

## Research Article

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# Tolerance of three grain legume species to transient waterlogging

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**Abstract.** Waterlogging reduces the yield of food crops. Tolerance to waterlogging could vary between and within species. This study aimed to quantify tolerance to soil waterlogging in two divergent genotypes of pea (*Pisum sativum*), two of lentil (*Lens culinaris*) and a grasspea (*Lathyrus sativus*) control at germination and during vegetative growth. Following germination, seeds were grown for 14 days in soil waterlogged with the water table 10 mm below the surface, and then by draining the pots and allowing to recover for 21 days—to be compared with 35 days of continuous waterlogging. In both pea and lentil, the pair of genotypes contrasted widely with large-seeded pea genotype Kaspera and lentil genotype Nugget showing higher (2-fold) root porosity and less effect on shoot nitrogen content under waterlogging than the other genotypes (NPE and ATC). During recovery, the same two genotypes—Kaspera pea and Nugget lentil—also recovered better than their smaller-seeded species pairs. Soil waterlogging at 10 mm depth had no significant effect on shoot and root dry mass after 14 days. Root penetration into waterlogged soil was restricted to ~100 mm depth and its distribution altered for pea and lentil genotypes but not for grasspea. Within the small sample studied, we demonstrated a significant genetic variation in both pea and lentil in tolerance to waterlogging after germination and subsequent recovery for the first time. Screening of additional pea and lentil germplasm for waterlogging conditions is clearly warranted.

**Keywords:** Chlorophyll; intra-species variation; legume; porosity; waterlogging.

## Introduction

Soil waterlogging is a common abiotic stress that impacts on crop production (Jackson and Colmer 2005). Seeds of some species can withstand waterlogged conditions (i.e. prolonged soaking) more than others (Crawford 1977). The effects of hypoxia and/or anoxia during vegetative growth of dryland crops are well documented; shoot

and root growth decrease and nutrient uptake becomes inhibited (pea, Cannell *et al.* 1979; lupin, Davies *et al.* 2000; wheat, Malik *et al.* 2002; barley, Pang *et al.* 2004; chickpea, Palta *et al.* 2010). Photosynthesis is also severely inhibited in waterlogging-sensitive plants resulting in reduced dry matter accumulation (wheat, Malik *et al.* 2001; barley, Pang *et al.* 2004). Inhibition of nitrogen

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nutrition reduces chlorophyll concentration of leaves resulting in premature leaf senescence (wheat, Malik et al. 2002; soya bean, Youn et al. 2008). Increased porosity through the development of aerenchyma tissue in root systems enhances the tolerance to waterlogging by facilitating oxygen movement via diffusion from shoots to roots (Colmer 2003). However, the response of a crop to waterlogging depends on the timing (developmental stage of the plant when stress was imposed), duration (number of days) and genotypic variation in waterlogging tolerance (Setter and Waters 2003).

Food legumes, a major dietary protein source (Erskine 2009), are grown on 77 million hectare worldwide [Food and Agriculture Organization (FAO) 2013] and often affected by waterlogging. Legume crops such as lupin (*Lupinus angustifolius*), chickpea (*Cicer arietinum*), lentil (*Lens culinaris* subsp. *culinaris*) and field pea (*Pisum sativum*) are susceptible to waterlogging at vegetative stages (Siddique et al. 1993; Siddique and Sykes 1997; Yu and Rengel 1999; Palta et al. 2010). In a detailed comparison of the differing responses of food legumes to waterlogging during vegetative growth, Solaiman et al. (2007) found faba bean (*Vicia faba*) as the most tolerant and field pea the least; with yellow lupin (*Lupinus luteus*), grasspea (*Lathyrus sativus*), narrow-leaved lupin, chickpea and lentil as intermediate (Solaiman et al. 2007). Within-species genetic variation in waterlogging tolerance is present in lupin (Davies et al. 2000), faba bean (Solaiman et al. 2007), soya bean (Henshaw et al. 2007), lotus (Real et al. 2008) and chickpea (Palta et al. 2010).

Waterlogged soil restricts food legume establishment in rice-based cropping systems in the Eastern Gangetic Plains of Bangladesh, Eastern India and Nepal (Ali et al. 2009). In such systems, pea, lentil, chickpea, grasspea and soya bean (*Glycine max*) are sown on residual soil moisture after paddy rice (Awadhwal et al. 2001). Relay sowing, the practice of broadcasting seed into a standing rice crop prior to harvest, predominates in Nepal for lentil and in Bangladesh for grasspea, and relay sown pea is also found (A. I. Malik, pers. obs.). Malik et al. (2015) recently found that the substitution of relay sown lentil for fallow was a useful option to intensify cropping in the Eastern Gangetic Plain. At the time of relay sowing, excess soil moisture and puddles are common features in puddled soil and such crops face transient soil waterlogging from an early developmental stage. Crawford (1977), Jackson (1979) and Sarlistyaningsih et al. (1995) demonstrated variation in germination of pea, bean and lupin seeds under different durations of waterlogging or anoxic conditions. Poor germination in waterlogged soil is a major impeding factor for crop establishment (Ramakrishna et al. 2000). Setter and Waters (2003) emphasized the need for future research on waterlogging

tolerance during germination. Knowledge on waterlogging tolerance from germination to crop establishment in lentil and pea is scant. This study aimed to assess waterlogging tolerance in contrasting pairs (seed size and origins—Table 1) of pea and lentil genotypes—compared with a grasspea, considered waterlogging tolerant (Purseglove 1968), control—in the period after germination to vegetative growth and during subsequent recovery.

