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Effects of transient subsurface waterlogging on root growth, plant biomass and yield of chickpea

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ABSTRACT

Root growth and proliferation are important for achieving the yield potential of chickpea in soils prone to waterlogging. Root growth characteristics and seed yield of the desi cultivar Rupali and the kabuli cultivar Almaz that differ in seed size and early vigour were investigated under well-drained and transiently-waterlogged conditions in glass-walled root boxes in a controlled-temperature glasshouse. Rooting parameters and detailed measurements of root growth and proliferation were made at 2-day intervals using a root mapping technique and by sampling the roots from the soil 14 days after the transient waterlogging ended. Although the roots of the kabuli cultivar Almaz had greater dry matter and length than the desi cultivar Rupali, the subsurface waterlogging promptly stopped the root growth of both genotypes. Root dry matter in both types of chickpea was reduced by two-thirds, 14 days after the cessation of the 12-day waterlogging treatment. The reduction resulted from an inhibition in root growth and proliferation, which led to a lower root length density down the soil profile, particularly in the top 0.6 m of the waterlogged plants. While root length and root dry matter was higher in the kabuli cultivar Almaz than in the desi cultivar Rupali after waterlogging, they were not associated with a greater above-ground dry matter or seed yield at maturity. The transient waterlogging reduced the seed yield by 54% in the kabuli cultivar Almaz and by 44% in the desi cultivar Rupali. The reduction in seed yield in the kabuli cultivar Almaz resulted from 50% decline in the number of seeds per pod while in the desi cultivar Rupali it was a consequence of less pods and seeds per pod. Subsurface waterlogging changed the rooting pattern in chickpea, inhibiting root branching and the growth of the tap root and severely reducing the growth of root branches. The release from the waterlogging induced the production of new roots rather than regrowth of existing roots.

1. Introduction

Chickpea (*Cicer arietinum* L.) is mainly grown as a rainfed crop across a wide range of environments, from the subtropics of India and north-eastern Australia to regions with a Mediterranean-type climate (Berger and Turner, 2007). In the Mediterranean-climatic region of southern Australia it is sown in the autumn and grows during the cool wet months of winter and spring. The combination of high rainfall in the June-to-August period and a fine-textured subsoil in the sandy-surfaced duplex soil leads to a transient saturated zone perched on the clay layer (Tennant et al., 1992). This condition, which often occurs without water being seen at the soil surface is called “subsurface waterlogging” (McFarlane and Cox, 1992), causes significant losses in chickpea yields (Siddique et al., 1993; Solaiman et al., 2007) and a recommendation that chickpea should not be grown on shallow duplex soils (Gregory, 1998). Even on the neutral-to-acid fine-textured soils that are recommended for chickpea production (Siddique et al., 2000), waterlogging can occur in winter and early spring.

Waterlogging affects the seed yield of grain legumes such as chickpea (Cowie et al., 1996b; Siddique et al., 1993; Solaiman et al., 2007) through a reduction in shoot growth and development, and a lower accumulation of above-ground dry matter. The effects of waterlogging on the above-ground dry matter are secondary as they essentially arise from damage to the root system (Malik et al., 2002; Bramley et al., 2007). Under waterlogging the root system is directly exposed to the changes of the soil environment such as a reduction in oxygen level and increase in CO_2 and ethylene concentration (Ponnamperuma, 1984). The effect of oxygen deficiency

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in reducing root respiration is reversible but the accumulation of CO_2 also inhibits root respiration and the effects are not reversible (Palta and Nobel, 1989). The combined result is decreased nutrient uptake (Setter and Belford, 1990) and reduced root growth (Jackson and Drew, 1984). Reciprocal grafts between a waterlogging susceptible lupin (*Lupinus angustifolius*) and a waterlogging tolerant lupin (*L. luteus*) clearly showed that the waterlogging tolerance resided in the root, not the shoot (Davies et al., 2000). Waterlogging also reduces the formation and longevity of root nodules and rates of nitrogen fixation (Matsunami et al., 2005).

Among grain legumes, chickpea is one of the least tolerant to waterlogging (Siddique et al., 2000; Solaiman et al., 2007). Its sensitivity depends on the duration of the waterlogging, the developmental stage at which it occurs and putatively the chickpea type (Cowie et al., 1996a; Solaiman et al., 2007). Growth reduction in chickpea is more detrimental when waterlogging occurs at seedling and flowering than at others stages (Cowie et al., 1996b). The kabuli types of chickpea are more sensitive to terminal drought than the desi types, irrespective of seed size (Leport et al., 1999, 2006; Yadav et al., 2006). However, the kabuli types are better adapted to medium-heavy textured soils that are prone to transient waterlogging, than the desi types (Siddique et al., 2000) and are putatively more tolerant to waterlogging (Cowie, 1993). Moreover, the kabuli chickpea have more vigorous early growth than desi chickpea (Leport et al., 1999) and this has been associated with greater waterlogging tolerance in other species (Jean, 1996; Nichols and Barbetti, 2005). This study compared the tolerance of the desi cultivar Rupali and the kabuli cultivar Almaz of chickpea to subsurface waterlogging imposed at a time when it is likely to occur in the field, that is, when the plants had four branches (Siddique et al., 2001). It evaluated the early growth and root characteristics of the two types of chickpea when waterlogged to determine whether differences in early vigour and root characteristics between the desi and kabuli types are associated with tolerance to the effect of subsurface waterlogging on root and shoot growth and seed yield.

