The importance of plant growth rate during and after waterlogging for lentil

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Abstract

Transient waterlogging caused by duplex or poorly drained soils, high rainfall, poor agricultural practices, or a combination is a major source of yield loss in lentil. We screened 111 lentil lines for response to waterlogging in 2019 and 2020 using a pot assay outdoors. At 484 ºCd after emergence (38 d) in 2019 and 452 ºCd after emergence (42 d) in 2020, plants were waterlogged for 184 ºCd (11d, 2019) and 167 ºCd (14 d, 2020) and allowed to recover for 323 ºCd (20 d, 2019) and 307 ºCd (26 d, 2020). Biomass at the end of recovery in the waterlogged plants varied 2.6-fold with genotype, and genotypic and phenotypic correlations showed associations with plant growth rate both during ($r_g = 0.92$; $r_p = 0.74$) and after waterlogging ($r_g = 0.75$; $r_p = 0.72$); there was no trade-off between maintenance of growth during waterlogging and growth during recovery. Biomass at the end of recovery of waterlogged plants relative to controls was associated with growth rate during recovery ($r_g = 0.88$, $r_p = 0.65$) and biomass at the end of waterlogging ($r_g = 0.61$, $r_p = 0.67$). Broad-sense heritability was 0.27 for growth rate during waterlogging, 0.37 for growth rate during recovery, 0.51 for biomass at the end of waterlogging, and 0.47 for biomass at the end of recovery. High biomass at the end of recovery correlated with cooler canopies but correlations varied with season and measurement date, and heritability of canopy temperature was low. We identified genotypes with consistently higher tolerance to waterlogging and provide an improved understanding of the physiological response of lentil to hypoxia highlighting the importance of growth rate not only during waterlogging but also during recovery.

Keywords

Biomass; canopy; grain legume; hypoxia; stress, temperature, phenotyping.

Introduction

A major limitation to lentil production is transient waterlogging (Solaiman et al., 2007; Malik et al., 2015; Wiraguna et al., 2017). High rainfall and soils with low infiltration rate, e.g., compacted or duplex soils where water accumulates in the sandy topsoil above the compacted clay layer, favour waterlogging. Cool season grain legumes are less tolerant to waterlogging than cereals and canola (Solaiman et al., 2007; Lapaz et al., 2020; Ploschuk et al., 2020). Of the cool season grain legumes, tolerance ranks faba bean ≈ lupin > chickpea > lentil > field pea (Jayasundara et al., 1997; Solaiman et al., 2007). Currently no reliable sources of waterlogging tolerance have been identified in lentil. The aim of this study was to screen 111 lentil lines to determine variation in response to 14-d of waterlogging at the mid-late vegetative stage. We focus on high-throughput phenotyping to quantify traits putatively associated with adaptation to water logging: growth rate and canopy temperature during and after waterlogging. Aerenchyma formation was investigated in two commercial lines with putative tolerance to waterlogging.

Methods

Plant material, environments and experimental design

The experiments were carried out outdoors at the Waite Campus, Adelaide, during two seasons. We tested 111 lines from the Australian lentil breeding program including 22 commercial varieties and 89 advanced breeder selections. Free-draining plastic pots were filled with 6-l of fully fertilised potting mix and each pot was placed inside a 10-l plastic bucket. Seven seeds per pot were sown at a depth of 5 cm on the 12th of August 2019 and 5th June 2020.

The experiment was a full factorial with 111 lines and two treatments, control and waterlogged, with three replicates. Treatments were laid out in a randomised complete block design with water regime allocated to block and line randomised within block. Soil water content in controls was maintained by regular irrigation up to field capacity. Waterlogging was achieved by filling buckets containing pots with water to the level of the soil surface, where it was maintained for the duration of the treatment. We began the waterlogging treatment in the mid vegetative period, at 484 ºCd after emergence (38 d after emergence, dae) in 2019 and 452 ºCd after emergence (42 dae) in 2020. Waterlogging was maintained for 184 ºCd (11 d) in 2019 and 167 ºCd (14 d) in 2020. Pots were drained and grown for a further 323 ºCd (20 d) in 2019 and 307 ºCd (26 d) in 2020.