## Methods

### Experimental design

The experimental design was factorial with treatments of genotypes (5) (Table 1) × waterlogging (3) in a completely randomized block design with four replications. A pot was the experimental unit. Genotype pairs (pea and lentil) were selected for their contrasting seed size and origins (Table 1).

Genotypes were sown in pots either free-drained (control) or waterlogged to 10 mm below the soil surface at the start of the experiment. This water table depth best represents relay sowing conditions in the field (Ali 2011). Fourteen days after sowing (DAS), waterlogged pots were either continued as waterlogged or allowed to drain. This gave the three treatments: (i) drained control, (ii) waterlogged (i.e. continuously waterlogged for 35 days) and (iii) recovery (14 day of waterlogging followed by 21 days recovery).

After 14 DAS, 4 waterlogged and 4 control pots were harvested (H1); and 4 pots were allowed to drain from each genotype. The pots were randomly re-positioned every 7 days to minimize glasshouse positional effects.

The experiment was conducted during the winter (July–August) of 2012 in the glasshouse of the University of Western Australia Plant Growth Facility. For the period of the experiment, minimum and maximum temperatures during the day were 16.9 and 23.9 °C, respectively; and photoperiod 10.5 h, light intensity 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .

**Plant culture.** Seeds of two pea, two lentil and a grass pea genotype(s) (Table 1) were surface sterilized with 1 %

**Table 1.** Names, country of origin and 100 seed weight of five legume genotypes.

Crop	Name	Origin	100 seed weight (g)
Pea	Kaspa	Australia	27.3 ± 1.4
	NPE 1191.515	Pakistan	7.2 ± 0.3
Lentil	Nugget	Australia	4.1 ± 0.5
	ATC 70856	Bangladesh	1.8 ± 0.6
Grasspea	Ceora	Australia	11.4 ± 1.1

commercial bleach (active ingredients NaOCl 40 mg L<sup>-1</sup>) for 1 min, washed 3–5 times with deionized water and placed on moist filter paper (Whatman no. 1) in 90 mm Petri dishes in a dark cabinet at room temperature overnight to imbibe. Seeds of grasspea were imbibed 3 days before the other genotypes (grasspea took longer to imbibe in a preliminary trial).

Four imbibed seeds were sown in each pot for lentil and grasspea and two seeds for pea genotypes. Seeding rate in the pots was decided according to reported field rates [i.e. 100–120 seeds m<sup>-2</sup> for lentil and 45–55 seeds m<sup>-2</sup> for pea (White et al. 2005)]. Seeds were placed on the surface of free-draining plastic pots (height 140 mm, diameter 120 mm) and each pot was placed in a closed-bottom plastic pot (height 180 mm, diameter 200 mm). Waterlogged treatment was imposed by filling the closed-bottom pots with DI-H<sub>2</sub>O up to 10 mm below the soil surface of free-draining pots. Water table was maintained by adding water every day. Control pots were weighed to 80 % of the field capacity at the start and retained at that weight by adding water every second day. Pots contained gravel at the bottom and 1.2 kg of mixed washed river sand and potting mix (1 : 1) (pH = 6.3 and EC (1 : 5 weight/volume in water extract) = 0.197 dS m<sup>-1</sup>). The potting mix contained 5 : 2 : 3 composted pine bark, coco peat and brown river sand. Rhizobium was not added to substrate. While filling the pots, platinum (Pt) electrodes were inserted in the substrate in 15 pots at a depth of 100 mm for redox measurement with three treatments for each genotype. Potting mix and river sand contained NH<sub>4</sub><sup>+</sup> 0.004 and 0.018, NO<sub>3</sub><sup>-</sup> 0.062 and 0.04, K<sup>+</sup> 0.0005 and 0.0009 μmol kg<sup>-1</sup> substrate, respectively.

The following nutrients were applied once 15 DAS which allow plants to establish on seed reserve prior to fertilization. Nutrients (μmol kg<sup>-1</sup> substrate): KNO<sub>3</sub> 0.15, Ca(NO<sub>3</sub>)<sub>2</sub> · 4H<sub>2</sub>O 0.375, KH<sub>2</sub>PO<sub>4</sub> 0.191, MgSO<sub>4</sub> · 7H<sub>2</sub>O 0.025, KCl 0.0030, H<sub>3</sub>BO<sub>3</sub> 0.0012, MnSO<sub>4</sub> · H<sub>2</sub>O 0.0002, ZnSO<sub>4</sub> · 7H<sub>2</sub>O 0.0005, CuSO<sub>4</sub> · 5H<sub>2</sub>O 0.00008, Na<sub>2</sub>MoO<sub>4</sub> · 2H<sub>2</sub>O 0.00008 and NiSO<sub>4</sub> · 6H<sub>2</sub>O 0.00025.