2. Materials and methods

2.1. Plant material

Chickpea (*Cicer arietinum* L.) cultivar Rupali, a small-seeded desi type, and the cultivar Almaz, a large-seeded kabuli type, were grown in glass-walled boxes filled to a depth of 1.0 m with soil, as described by Liao et al. (2006). The soil was a reddish-brown sandy clay loam from the A-horizon of a field site at Merredin, Western Australia (USDA, Calcic Haploxeralf), pH 7.0. The soil was put through a 2 mm sieve and then mixed 9:1 with yellow sand (pH 5.6, classified Uc5.22 by Northcote et al., 1975) to reduce compaction and improve drainage. The soil was packed to a bulk density of approximately 1.53 g cm^{-3}. The seeds were inoculated with a commercial group N *Bradyrhizobium* preparation before seeding. Eight seeds were sown in a row close to the glass-wall of each box. At seeding, on 1 April 2005, 0.16 g of Richgro^{TM} trace elements (with K, Fe, Ca, Mg, Cu, Zn, Bo, Mo), 1.22 g of potassium nitrate, 1.15 g of ammonium nitrate, 1.73 g of calcium nitrate and 2.57 g of triple superphosphate were mixed into the top 0.1 m of soil in each box. Two weeks after sowing the seedlings were thinned to 4 plants per box. Each glass-walled growth box (four plants) served as a replicate, and there were 16 boxes for each genotype. The boxes were arranged randomly in a naturally-lit, temperature controlled glasshouse in Perth, Western Australia, with day/night temperatures of 22/10 °C, and natural photoperiod between 10 and 11.5 h. Details of the glass-walled root boxes have been described in Liao et al. (2006) and Palta et al. (2007). Briefly they were constructed from polyvinyl chloride (PVC), 0.24 m in length, 0.10 m wide and 1.0 m deep with one glass side. The glass side was covered with a black PVC sheet to avoid any exposure to light. The boxes were placed on steel stands at an angle of 30° from the vertical and spaced 0.05 m apart. The plants were watered daily by hand to maintain the soil water content close to field capacity and to avoid drainage of excess water. Watering was maintained until 26 days after sowing (DAS).

2.2. Treatments

At 26 DAS when the tap roots reached 0.5 m below the soil surface, the 16 root boxes of each genotype were randomly divided into 2 groups of 8. One group was maintained free of waterlogging (well-drained). The other group of boxes was waterlogged slowly by adding water continuously for 10 h to the bottom of each box, until the water table reached 0.1 m below the soil surface. The waterlogging level was maintained at about 0.1 m from the soil surface for 12 days (until 38 DAS) by adding small amounts of water three times per day to replace the water lost by evapotranspiration. After 12 days of waterlogging, water was allowed to drain for 12 h from the root boxes. Plants were then allowed to recover for 14 days, until 52 DAS, when the plants from four boxes of each waterlogging treatment of each genotype were harvested. The remaining boxes were grown until maturity (119 DAS) after which they were harvested.

2.3. Measurements

The developmental stages (phenostages) were checked at 3-day intervals and the phenostage noted when 50% of the plants had reached the particular stage. Stages recorded were: branching, flowering, 50% pod set (protrusion of the pod beyond the petals, about 8 mm long) and physiological maturity.

The growth of the roots was measured through the glass-wall in each growth box every 2 days from the time the seedlings were at the 1-leaf stage (11 DAS) until the first tap root in the well-drained treatment reached the bottom of the box (51 DAS). Because root growth recommenced 11 days after the waterlogging was ended, measurements of the recovery of root growth were extended for 29 days, until 67 DAS, using plants from the four boxes per genotype from the waterlogged treatment that were grown until maturity (119 DAS). Each time that root growth was measured through the glass-wall in each growth box, the black PVC cover sheet was removed and replaced with a transparent plastic film and all the visible new roots were traced on the transparent film using a waterproof permanent pen. After removal of the transparent film from the glass-wall, all the visible new roots were also marked on the glass-wall. In this way, it was possible to identify the new root growth at the subsequent measurement time. The glass-wall was then covered with the black PVC cover-sheet.