Measurements and data analysis

During waterlogging we measured soil oxygen with a Galvanic-cell soil oxygen sensor placed approximately 7 cm below the soil surface. The plant biomass and growth rate were measured non-destructively using the method of Lake and Sadras (2021) combining 2D green canopy cover and plant height to work out a 3D measurement. Biomass was measured weekly from emergence until the start of waterlogging and twice-weekly during waterlogging and recovery. After the recovery period, shoots were harvested and oven dried until constant weight.

We measured canopy temperature on cloudless days once during waterlogging and twice after waterlogging with a FLIR C3 thermal imaging camera. To account for time-trends in ambient temperature during the measurement period, we report canopy temperature of waterlogged treatments as the difference in individual pot canopy temperature and the average temperature of the preceding 24 pots.

In 2020 we probed for aerenchyma in roots of PBA Greenfield and PBA Ace, which were identified as amongst the best performing commercial lines in response to waterlogging in 2019. The experimental design was a factorial with two lines, two treatments (control and waterlogged), and three durations of waterlogging with three replicates. Pots were sown with three seeds on the 20th July 2020 and waterlogged on the 25th of August for 7, 14 and 21 d. At the end waterlogging we sampled roots 4 cm below the cotyledon and analysed under Nikon Ni-E compound microscope at 40x objective and analysed for presence of aerenchyma.

We used ANOVA (Genstat) to test for the effects of year, treatment, line and their interaction on biomass at the end of recovery and related traits. Genetic and phenotypic correlations and broad sense heritability were calculated with META-R for Windows. From ANOVA and regressions, we report p-value as a continuous quantity, and Shannon information transform [$s = -\log_2(p)$] as a measure of the information against the tested hypothesis (Greenland, 2019).

Results

Shoot biomass at the end of waterlogging and end of recovery

Year, waterlogging treatment and line all affected biomass at the end of waterlogging ($p < 0.001$, $s > 9.9$), with interactions between year and waterlogging ($p < 0.001$, $s > 9.9$), waterlogging and line ($p = 0.009$, $s = 6.8$), and between year, line and waterlogging ($p = 0.015$, $s = 6.1$). Pooled across lines, biomass at the end of waterlogging was 2.1-fold larger in controls than in waterlogged plants. For waterlogged plants it ranged from 0.19 to 0.66 g p^{-1} between lines (Table 1). Heritability of biomass at the end of waterlogging was 0.51 for waterlogged plants and 0.50 for controls (Table 1). Shoot biomass at the end of recovery varied with line, waterlogging treatment and year (all $p < 0.001$, $s > 9.9$) and with the interactions between line and waterlogging ($p = 0.007$, $s = 7.2$), between year and waterlogging ($p = 0.001$, $s > 9.9$) and between year and line ($p = 0.054$, $s = 4.2$); the three way interaction had $p = 0.186$, $s = 2.4$. Heritability of biomass at the end of recovery was 0.47 in the waterlogged plants and 0.50 in controls. Across lines, treatments and years, shoot biomass at the end of recovery varied almost 4.5-fold from 0.41 to 1.85 g p^{-1}. Shoot biomass at the end of recovery varied with line from 0.41 to 1.07 g p^{-1} under waterlogging and from 1.10 to 1.85 g p^{-1} in controls.

Biomass at the end of recovery, as a percentage of the controls, varied with year and line ($p < 0.001$, $s > 9.9$), and with the interaction ($p = 0.014$, $s = 6.2$). It ranged from 27% in B35 to 72% in B40. For data pooled across seasons, the two top preforming commercial lines were PBA Greenfield, which averaged 62% and PBA Ace, which averaged 58%.

Table 1. Shoot biomass, plant growth rate and relative canopy temperature of 111 lentil lines grown under control and waterlogged conditions over two seasons.