## Measurements

**Redox.** Redox potential was measured daily in 15 pots (i.e. 6 waterlogged, 6 recovery and 3 control) with Pt electrodes and a silver/silver chloride reference electrode attached to a millivolt-meter. The reading was corrected as described by Patrick et al. (1996).

**Growth.** Harvested plants were divided into shoots and roots. All plant parts were dried for 72 h at 59 °C. Plant biomass at H1 and final harvest was measured by weighing the dry mass of the shoots and roots. The root

lengths of main axis and the longest lateral roots were measured with a ruler at harvest.

**Porosity.** Porosity (% gas space per volume) of main and lateral roots was determined at in the final harvest following the principle described by Raskin (1983) and as modified by Thomson et al. (1990). Briefly, roots were lined up and cut into 50 mm segments on a tray containing water to avoid root drying. Roots were weighed submerged and then vacuum infiltration was carried out by subjecting the submerged tissue to low pressure with a vacuum pump three times to ensure the exit of all air from the roots. The fresh mass of the roots in the air was recorded and corrected using the equation of Thomson et al. (1990).

**Nitrogen, chlorophyll concentration and chlorophyll fluorescence.** Oven-dried pulverized samples were analysed for total nitrogen (N) in shoots using an auto analyser (Elementer, Model: Vario Macro, Hanau, Germany) against ethylenediaminetetraacetic acid and rice flour as standards. Total N was calculated on shoot dry mass basis. Relative changes in chlorophyll concentration were assessed thrice during the experiment at 21, 27 and 34 DAS on the youngest fully-expanded leaves of all plants in each pot with a hand-held chlorophyll meter (Minolta SPAD 502, Osaka, Japan).

Chlorophyll fluorescence was measured at 34 DAS on the youngest fully-expanded leaves of 1 plant in each pot after keeping the leaves in the dark for 30 min prior to measurement by using plant efficiency analyser (PEA) (Hansatech Instrument Ltd, UK). Measurements were taken at 50 % light intensity and the exposure time was set at 5 s. To obtain optimum light saturation point, a series of measurements taken in a preliminary experiment to ensure photosynthetic apparatus response to light and the accuracy of measurement for fluorescence. The  $F_0$  (minimal fluorescence),  $F_m$  (maximal fluorescence),  $F_v$  (variable fluorescence) and  $F_v/F_m$  were recorded.

## Statistical analysis

Two-way analyses of variance (ANOVAs) were performed using GenStat 14th edition (VSN International) to determine the effects of plant genotype (2 pea, 2 lentil and 1 grass pea), waterlogging (well drained, waterlogged for 14 days, waterlogged for 35 days) and their interaction on the following response variables: main root length, main root porosity, shoot chlorophyll and nitrogen concentration, shoot and root mass. If main effects or interactions were significant, we then proceeded with multiple comparison tests to compare differences among means using Tukey's test. Separate analyses were performed on data collected by Day 14 and on data collected after 35 days of treatment.

**Table 2.** Degrees of freedom (df), *F* values and probabilities of two-way ANOVA. <sup>1</sup>Values in parentheses indicate number of missing plots.

Character	Source of variation	Genotype (G)	Treatment (T)	G × T	Residual
Waterlogging 14 days					
Shoot mass	df	4	1	4	25 (5) <sup>1</sup>
	<i>F</i> value	9.11	0.05	1.24	
	Probability	<0.001	0.825	0.318	
Root mass	<i>F</i> value	9.31	2.82	0.86	
	Probability	<0.001	0.105	0.502	
Main root length	<i>F</i> value	1.31	29.28	3.12	
	Probability	0.295	<0.001	0.033	
Shoot nitrogen	df	4	1	4	22 (8) <sup>1</sup>
	<i>F</i> value	18.31	21.60	0.49	
	Probability	<0.001	0.002	0.746	
Waterlogging 35 days					
Shoot mass	df	4	2	8	42 (3) <sup>1</sup>
	<i>F</i> value	88.01	0.56	0.30	
	Probability	<0.001	0.574	0.961	
Root mass	<i>F</i> value	21.85	1.13	0.26	
	Probability	<0.001	0.331	0.976	
Main root length	<i>F</i> value	4.30	19.54	1.09	
	Probability	0.005	<0.001	0.388	
Shoot nitrogen	<i>F</i> value	2.55	30.93	3.17	
	Probability	0.053	<0.001	0.007	
Chlorophyll concentration	<i>F</i> value	31.63	341.93	29.01	
	Probability	<0.001	<0.001	<0.001	
Chlorophyll fluorescence	<i>F</i> value	2.09	4.59	1.60	
	Probability	0.100	0.016	0.153	
Root porosity	df	4	2	8	30 (15) <sup>1</sup>
	<i>F</i> value	8.59	6.07	0.99	
	Probability	<0.001	0.006	0.463	

## Results

### Redox potential

Redox potential was  $365 \pm 23$  mV in the drained control pots and  $200 \pm 4$  mV in the waterlogged pots at the start of the experiment. After 14 days the redox potential had declined to  $173 \pm 40$  mV and it was  $70 \pm 10$  mV by 35 days in waterlogged pots at the end of the experiment. After draining waterlogged pots on 14 DAS, the redox potential increased to  $248 \pm 55$  mV after 2 days and to  $403 \pm 21$  mV after 7 days of recovery. The redox potential of control pots remained close to 400 mV throughout the experimental period (data not shown).