The transparent film for each mapping day was cut into 0.1 m sections starting at 0–0.1 m, and each section was scanned at 600 pixels per mm using a ScanJet, Hewlett Parkard scanner. The images were analyzed for the number and length of the roots in each section and for each mapping day using the computer software ROOTEDGE (Rootedge, 1999). Since not all the roots grown in the root box were displayed on the glass-wall, root length density was calculated as the root length in each section divided by the visual soil volume of the corresponding section. According to Hurd (1967) and Hurd and Spratt (1975) the visual soil volume is the space of soil where root growth is visually through a section of the glass-wall. The visual soil volume was obtained by multiplying the surface area of the section of the glass-wall (24 cm × 10 cm) by the horizontal soil width of 5 mm (Hurd, 1967; Hurd and Spratt, 1975; Liao et al., 2006).

Table 1
Leaf area, leaf dry weight, specific leaf area, number of branches and shoot dry matter at 52 days after sowing (14 days after subsurface waterlogging for 12 days or kept well-drained) in the desi cultivar Rupali and the kabuli cultivar Almaz. Means in each column followed by a different superscript letter are significantly different ($P < 0.05$).

Genotypes	Leaf area (cm^2/plant)	Leaf dry weight (g/plant)	Specific leaf area (mg/cm^2)	Number of branches	Shoot dry matter (g/plant)
Well-drained					
Desi cultivar Rupali	850.9^a	2.5^a	2.9^a	16.8^a	4.4^a
Kabuli cultivar Almaz	942.4^a	3.0^a	3.2^a	12.2^b	5.3^b
Subsurface waterlogged					
Desi cultivar Rupali	218.0^b	0.6^b	2.8^a	6.8^c	1.3^c
Kabuli cultivar Almaz	303.1^b	1.0^b	3.3^a	6.3^c	2.3^d

2.4. Sampling

When the tap root in the well-drained treatment reached the bottom of the boxes at 26 DAS, the plants from four boxes in the well-drained and waterlogged treatments were harvested. At this time plants were at flower initiation as 50% of the plants had visible flower buds. In this harvest above-ground and below-ground dry matter was measured in both genotypes and treatments. Shoots were harvested as a replicate by cutting the shoots from the roots at the crown. The number of branches was recorded and the shoots dried at 70 °C and weighed. Immediately after the shoots were harvested, the glass-walled root boxes were opened by removing the glass-wall and the soil in each growth box was sampled in 0.1 m sections. The roots in each section were recovered from the soil by repeated sieving on a 1.4 mm sieve to produce a clean sample as described by Palta and Fillery (1993). After the roots were recovered from a section of the soil, they were placed in a plastic bag and stored at 4 °C until they were measured, usually within 2 days. The root length in each sample was measured by staining the roots for 30 min with a 0.1% solution of methylene blue and placing them in a glass tray (0.2 by 0.3 m) with about 3 mm of water and untangling the roots with a plastic spatula to minimize overlapping. The glass tray was placed on the scanner, and the roots were scanned and the images analyzed as above. The root material was then dried and weighed. Root length density was calculated as root length (cm) per cm^3 of soil. Means and standard errors were calculated with the Genstat (8th edition: VSN International Ltd., Hemel Hempsted, UK) means program and tests for differences among genotypes and treatments were performed using a one- and a two-way ANOVA, respectively. Significant differences ($P = 0.05$) between means were identified with the least significance difference (LSD) test.

3. Results

3.1. Phenology

The two chickpea genotypes emerged 7 DAS and were branching within 2 days of each other at 16 DAS. When subsurface waterlogging was imposed at 26 DAS the desi cultivar Rupali had 4 branches and the kabuli cultivar Almaz had 6 branches. Subsurface waterlogging for 12 days during vegetative growth had no effect on the subsequent phenostages of flowering, podding and maturity. Fifty percent flowering in the desi cultivar Rupali occurred as early as 54 ± 3.5 DAS or 864 degree-days (base temperature 0 °C), 16 days ahead of flowering in the kabuli cultivar Almaz. Fifty percent podding occurred at 62 ± 3.8 DAS in the desi cultivar Rupali and 80 ± 4.6 DAS in the kabuli cultivar Almaz. Physiological matu-

