T1 and T2 (0.1 and -0.002 g/days, respectively).

The results of reproductive dry matter (RDM) were similar to these for TDM. The ANOVA indicates that RDM/plant was significantly different for genotypes, water treatments and they interaction (p<0.001) (Table 1). Spearfelt had the highest RDM at both treatments: T1 (12.9 g/plant) and T2 (6.34 g/plant) (Table 1 and Fig. 6). The lupin (control) was the lowest genotype in RDM at both T1 (5.8 g/plant) and T2 (3.2 g/plant). Overall, RDM at T1 was higher than T2 for all genotypes.

WUE was significantly different among genotypes, water treatments and the interaction (p<0.001). The WUE results at physiological maturity for the genotypes and water treatments interaction are plotted against the genotypes in Fig. 7. Differences among genotypes (including control) were non significant under fully watered conditions (T1).
But under moisture stress (T2) major differences emerged among all genotypes (Table 1). Dongara and Spearfelt had the highest WUE at T1 and T2 (1.2 and 7.6 g/ML-2), where Arwon and Kariba the lowest (0.9 and 1.6 g/ML-2), respectively. Lupin (control) had lowest and third WUE at T1 and T2 (0.5 and 5.3 g/ML-2, respectively).

5. Discussion

This study was conducted to identify the variation in water-use efficiency (WUE) among four genotypes of *P. vulgaris* under cool conditions compared with the control species, narrow-leaved lupin (*Lupinus angustifolius* cv. Mandelup) with respect to post-anthesis drought tolerance. These genotypes were studied in a glasshouse through three stages of plant growth: germination, pre-anthesis to early post-anthesis growth under well-watered conditions, and early post-anthesis to physiological maturity growth under well-watered (T1), 100% field capacity, and water-stressed conditions (T2), 30% field capacity. The growing season in the WA wheatbelt is during the annual rainfall period (May-October) (Thomson and Siddique 1997). The long-term mean minimum and maximum temperatures of the WA wheatbelt region in May are 12.9 and 24.1°C, respectively (Australian Government Bureau of Meteorology 2012). The glasshouse trial of four *P. vulgaris* genotypes and lupin (control) was grown for its first month of growth with cool temperatures (11.1 min. and 23.2°C max.) and the mean temperature during the period of pre-anthesis to early post-anthesis was 13.3-23.6°C. This is lower than the ideal temperature for the common bean (17.5–25°C) as reported by Gepts (1998). De Almeida (2012) found also the temperature range 18-25°C was suitable for common bean growth and production, when compared with two different cooler temperature regimes. The results of the current study indicate that there is variation in WUE among the *P. vulgaris* genotypes and between them and lupin (the control species). Consequently, the hypotheses of this study may be accepted.

The differences between common bean genotypes and the control species

Narrow-leaved lupin, a cool season legume, differed from the genotypes of common bean, a warm season legume, from pre-anthesis to early post-anthesis. In addition, the current study showed lupin had the lowest total dry matter (TDM) (Figure 8) and R/S among the four *P. vulgaris* genotypes. It ranked also the lowest in RGR in the same period. RDM and WUE for lupin were higher than only one variety of *P. vulgaris* genotype (Kariba) in pre-anthesis to early post-anthesis growth.

During the early post-anthesis to physiological maturity growth period, lupin (control) again had the lowest TDM under both T1 and T2, and R/S under T2 among the four *P. vulgaris* genotypes. A study carried out by Siddique et al. (2001) compared the efficiency of water use of seven cool season grain legumes, and showed that *V. faba* (faba bean cv. Fiord) and *P. sativum* (field pea cv. Dundale) had higher TDM, reproductive dry matter (RDM) and WUE than narrow-leaved lupin. Furthermore, Markhart (1985) indicated that TDM in Tepary bean (*Phaseolus acutifolius*) decreased significantly more than TDM in the common bean for both treatments, well watered and stress-watered. The current study shows that there is a significant correlation between TDM and RDM. This finding agrees with the study by Anderson (1980), who reported that TDM was positively correlated with RDM in lupin. Otherwise for R/S, lupin was higher than only two genotypes of common beans (Kariba and Dongara) under T1. The current study also found that the values of R/S in common bean genotypes during early post-anthesis to physiological maturity growth under moisture stress (T2) were higher than R/S under fully-watered condition (T1), but the opposite occurred in lupin. The result of common bean agrees with Markhart (1985), who showed increased R/S in both the common bean and the *P. acutifolius* to the same extent when they were water-stressed.

Relative growth rate (RGR) in the current study varied between the common bean and lupin. During early post-anthesis to physiological maturity growth, RGR was higher under well-watered conditions than drought conditions in all genotypes. This can also be inferred from Costa Franca et al. (2000), who indicated that the RGR of two common bean genotypes (Ouro Negro and Xodo) under water-stressed conditions was lower than in well-watered conditions. According to Dracup and Kirby (1996) and Freneh and Turner (1991), lupin grows faster from vegetative to reproductive stage, and the duration of pod and seed-filling is shorter, when the species is exposed to water-stressed conditions compared to well-watered conditions. In this study, lupin ranked the third among genotypes in RGR during early post-anthesis to physiological maturity growth under both T1 and T2. This indicates that some common bean genotypes, such as Spearfelt, accelerate their growth when exposed to water-stressed conditions more than lupin. In the future, the comparison of beans with lupin needs to be repeated in the field.