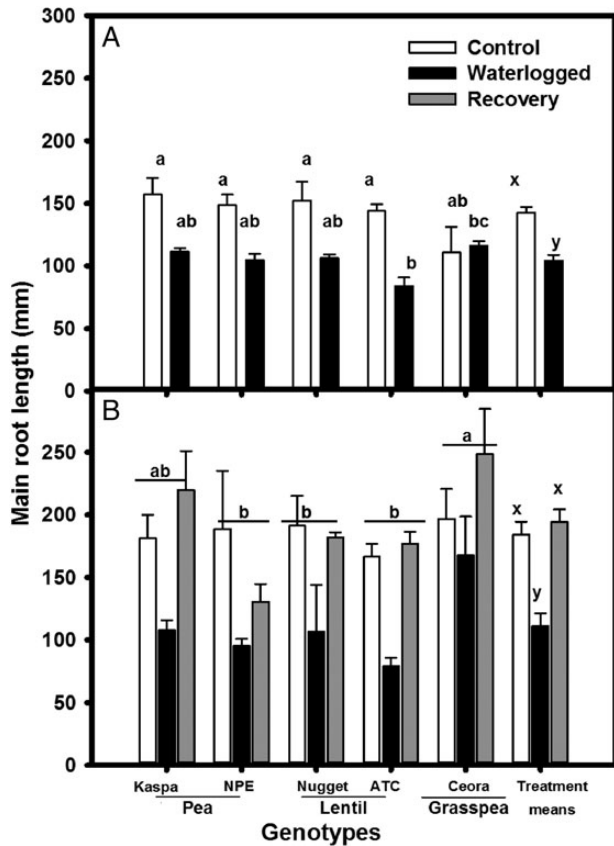
### Root growth

Roots of plants grown in drained soil reached the base of pots after 14 days of treatment for all genotypes. Overall root length was significantly ( $P < 0.001$ , Table 2) shorter in plants grown in waterlogged soil for 14 days than in the drained control in all pea and lentil genotypes (Fig. 1A). However, root length in grasspea was unaffected by waterlogging (Fig. 1A).

Again with the exception of grasspea, the extended waterlogging treatment stopped main root extension in all genotypes when the duration of waterlogging was prolonged from 14 to 35 days (Fig. 1A and B). On those

pots, where the waterlogging stress was ceased after 14 days, main roots continued to elongate and reach the length of roots on continuously drained pots in all genotype except pea—NPE (Fig. 1B).

As with root length, root dry mass differed significantly ( $P < 0.001$ , Table 2) among legume genotypes when



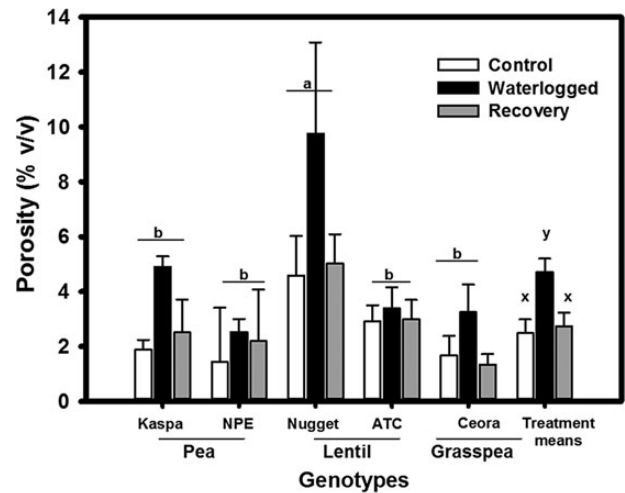
**Figure 1.** Main root length (mm) of legume genotypes (A) after 14 days and (B) after 35 days of waterlogging. Treatments were drained control (white bars), continuously waterlogged (black bars), 14 days waterlogged and subsequent 21 days of recovery (grey bars). Values are the means of four replicates standard errors. Overall treatment (+SE) means are shown grouped on the right. Means associated with different letters are significantly different ( $P < 0.05$ ) by Tukey's test.

grown in drained soil; however, there was no effect of waterlogging on root dry mass (Table 3).