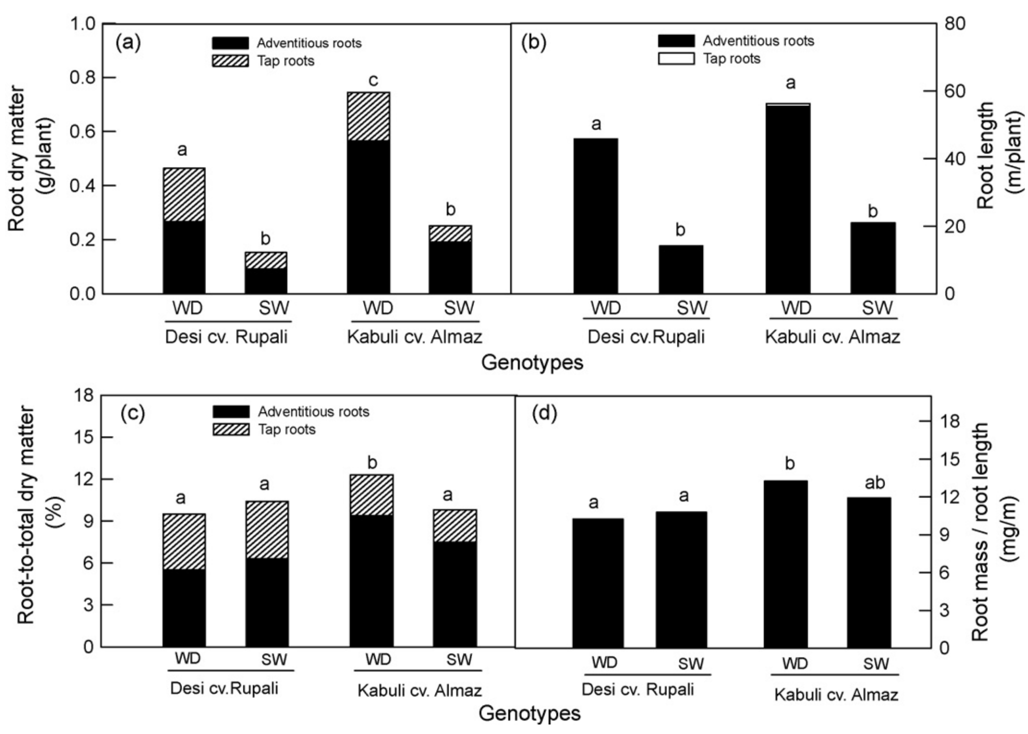

Fig. 1. (a) Root dry matter, (b) root length, (c) the root-to-total dry matter and (d) the root mass per unit of root length of adventitious roots (closed bars), and tap roots (cross-hatched bars) of the desi cultivar Rupali and kabuli cultivar Almaz when kept well-drained (WD) or subjected to subsurface waterlogging (SW) for 12 days. Measurements were 14 days after the subsurface waterlogging ended (52 DAS). Bars followed by a different letter are significantly different ($P < 0.05$).

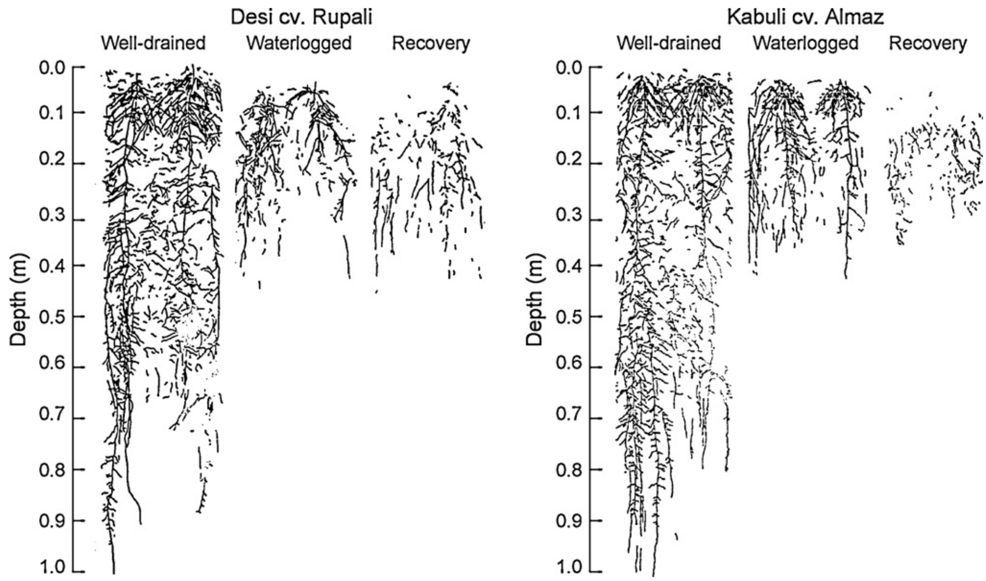

Fig. 2. Visible roots of the desi cultivar Rupali and the kabuli cultivar Almaz under well-drained and subsurface-waterlogging conditions 14 days after the subsurface waterlogging ended (52 DAS) and after recovery from waterlogging 29 days after the subsurface waterlogging was ended (67 DAS). Measurements were made by root mapping though the glass-wall of the boxes. The root images presented correspond to two plants, but there were four plants per box.

rity was reached earlier in the desi cultivar Rupali (114 ± 6.4 DAS) than in the kabuli cultivar Almaz (132 ± 7.5 DAS).