Trait	Timing/period	Source of variation				Broad sense heritability[3]	
		Waterlogging		Line			
		Control[1]	Waterlogged[1]	Control[2]	Waterlogged[2]	Control	Waterlogged
Biomass (g p^{-1})	Start of waterlogging	0.30 ± 0.004	0.27 ± 0.004	0.17 - 0.44	0.16 - 0.47	0.62	
	End of waterlogging	0.79 ± 0.01	0.37 ± 0.007	0.46 - 1.10	0.19 - 0.66	0.50	0.51
	End of recovery	1.4 ± 0.01	0.71 ± 0.01	1.10 - 1.85	0.41 - 1.07	0.50	0.47
Plant growth rate (mg p^{-1} $^{o}Cd^{-1}$)	Before onset of waterlogging	0.64 ± 0.0009	0.60 ± 0.0043	0.37 - 0.94	0.34 - 1.00	0.62	
	During waterlogging	3.1 ± 0.043	0.62 ± 0.014	1.61 - 4.12	0.07 - 1.55	0.19	0.27
	During recovery	3.0 ± 0.029	1.0 ± 0.014	1.18 – 2.87	0.64 – 1.73	0.18	0.37
Canopy temperature difference (°C)	During waterlogging	1.4 ± 0.08	0.4 ± 0.09	-0.90 - 3.20	-1.80 - 1.56	Both < 0.01	Both < 0.01
	Early recovery	0.6 ± 0.06	1.2 ± 0.06	-2.17 - 2.70	-1.91 - 2.46	0.17, 0.05	0.21, <0.01
	Late recovery	0.17 ± 0.087	1.5 ± 0.08	-3.25 - 1.91	-3.07 - 3.41	<0.01, 0.14	0.22, < 0.01

[1] Mean ± s.e. [2] Range [3] Broad sense heritability is for the data pooled for two seasons except for canopy temperature, for which we report 2019, 2020 separately.

Plant growth rate during waterlogging and recovery

Line and waterlogging affected plant growth rate during waterlogging ($p < 0.001$, $s > 9.9$) but year did not ($p = 0.221$, $s > 2.2$). There were two way interactions – line and waterlogging, year and waterlogging (both $p < 0.001$, $s > 9.9$) – and a three way interaction ($p < 0.001$, $s > 9.9$), with interaction between year and line returning $p = 0.068$, $s = 3.9$. For data pooled across years, plant growth rate averaged 0.62 mg p^{-1} $^{o}Cd^{-1}$ for waterlogged plants and 3.1 mg p^{-1} $^{o}Cd^{-1}$ in controls, with heritability of 0.19 for control and 0.27 for waterlogged treatments (Table 1). Line, waterlogging and year affected plant growth rate during recovery ($p < 0.001$, $s > 9.9$, Fig. 1c). There were interactions between line and waterlogging ($p = 0.011$, $s = 6.5$), line and year ($p = 0.051$, $s = 4.3$), but not waterlogging and year ($p = 0.311$, $s = 1.7$) or three way interaction ($p = 0.462$, $s = 1.1$). For the pooled data the difference between controls and waterlogged treatments was reduced to 3-fold during the recovery with controls growing at 3.0 mg p^{-1} $^{o}Cd^{-1}$ and the waterlogged plants growing at 1.0 mg p^{-1} $^{o}Cd^{-1}$. For the waterlogged treatment heritability was 0.37 and 0.18 for controls (Table 1).