**Porosity of main and lateral roots**

In drained soil, main root porosity was highest (i.e. 4.6 %) for lentil—Nugget; and this value was ~2-fold higher than any other legume genotype in the study. Thirty-five days of waterlogging significantly ( $P > 0.001$ , Table 2) increased main root porosity in all legume genotypes (Fig. 2). Porosity increased for Kaspa, Nugget and grasspea by 2-fold, NPE by 1.1-fold and ATC by 1.4-fold in plants grown in waterlogged soil compared with the drained control.

Main root (i.e. tap root) porosity of plants that were allowed to drain after 14 days of waterlogging returned towards the control value in all genotypes (Fig. 2A) due



**Figure 2.** Effect of waterlogging treatment on root systems of legume genotypes main root porosity (% gas per unit volume after 35 days of growth). Treatments were drained control (white bars), continuously waterlogged (black bars), 14 days waterlogged and subsequent 21 days of recovery (grey bars). Values are the means of four replicates standard errors. Overall treatment (+SE) means are shown grouped on the right. Means associated with different letters are significantly different ( $P < 0.05$ ) by Tukey's test.

**Table 3.** Root mass of five legume genotypes after 14 and 35 days of treatment in control, fully-drained soil; waterlogged, water table 10 mm below soil surface; recovery, after 14 days of waterlogging pots were allowed to drain. Means associated with different letters are significantly different ( $P < 0.05$ ) by Tukey's test. Values are the means of four replicates.

Crop	Name	Root mass (mg plant <sup>-1</sup> ) 14 days			Root mass (mg plant <sup>-1</sup> ) 35 days			
		Control	Waterlogged	Mean	Control	Waterlogged	Recovery	Mean
Pea	Kaspa	114 ± 22	91 ± 20	102.5 ac	359 ± 12	391 ± 30	348 ± 30	332.5 a
	NPE	42 ± 10	40 ± 9	40.9 ab	432 ± 14	384 ± 110	433 ± 90	416.3 a
Lentil	Nugget	55 ± 10	38 ± 8	46.6 ab	177 ± 23	133 ± 11	169 ± 32	159.6 bc
	ATC	20 ± 7	27 ± 8	23.3 c	97 ± 21	46 ± 1	84 ± 3	75.6 c
Grasspea	Ceora	90 ± 20	48 ± 4	69.6 ab	151 ± 110	192 ± 20	244 ± 24	195.8 b

**Table 4.** Shoot mass of five legume genotypes after 14 and 35 days of growth in control, fully-drained soil; waterlogged, water table 10 mm below soil surface; recovery, after 14 days of waterlogging pots were allowed to drain. Means associated with different letters are significantly different ( $P < 0.05$ ) by Tukey's test. Values are the means of four replicates.

Crop	Name	Shoot mass ( $\text{mg plant}^{-1}$ ) 14 days			Shoot mass ( $\text{mg plant}^{-1}$ ) 35 days			
		Control	Waterlogged	Mean	Control	Waterlogged	Recovery	Mean
Pea	Kaspa	84 ± 21	59 ± 20	71.3 a	1680 ± 140	1406 ± 140	1475 ± 250	1496 a
	NPE	42 ± 4	60 ± 20	50.8 ab	1157 ± 110	1014 ± 110	1060 ± 200	1077 b
Lentil	Nugget	21 ± 4	23 ± 2	21.7 b	293 ± 40	232 ± 52	274 ± 23	266 c
	ATC	25 ± 10	32 ± 11	28.6 b	194 ± 31	155 ± 24	202 ± 33	184 c
Grasspea	Ceora	20 ± 3	26 ± 2	21.9 b	341 ± 110	427 ± 32	429 ± 53	399 c

to resumed growth after drainage of the pots. Lateral root porosity demonstrated a similar pattern to main roots when grown in drained and in waterlogged soil as well as at recovery (data not shown). Waterlogging increased (both main and lateral) porosity over the control due to the formation of aerenchyma (visual observation under microscope—data not shown).

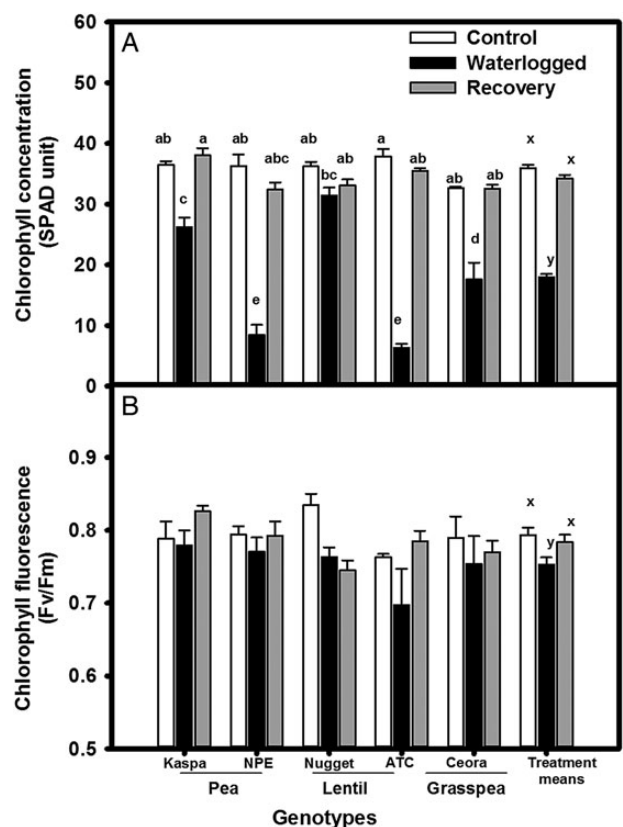
### Shoot growth and chlorophyll concentration