3.2. Effects on leaf area and shoot dry matter

Compared with the well-drained plants, subsurface waterlogging during vegetative growth reduced the leaf area of both cultivars by about 70% ($P < 0.05$) (Table 1). The specific leaf area was not affected by the subsurface waterlogging because leaf dry weight and leaf area were similarly reduced. Accumulation of shoot dry matter under subsurface waterlogging was reduced ($P < 0.05$) by 70% in the desi cultivar Rupali and 56% in the kabuli cultivar Almaz. The reduction in shoot dry matter was largely due to a 50% decline in the number of branches ($P < 0.05$) (Table 1).

3.3. Effects on root growth and proliferation

At 52 DAS, the kabuli cultivar Almaz had produced 38% more root dry matter than the desi cultivar Rupali when grown under well-drained conditions ($P < 0.05$) (Fig. 1a). The greater root dry matter in the kabuli cultivar Almaz resulted from greater adventitious root dry matter (52%) rather than from tap root dry matter (8%). Transient subsurface waterlogging reduced the root dry matter of both types of chickpea by 67% ($P < 0.05$). The reduction in root dry matter in both chickpea types was due to reductions in adventitious and tap roots (Fig. 1a). The total root length in the kabuli cultivar Almaz was 20% greater than in desi cultivar Rupali (Fig. 1b) when plants were grown free of waterlogging due to differences in adventitious root length (18%) rather than from differences in tap root length (7%). Total root length was reduced by the transient subsurface waterlogging by about two-thirds in both chickpea types ($P < 0.05$) due to reductions in the length of adventitious and tap roots (Fig. 1b). The kabuli cultivar Almaz had a greater ($P < 0.05$) proportion of root-to-total plant dry matter and had a higher proportion of root dry matter allocated into the adventitious roots rather than into the tap root than the desi cultivar Rupali when grown under well-drained conditions (Fig. 1c). Subsurface waterlogging significantly reduced ($P < 0.05$) the proportion of root-to-total plant dry matter in the kabuli cultivar Almaz, but not the desi cultivar Rupali. The root mass per unit of root length, which is an indirect measurement of root thickness, was greater in the kabuli cultivar Almaz ($P < 0.05$) than in the desi cultivar Rupali and was not affected by the transient subsurface waterlogging (Fig. 1d).

The patterns of root growth and proliferation were changed similarly in both chickpea types by subsurface waterlogging (Fig. 2). The extension of both tap and adventitious roots and the production of new adventitious roots were markedly reduced 2 days after the waterlogging commenced while those of the well-drained chickpea continued unhindered (Fig. 3). After water was drained from the waterlogged pots, no extension in the existing tap or adventitious roots was observable (Fig. 2). Instead, both chickpea types started producing new roots from the highest part of the tap root (crown) 5-6 days after the waterlogging event was ended. The new roots were twice as thick as the existing adventitious roots. The growth and proliferation of the new formed roots during the recovery period was confined to the top 0.40 m of the soil profile (Fig. 2). The maximum rooting depth, measured daily by root mapping, was similar in both chickpea types (Fig. 2). Total root length down the soil profile was greater in the kabuli cultivar Almaz than the desi cultivar Rupali. The differences in total root length became significant 30 DAS (Fig. 3). The tap roots in both chickpea cultivars commenced branching at 20 DAS, but more branches were produced in the kabuli cultivar Almaz (data not shown). The lateral extension of the branches in the kabuli cultivar Almaz was faster than in the desi cultivar Rupali (Fig. 2). Under well-drained conditions root length per plant increased faster in the kabuli cultivar Almaz (22.8 cm/day, $P < 0.05$) than in the desi cultivar Rupali (20.4 cm/day) type. Root length per plant under subsurface waterlogging increased slowly but the rates were faster in the kabuli cultivar Almaz (3.4 cm/day, $P < 0.05$) than in the desi cultivar Rupali (2.0 cm/day) chickpea (Fig. 3). When the subsurface waterlogging was terminated, root length per plant in both cultivars increased slowly at rates of about 3.5 cm/day during the first 12 days and then at rates of about 11 cm/day.

There was a strong linear correlation ($y = -0.288893 + 5.5127127x$; $r^2 = 0.91$, $P < 0.01$) between the root length measured when the plants were harvested at 52 DAS and the total root length measured by root mapping down the soil profile at the same time (Fig. 4). This correlation indicates that root length measured by root mapping can be used, after calibration, to