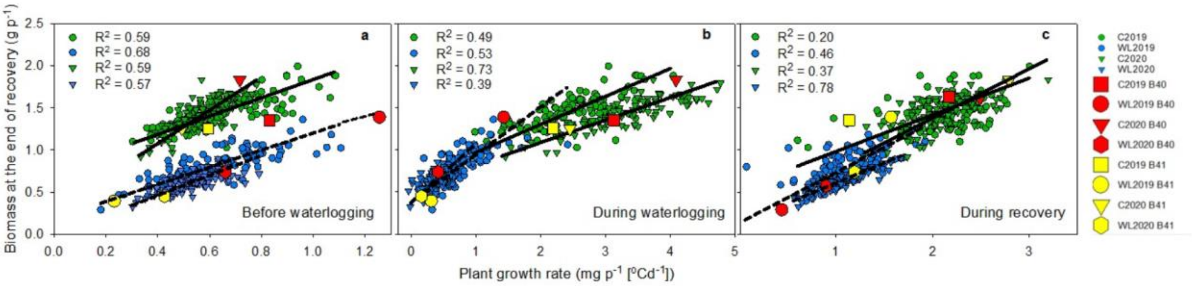

Fig. 1. Relationships between biomass at the end of recovery and plant growth rate before, during and after waterlogging. Symbols are control (green), waterlogging (blue), 2019 (circle) and 2020 (triangle); contrasting B40 (red) and B41 (yellow) lines are also indicated for each treatment and year. Dashed lines indicate waterlogging and solid lines are controls. In all cases, regressions returned $p < 0.001$ and $s > 9.9$.

Associations between biomass at the end of recovery and plant growth rate

Across years and treatments, biomass at the end of recovery associated with plant growth rate before, during and after waterlogging (Fig. 1). Biomass at the end of recovery in waterlogged treatments associated more strongly with plant growth rate ($0.39 < R^2 < 0.78$) than with relative growth rate ($0.03 \leq R^2 \leq 0.65$). Fig. 1 highlights extreme lines, B40 and B41. B40 was in the top 10% for growth rate during waterlogging, biomass at the end of waterlogging and biomass at the end of recovery in both absolute terms and relative to control. Line B41 was consistently in the bottom 10% for these traits. PBA Greenfield ranked in the top 13% for growth rate during waterlogging, top 6% for biomass at the end of waterlogging, and for biomass at the end of recovery in absolute terms top 15% and top 8% relative to control. PBA Ace ranked in the top 25% for growth rate during waterlogging, top 20% for biomass at the end of waterlogging, and for biomass at the end of recovery in absolute terms top 27% and 20% relative to control.

Canopy temperature and aerenchyma

Canopy temperature, biomass at the end of recovery and their relationship are presented in Fig. 2. In 5 out of 6 cases resulting from the combination of measurement period and year, higher biomass at the end of recovery associated with cooler canopies; the exception was the lack of association during waterlogging in 2019. However heritability of canopy temperature was low and inconsistent (Table 1). We found no evidence of aerenchyma formation in PBA Greenfield and PBA Ace under waterlogging (not shown).

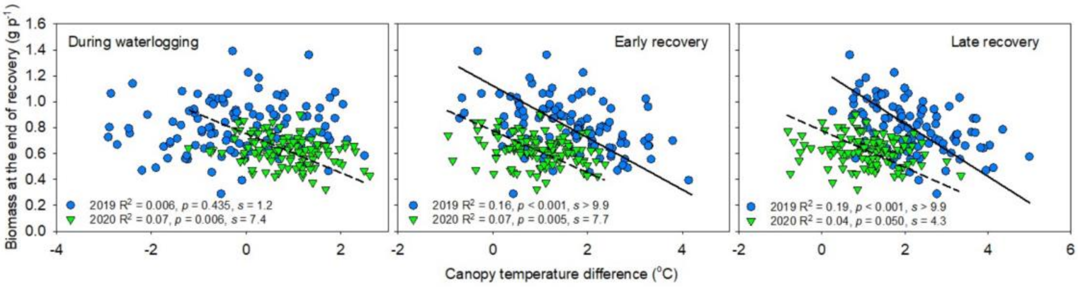

Fig. 2. Relationships between biomass at the end of recovery of waterlogged plants and canopy temperature difference during waterlogging, early and late recovery. Data are for 2019 (blue circle) and 2020 (green triangle). Solid lines indicate 2019 and dashed lines are 2020.

Conclusion

We found genotypic differences in tolerance to hypoxia associated with plant growth rate both during and after waterlogging, with no trade-off. Selection for growth rate during or after waterlogging are promising traits; however, heritability was higher during recovery. Heritability was also high for plant biomass at the end of waterlogging, which associated with biomass at the end of recovery. Cooler canopies primarily in the recovery period were associated with greater biomass at the end of recovery but the heritability of this trait was low limiting its application for selection.

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