Shoot dry mass differed significantly among legume genotypes grown in drained soil. Waterlogging had no effect on shoot dry mass compared with the drained control (Table 4). However, waterlogging reduced leaf chlorophyll concentration significantly in all genotypes when compared with the drained control ( $P < 0.001$ , Table 2) (Fig. 3A). At the end of the waterlogging treatment period (35 DAS), there was a significant interaction ( $P < 0.001$ , Table 2) of treatment  $\times$  within-species variation, indicating that chlorophyll concentration in small-seeded pea (NPE) and lentil (ATC) were markedly reduced compared with the larger-seeded pea Kaspa and lentil Nugget. After only 7 days of recovery, chlorophyll concentration started to recover (data not shown) and recovered to the control value by the end of the experiment (Fig. 3A).

Chlorophyll fluorescence (maximum quantum efficiency  $F_v/F_m$ ) on the youngest fully developed leaves was reduced by waterlogging in all legume genotypes compared with drained control ( $P < 0.05$ , Table 2) (Fig. 3B). The reduction was more pronounced in lentil than in pea. The chlorophyll fluorescence of the youngest fully-expanded leaf of plants previously waterlogged recovered to the control value for all legumes except for lentil Nugget (Fig. 3B).

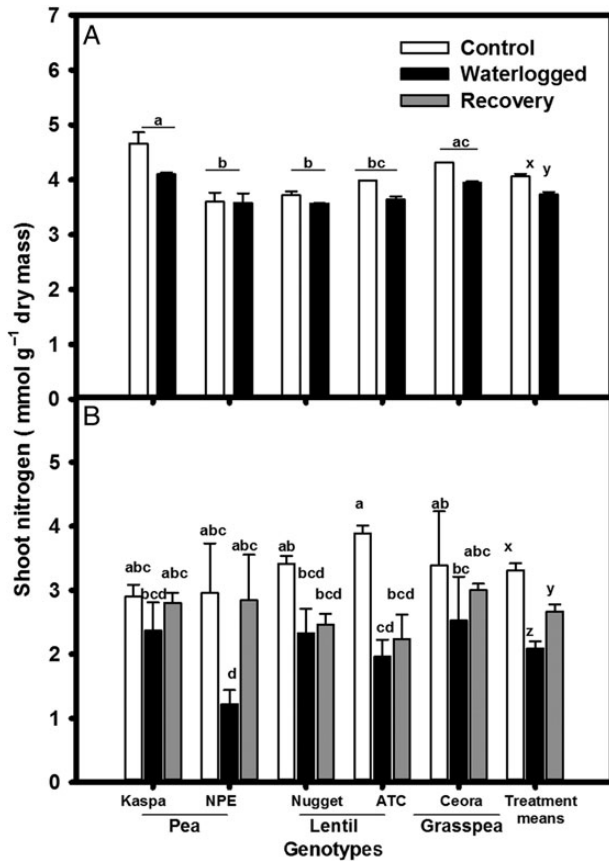
### Nitrogen concentration

Total shoot nitrogen concentration decreased over time in all plants grown in drained conditions (Fig. 4A and B). Fourteen days of waterlogging significantly ( $P < 0.01$ , Table 2) reduced nitrogen concentration on average



**Figure 3.** Effect of waterlogging treatment on youngest fully-expanded leaves. (A) Chlorophyll concentration (SPAD unit) and (B) chlorophyll fluorescence ( $F_v/F_m$ ) of legume genotypes after 35 days of growth. Treatments were drained control (white bars), continuously waterlogged (black bars), 14 days waterlogged and subsequent 21 days of recovery (grey bars). Values are the means of four replicates standard errors. Overall treatment (+SE) means are shown grouped on the right. Means associated with different letters are significantly different ( $P < 0.05$ ) by Tukey's test.

(Fig. 4A), which decreased further when the treatment was prolonged to 35 days (Fig. 4B). The reduction of total shoot nitrogen varied after 35 days of treatment



**Figure 4.** Effect of waterlogging treatment on shoot nitrogen ( $\text{mmol g}^{-1}$  dry mass) of legume genotypes (A) after 14 days and (B) after 35 days of growth. Treatments were drained control (white bars), continuously waterlogged (black bars), 14 days waterlogged and subsequent 21 days of recovery (grey bars). Values are the means of four replicates standard errors. Overall treatment (+SE) means are shown grouped on the right. Means associated with different letters are significantly different ( $P < 0.05$ ) by Tukey's test.

and the interaction of treatments with both between-species and within-species reached significance. This reduction was greatest in the small-seeded pea NPE and lentil ATC with 35 days of waterlogging. With 21 days of recovery following waterlogging, the nitrogen level in both pea genotypes and grasspea had recovered to the level of plants grown continuously in drained soil (Fig. 4B). However, the lentil genotypes did not recover similarly.