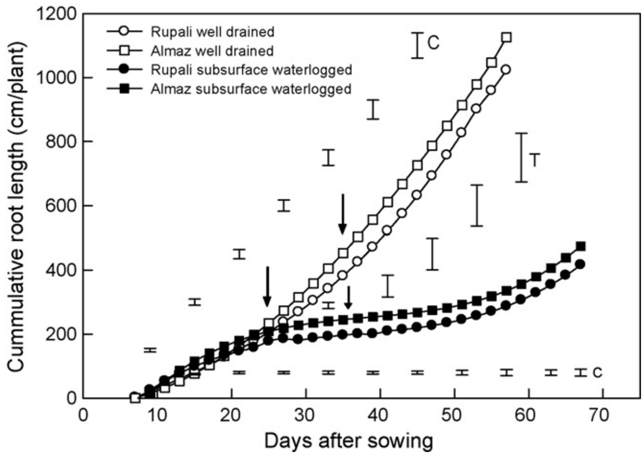

Fig. 3. Cumulative total root length of the desi cultivar Rupali and the kabuli cultivar Almaz on the glass face of the root boxes under well-drained and subsurface-waterlogging conditions, and during the recovery from waterlogging. Measurements were made at 2-day intervals from the time the seedlings were at the 1-leaf stage (11 DAS) until the tap roots in the well-drained treatment reached the bottom of the box at 52 DAS. The arrows represent the beginning and end of the transient subsurface waterlogging. Vertical bars with the letter C represent the LSD ($P = 0.05$) for comparison between chickpea genotypes and vertical bars with the letter T represent the LSD ($P = 0.05$) for comparison between treatments.

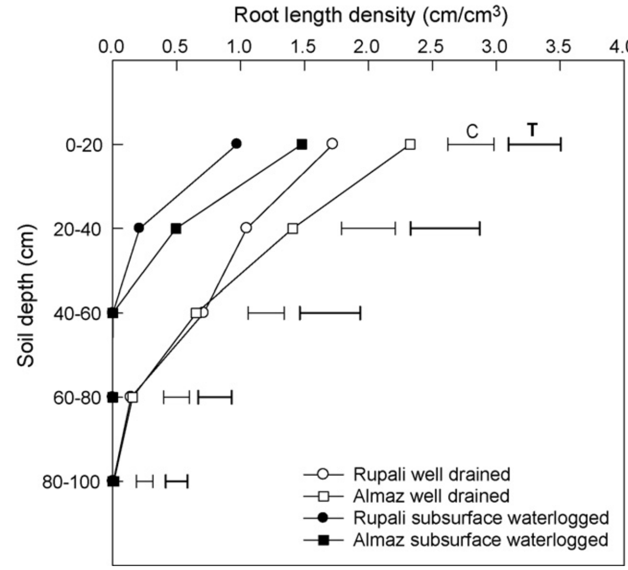

Fig. 5. Root length density down the soil profile of the desi cultivar Rupali and the kabuli cultivar Almaz under well-drained and subsurface-waterlogged conditions. Horizontal bars with the letter C represent the LSD ($P = 0.05$) for comparison between chickpea genotypes and horizontal bars with the letter T represent the LSD ($P = 0.05$) for comparison between treatments.

reliably estimate the root length down the soil profile in chickpea. Root length density measured when the plants were harvested at 52 DAS was higher in the well-drained than in the subsurface-waterlogged chickpea throughout the soil profile (Fig. 5). Below 0.6 m, there were no roots in the waterlogged treatment. The root length of well-drained and subsurface-waterlogged kabuli cultivar Almaz was higher than in the desi cultivar Rupali in the top 0.4 m of the soil profile (Fig. 5).

Root growth in the top 0.2 m of the soil profile in well-drained chickpea increased linearly in both types at a rate of 9.3 cm/day to a maximum of nearly 400 cm at 45 DAS, after which it no longer increased (Fig. 6a). In the 0.2–0.4 m of the soil profile roots of both chickpea cultivars grew slowly in the first 21 DAS at a rate of 1.3 cm/day and then rapidly. The root growth after 21 DAS was faster in the kabuli cultivar Almaz (9.3 cm/day) than in desi cultivar Rupali (7.9 cm/day) and by 57 DAS, when the measurements were ended, the roots were 361 cm/plant in the kabuli cultivar Almaz and 315 cm/plant in the desi cultivar Rupali (Fig. 6a). Roots in the 0.4–0.6 m of the soil profile were noticeable in both chickpea types

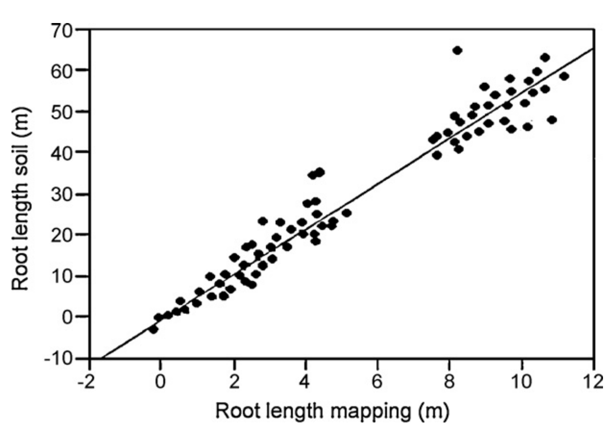

Fig. 4. The relationship between root length measured in the soil when the plants were harvested at 52 DAS and the cumulative root length measured by root mapping though the glass-wall of the root boxes at 52 DAS. The linear relationship ($r^2 = 0.92$) is $Y = -0.288893 + 5.5127127x$; ($P < 0.01$). Data are from both cultivars and both treatments.