Thus lupin had the lowest reproductive dry matter value under all moisture conditions at physiological maturity, and the lowest WUE under T1. Robertson et al. (2000) agreed with this finding, reporting that when the common bean is exposed to two treatments—water-stressed and well-watered conditions—it showed high in RDM and WUE. Otherwise, lupin was higher than Kariba and Dongara in WUE under T2. This finding disagrees with a study carried out by Singh and Reddy (1988) who found there is no significant difference in WUE among a warm season crop (*Sorghum bicolour* L.) and two cool-season crops; chickpea (*Cicer arietinum* L.) and Safflower (*Carthamus tinctorius* L.). However, both the lupin and *P. vulgaris* genotypes had lower RDM under moisture stress condition (T2) than under fully-watered condition (T1). Lizana et al. (2006), who found that the number of pods in a common bean genotype (Arroz) under water-stressed conditions was 72 per cent lower per plant than under well-watered conditions, supports these results. In addition, Anderson
higher WUE, which can be an alternative legume crop for cool-season legumes such as lupin in both the glasshouse and field. Hence, it is recommended to screen more genotypes of common bean compared with potentially for other regions. Therefore, common bean genotypes clearly vary in their tolerance to water stress. In conclusion, variation was observed among the four common bean genotypes and lupin (control) during pre-anthesis to early post-anthesis growth, and early post-anthesis to physiological maturity growth when exposed to different water regimes (T1 and T2) under cool temperatures. Arwon was the highest in TDM, R/S, RGR and WUE during pre-anthesis to early post-anthesis growth, while Spearfelt and Arwon were the most common bean genotypes have shown good results in these measurements during the early post-anthesis to physiological maturity. There is potential for other common beans genotypes with higher WUE, which can be an alternative legume crop for rain-fed and mild winter growing in Western Australia and potentially for other regions. Hence, it is recommended to screen more genotypes of common bean compared with cool-season legumes such as lupin in both the glasshouse and field.

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References


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Figures

**Figure 1:** Geraldton (Airport) long term averages temperature and rainfall adapted from www.bom.gov.au/climate. Arrow indicates field sowing time for lupin. H (°C) is the mean highest temperature of the current glasshouse experiment and L (°C) is mean lowest temperature in months after the ‘sowing’ arrow on the diagram.

**Plant Growth Stage**

![Plant Growth Stage Diagram](image)

**Figure 2:** Three harvests, first harvest (H1), second harvest (H2) and third harvest (H3) were covering three stages of plant growth, germination, flowering and reproductive. In addition, the two treatments, 100% field capacity (T1) and 30% field capacity (T2) were imposed early post-anthesis to physiological maturity. (DAS; Days after sowing).
Figure 3: Mean of total dry matter per plant (roots and shoots) of genotypes of pre-anthesis to early post-anthesis growth as at the second harvest (H2) in plants grown at 100% field capacity (T1) of four *P. vulgaris* genotypes and lupin (*Lupinus angustifolius* cv. Mandelup). Statistical significance, compared the four *P. vulgaris* genotypes with lupin, was determined by ANOVA test (p<0.05) and l.s.d. (p=0.05) is indicated as a bar.

Figure 4: Mean of the reproductive dry matter per plant of the four *P. vulgaris* genotypes and lupin (*Lupinus angustifolius* cv. Mandelup) in the second harvest (H2) at 100% field capacity (T1) and l.s.d. (p=0.05) is indicated as a bar.

Figure 5: Mean of total dry matter per plant (roots and shoots) of four *P. vulgaris* genotypes and lupin (*Lupinus angustifolius* cv. Mandelup) in the third harvest (H3) at 100% field capacity (T1) and 30% field capacity (T2). Statistical significance, compared the four *P. vulgaris* genotypes with lupin, was determined by ANOVA test (p<0.05) and l.s.d. (p=0.05) is indicated as a bar, where g, genotype and i, interaction.
Figure 6: Mean of the reproductive dry matter (RDM) per plant of the four *P. vulgaris* genotypes and lupin (*Lupinus angustifolius* cv. Mandelup) at 100% field capacity (T1) and 30% field capacity (T2) for the early post-anthesis to physiological maturity growth period and l.s.d. (p=0.05) is indicated as a bar, where g; genotype and i; interaction.

Figure 7: Mean of water use efficiency (WUE) of the four *P. vulgaris* genotypes and lupin (*Lupinus angustifolius* cv. Mandelup) at 100% field capacity (T1) and 30% field capacity (T2) for the early post-anthesis to physiological maturity growth period and l.s.d. (p=0.05) is indicated as a bar, where g; genotype and i; interaction.