## Discussion

The results demonstrated a significant variation in waterlogging tolerance within legumes. In contrast to other studies, in which seedlings of food legumes were exposed to waterlogging (Davies et al. 2000; Solaiman et al. 2007; Palta et al. 2010; Bramley et al. 2011), the current study focussed on transient waterlogging tolerance: after aerobic

imbibition where seeds were germinated on the surface of waterlogged soil, and their growth in waterlogged soil and recovery from waterlogging stress were assessed.

### Variation in waterlogging tolerance among legume genotypes

Genetic variation in waterlogging tolerance within legume species has been demonstrated in lupin (Davies et al. 2000), faba bean (Solaiman et al. 2007), soya bean (Henshaw et al. 2007; Youn et al. 2008), lotus (Real et al. 2008), chickpea (Palta et al. 2010) and in the current study in both lentil and pea. The genotypes for this experiment were selected as contrasting pairs of pea and lentil with one small-seeded genotype each from South Asia, where there is a history of relay-sowing and germination under waterlogged conditions, and the other genotype—a larger-seeded Australian cultivar.

Following germination, important traits for waterlogging tolerance are the ability to increase root porosity during waterlogging, and for roots to recover during transient waterlogging; and to maintain shoot nitrogen and leaf chlorophyll (Malik et al. 2001). Considering these traits, the large-seeded genotypes from Australia (pea–Kaspa and lentil–Nugget) demonstrated greater waterlogging tolerance than their contrasting small-seeded pairs originating from South Asia (pea–NPE and lentil–ATC), presumably, due to greater carbohydrate pool and early vigour.

Enhanced root porosity contributes to better aeration in the root system in waterlogged soil (Colmer 2003). In both food (Solaiman et al. 2007) and pasture legumes (Gibberd et al. 1999) higher root porosity was observed in tolerant genotypes compared with sensitive genotypes. In the current experiment, tolerant legume cultivars showed enhanced root porosity by the formation of aerenchyma when grown in waterlogged substrate compared with sensitive genotypes. We confirmed the presence of aerenchyma by cross section of the main axis of the root (data not shown). Root porosity increased (up to 10%) with growth in waterlogged conditions; however, constitutive root porosity was very low ( $\sim 2\%$ ) and these values are much lower than those reported for waterlogging-tolerant legumes (Gibberd et al. 2001); all the species (and genotypes within species) responded to the treatment by increasing porosity with different degree of augmentation. Formation of adventitious roots is one trait that contributes to waterlogging tolerance; however, we did not observe adventitious root development in any of the legume genotypes; although, there is evidence for lentil to form adventitious roots when grown in waterlogged substrate (Erskine et al. 1994; Solaiman et al. 2007). In pea there are no reports of the development of adventitious roots (Jackson 1979). The discrepancy of the results from previous findings

could be due to the experimental conditions. In the current study, the seeds were germinated aerobically on waterlogged soil in contrast to earlier experiments where 12- to 21-day-old seedlings were exposed to waterlogging.

Despite enhanced root porosity, waterlogging restricted root penetration into the substrate of pea and lentil (present study), wheat (Malik et al. 2001, 2002), soya bean (Henshaw et al. 2007), lupin (Davies et al. 2000; Real et al. 2008; Bramley et al. 2011) and chickpea (Palta et al. 2010). Restricted main root length was due to inadequate O<sub>2</sub> diffusion to the root tip in waterlogged soil. According to the model of Armstrong (1979) with a given porosity (4–8 %, see Fig. 2) O<sub>2</sub> could only diffuse down to 80–160 mm without any radial loss of oxygen along the root length. This demonstrates that the radial leakage of O<sub>2</sub> along the root length and/or higher O<sub>2</sub> uptake rate restricted roots to achieve desired root length. In contrast, grasspea main root length was not restricted by waterlogging (Fig. 1A). Presumably, grasspea formed a barrier to radial loss of O<sub>2</sub>. Solaiman et al. (2007) demonstrated the greater waterlogging tolerance of grasspea compared with lentil and pea. Further research is warranted to confirm the hypothesis.