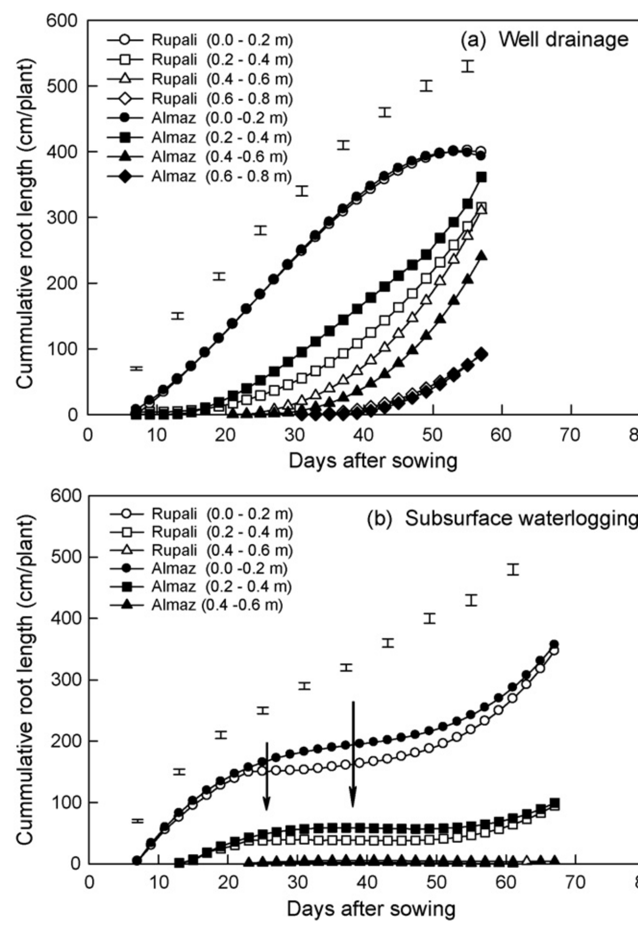

Fig. 6. The cumulative root length in different soil layers of the desi cultivar Rupali and the kabuli cultivar Almaz under (a) well-drained conditions and (b) subsurface-waterlogging and recovery from waterlogging. Vertical bars represent the LSD ($P = 0.05$) for comparison between chickpea genotypes.

Table 2
Effect of 12 days of subsurface waterlogging during the vegetative phase on shoot dry matter, yield, harvest index and the components of seed yield in the desi cultivar Rupali and the kabuli cultivar Almaz at maturity. Means in each column followed by a different superscript letter are significantly different ($P<0.05$).

Genotype	Shoot dry matter (g/plant)	Seed yield (g/plant)	Harvest index	Pods/plant	Seeds/pod	Seed weight (g/seed)
Well-drained						
Desi cultivar Rupali	32.2^{a}	12.5^{a}	0.39^{a}	70.7^{a}	1.16^{a}	0.16^{a}
Kabuli cultivar Almaz	32.3^{a}	8.1^{b}	0.25^{b}	32.8^{b}	0.68^{b}	0.37^{b}
Subsurface waterlogged						
Desi cultivar Rupali	21.2^{b}	7.0^{c}	0.33^{c}	53.7^{c}	0.78^{b}	0.17^{a}
Kabuli cultivar Almaz	21.3^{b}	3.7^{d}	0.17^{d}	30.5^{d}	0.34^{c}	0.35^{b}

after 20 DAS, but their growth was slow at a rate of 2.2 cm/day until 33 DAS after which they grew more rapidly. During the rapid phase of growth in this section of the soil profile the root growth was faster in the desi cultivar Rupali (6.5 cm/day) than in kabuli cultivar Almaz (4.7 cm/day) (Fig. 6a). Root growth in the 0.6–0.8 m of the soil profile began at 31 DAS in both genotypes and increased slowly at a rate of 1.3 cm/day until 43 DAS, after which root growth was similarly in both types and more rapid (3.5 cm/day).