Figure 8: Mean of total dry matter per plant (roots and shoots) of Arwon (highest) and D4.3.3.1 (lowest) comparing with lupin (*Lupinus angustifolius* cv. Mandelup) in the first harvest (H1), second harvest (H2) at pre-anthesis and third harvest (H3) at post-anthesis period growth for 100% field capacity 'well watered (T1) and 30% field capacity 'stress-watered'

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### Table 1: Time of flower (TF), Total dry matter (TDM), root/shoot ratio (R/S), relative growth rate (RGR), reproductive dry matter (RDM), water use efficiency (WUE) for the four *P. vulgaris* genotypes and lupin (*Lupinus angustifolius* cv. Mandelup) in the first harvest (H1), the second harvest (H2) at 100% field capacity (T1) and the third harvest (H3) at T1 and 30% field capacity (T2). Statistical significance was determined by ANOVA test (p<0.05).

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Variety</th>
<th>Geno mean</th>
<th>l.s.d. 5%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kariba</td>
<td>Dongara</td>
<td>Arwon</td>
</tr>
<tr>
<td>First harvest (H1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDM (g/plant)</td>
<td>0.5</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>TF (DAS)</td>
<td>47</td>
<td>46.6</td>
<td>55.6</td>
</tr>
<tr>
<td>TDM (g/plant)</td>
<td>10.7</td>
<td>11.9</td>
<td>16.1</td>
</tr>
<tr>
<td>R/S</td>
<td>0.43</td>
<td>0.33</td>
<td>0.26</td>
</tr>
<tr>
<td>RGR (g)</td>
<td>0.7</td>
<td>0.75</td>
<td>0.3</td>
</tr>
<tr>
<td>RDM (g/plant)</td>
<td>1.3</td>
<td>3.2</td>
<td>3.4</td>
</tr>
<tr>
<td>WUE (g/ML⁻²)</td>
<td>0.5</td>
<td>1.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Second harvest (H2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TDM (g/plant)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>14.1</td>
<td>11.7</td>
<td>22.6</td>
</tr>
<tr>
<td>T2</td>
<td>11.9</td>
<td>10.6</td>
<td>14.2</td>
</tr>
<tr>
<td>Treat mean</td>
<td>13</td>
<td>11.15</td>
<td>18.4</td>
</tr>
<tr>
<td>R/S</td>
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</tr>
<tr>
<td>T1</td>
<td>0.07</td>
<td>0.07</td>
<td>0.17</td>
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<tr>
<td>T2</td>
<td>0.11</td>
<td>0.11</td>
<td>0.21</td>
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<tr>
<td>Treat mean</td>
<td>0.08</td>
<td>0.09</td>
<td>0.19</td>
</tr>
<tr>
<td>RGR (g)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>T1</td>
<td>0.1</td>
<td>-0.006</td>
<td>0.17</td>
</tr>
<tr>
<td>T2</td>
<td>0.03</td>
<td>-0.03</td>
<td>-0.05</td>
</tr>
<tr>
<td>Treat mean</td>
<td>0.065</td>
<td>-0.018</td>
<td>0.06</td>
</tr>
<tr>
<td>RDM (g/plant)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>8.9</td>
<td>7.7</td>
<td>12.2</td>
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<tr>
<td>T2</td>
<td>6.31</td>
<td>5.3</td>
<td>6.1</td>
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<tr>
<td>Treat mean</td>
<td>7.61</td>
<td>6.5</td>
<td>9.15</td>
</tr>
<tr>
<td>WUE (g/ML⁻²)</td>
<td></td>
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</tr>
<tr>
<td>T1</td>
<td>0.99</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>T2</td>
<td>1.6</td>
<td>3.5</td>
<td>6.3</td>
</tr>
<tr>
<td>Treat mean</td>
<td>1.29</td>
<td>2.35</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Geno mean, genotype mean; Treat mean, treatments mean; ns, not significant; n/a, not applicable; *, ** and *** significant difference at 0.05%, 0.01% and 0.001% probability level, respectively.

### Table 2: Table of correlations for the four *P. vulgaris* genotypes and lupin for reproductive dry matter (RDM), root to shoot ratio (R/S), total dry matter (TDM) and water use efficiency (WUE) during early post-anthesis to physiological maturity growth for the third harvest (H3) at physiological maturity. Significant correlations (p<0.05) are in bold.

<table>
<thead>
<tr>
<th>Characters</th>
<th>RDM</th>
<th>R/S</th>
<th>TDM</th>
<th>WUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RDM</td>
<td>-</td>
<td></td>
<td></td>
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<tr>
<td>R/S</td>
<td>0.1758</td>
<td>-</td>
<td></td>
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<tr>
<td>TDM</td>
<td>0.9391</td>
<td>0.4826</td>
<td>-</td>
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<tr>
<td>WUE</td>
<td>-0.5398</td>
<td>0.6012</td>
<td>-0.2977</td>
<td>-</td>
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