Decreased shoot nitrogen in response to waterlogging has been well documented—wheat (Huang et al. 1994; Malik et al. 2001, 2002), soya bean (Riche 2004) and food legumes (Solaiman et al. 2007). Nitrogen reduction varied between legume genotypes under waterlogging due to effects on nitrogen fixation and the number of nodules (Cannell et al. 1979; Bacanamwo and Purcell 1999; Riche 2004; Bedard-Haughn 2009). The reduction of shoot nitrogen in waterlogged crops was due to a decrease in nutrient uptake (Malik et al. 2002; Solaiman et al. 2007; Palta et al. 2010). Waterlogging causes stelar anoxia to develop in the root (Aguilar et al. 2003), which reduced the loading of nutrients into the translocation stream, and restricted nitrogen supply to the shoot (Malik et al. 2001). In the current experiment, those genotypes able to enhance root porosity under waterlogging managed to maintain shoot N better—as in lotus (James and Sprent 1999; Striker et al. 2012, 2014) and soya bean (Shimamura et al. 2003; Thomas et al. 2005)—than those with low root porosity under waterlogging. Variation within species in chlorophyll retention in waterlogging conditions was reported in soya bean (Youn et al. 2008) and canola (Ashraf and Mehmood 1990), and tolerant genotypes with higher chlorophyll concentration identified (Talbot et al. 1987; Ashraf and Mehmood 1990; Pang et al. 2004; Youn et al. 2008). Once again, in this respect pea–Kaspa and lentil–Nugget showed better performance than other genotype pairs while the control grasspea also performed well.

### Effect of waterlogging on growth

Waterlogging reduced shoot growth in wheat (Malik et al. 2002), lotus (Mendoza et al. 2005) and chickpea (Palta et al. 2010). Other studies mentioned no significant effect of waterlogging on shoot growth in lupin, pea, lentil, faba bean, chickpea and grasspea during the treatment period (i.e. 7 days), but the effect of waterlogging appeared on plants during the recovery period (Yu and Rengel 1999; Solaiman et al. 2007). We did not find an effect of waterlogging on shoot and root mass in our experiment. In previous studies, seedlings of 12–21 days were exposed to waterlogging; however, in the current experiment seeds were germinated on waterlogged soil and exposed to soil waterlogging at initial establishment; and as the water table in the pots was 10 mm below the soil surface, roots accessed aerobic conditions on the top layer of the substrate. Increase in shoot dry weight in wheat has been reported when exposed to a short period (e.g. 8 days) of waterlogging due to carbohydrate accumulation (Trought and Drew 1980). Moreover, roots responded to waterlogging by altering their distribution pattern and producing numerous lateral roots (visual observation). These roots grew close to the soil surface to obtain oxygen under waterlogging conditions (Voeselek et al. 1999), as was found in various subspecies of maize (*Zea mays* ssp. *huehuetenangensis*) (Mano et al. 2005a, b) and in *Brassica napus* (Cannell and Belford 1980).

### Recovery from waterlogging

Root length recovers from waterlogging once allowed to grow in drained conditions. In wheat, stored carbohydrate was preferentially allocated to the re-growth of the root system during recovery (Malik et al. 2001, 2002). Root length recovered to the control value for tolerant legumes in the present experiment. This resulted in increasing net uptake of nitrogen transported to the shoot (Buwalda et al. 1988) and recovered leaf chlorophyll. Plants had to direct their energies into renewed pigment production, and re-greened chlorotic leaves at the onset of recovery (Smethurst et al. 2005). A previous study showed that pea and grasspea did not recover within 10 days after termination of waterlogging (Solaiman et al. 2007). Presumably, in our study, a longer recovery period led to the different result.

In the present study, relatively waterlogging-tolerant genotypes had an altered root distribution (i.e. near the soil surface) pattern while grown in waterlogged conditions as demonstrated by shallow root system—root length was short (~100 mm) in waterlogged plants. But the overall root dry weight was similar for both drained and waterlogged treatments. However, there are disadvantages to the formation of the lateral roots—Armstrong



et al. (1983) in pea and Malik et al. (unpubl. data) in wheat demonstrated that the lateral roots consumed O<sub>2</sub> which restrict O<sub>2</sub> movement through aerenchyma in the primary root; thus restrict root penetration into the deeper zone. However, in the current experiment plants maintained growth during the stress periods; presumably, the lateral roots become functional roots as demonstrated for pasture legumes (Gibberd et al. 1999). It is promising that during the recovery period the shallow root resumed growth and reached the same length as in the drained control, allowing access to soil moisture at depth as the soil profile dries later in the season.

## Conclusion

The present study with a limited number of legume genotypes identified variations in tolerance to transient waterlogging and its recovery between legume crops and also intra-species variation in pea and lentil—associated with seed mass. Waterlogging-tolerant legume genotypes had high root porosity, were relatively unaffected in shoot nitrogen content under waterlogging and in recovery could resume root growth and rapidly regain chlorophyll concentration to control levels. Clearly, there is substantial potential to select in a wider range of both pea and lentil germplasm for increased levels of waterlogging tolerance. Further investigation should first focus on evaluation of a large number of genotypes within each species of legumes to determine the genetic variation, followed by physiological assessment using contrasting genotypes.

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## Contributions by the Authors

T.I.A., A.I.M. and W.E. designed the research and analysed data; T.I.A. performed the experimentation in partial fulfilment of her MSc thesis project and A.I.M. drafted the manuscript. All Authors agreed to the final version of the manuscript.

## Conflict of Interest Statement

None declared.

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