Root growth in the top 0.2 m of the soil profile under subsurface waterlogging was slow at rates of 0.85 cm/day in the desi cultivar Rupali and 2.26 cm/day in the kabuli cultivar Almaz (Fig. 6b). After the waterlogging ended at 38 DAS, root growth in the top 0.2 m of the soil profile was slow until 50 DAS, after which it increased rapidly, but not as rapidly as in the well-drained plants. Root growth during and after waterlogging was higher in the kabuli cultivar Almaz than in the desi cultivar Rupali (Fig. 6b). The growth of roots in the 0.2–0.4 m of the soil profile was reduced by the waterlogging in both chickpea types (Fig. 6b). Root growth in this layer of the soil profile after the waterlogging was ended was slow until 55 DAS and then increased moderately until 67 DAS (Fig. 6b). Roots in the 0.4–0.6 m of the soil profile were visible 6 days before the waterlogging was imposed, but they did not grow during or after waterlogging (Fig. 6b). No roots were produced in the 0.6–0.8 m of the soil profile during or after waterlogging.

3.4. *Effects on root nodule*

The number of root nodules per plant in the well-drained treatment was 150 and 130 in the desi cultivar Rupali and kabuli cultivar Almaz (LSD $P=0.05$; 12.5), respectively. Subsurface waterlogging reduced the number of root nodules per plant to 80 in the desi cultivar Rupali, but no significant reduction occurred in the kabuli cultivar Almaz. Root-nodule dry weight under well-drained conditions was 90 mg/plant in both chickpea types, but subsurface waterlogging reduced the nodule dry matter to 20 mg/plant in the desi cultivar Rupali, whereas no significant reduction of the dry matter occurred in the kabuli cultivar Almaz.

3.5. *Effects on seed yield and yield components*

Under well-drained conditions the above-ground dry matter at maturity was similar for the two chickpea types (Table 2). Subsurface waterlogging similarly reduced final dry matter by a third in both chickpea types. Seed yield, harvest index, pod number and the number of seeds per pod were higher in the desi cultivar Rupali than in the kabuli cultivar Almaz under well-drained conditions and with subsurface waterlogging. The differences in seed yield between the two chickpea types under well-drained conditions resulted from differences in the number of pods and seeds per pod. The seed yield was reduced more by waterlogging in the kabuli cultivar Almaz (55%, $P<0.05$) than in the desi cultivar Rupali (42%) because of a reduction in the number of pods in the desi cultivar Rupali and due to a reduction in the number of empty pods in the kabuli cultivar Almaz (Table 2). The harvest index of both chickpea types was reduced by the transient subsurface waterlogging. Seed size was greater in the kabuli cultivar Almaz than in the desi cultivar Rupali under well-drained and waterlogged conditions; seed weight was not affected by the transient subsurface waterlogging (Table 2).

4. Discussion

Under well-watered and well-drained conditions, the desi cultivar Rupali yielded more than the kabuli cultivar Almaz. The 12 days of transient subsurface waterlogging did not change the yield advantage of the desi cultivar Rupali over the kabuli cultivar Almaz. This is surprising since the kabuli cultivar Almaz had greater early vigour, root dry matter and root length density than the desi cultivar Rupali and it has been suggested that genotypes with vigorous early shoot and root growth are better able to tolerate transient waterlogging (Hartley et al., 1993; Bejiga and Anbessa, 1995). The faster rates of increasing root length by the kabuli cultivar Almaz under subsurface waterlogging (3.4 cm/day; $P<0.05$) compared with those of the desi cultivar Rupali (2.0 cm/day) were not sufficient to lessen the persistent effect of the subsurface waterlogging on shoot growth and yield. Both the tap root and adventitious root dry matter were reduced as a result of a reduction in root length and branching. This led to a lower root length density, particularly in the top 0.6 m of the soil profile. These results highlight the severe effects that subsurface waterlogging has on root growth and proliferation even in genotypes with vigorous early growth.

Kabuli chickpeas rarely produce more than one seed per pod, whereas desi chickpea usually have a proportion of pods with two seeds (Davies et al., 1999). The reduction in seed yield from waterlogging arose from both a reduction in the number of branches and pods in the desi cultivar Rupali and a decrease in the number of seeds per pod and the number of empty pods in both the desi cultivar Rupali and the kabuli cultivar Almaz. Previous studies with desi and kabuli chickpeas have shown that terminal drought reduces the number of pods and number of seeds/pod in desi chickpea and the number of seeds/pod in kabuli chickpea and that the larger seed size of the kabuli type is not the main determinant of pod abortion in chickpea as small-seeded kabuli chickpea had higher pod abortion compared to similar-sized desi chickpea (Leport et al., 1999, 2006). Thus, it appears that kabuli chickpeas are more sensitive to water shortage and water excess, than desi chickpea and this is not associated with root growth characteristics.

Yield increases in chickpea resulting from increases in the number of pods have been associated with the availability of plant nitrogen (Palta et al., 2005). The yield reduction in the desi cultivar Rupali as a consequence of a reduction in the number of pods is consistent with this finding. Measurements of nitrogen content and rates of nitrogen fixation were not made in this study, but the large reduction in both root nodule number and dry matter in the desi cultivar Rupali indicated that subsurface waterlogging is likely to have reduced nitrogen fixation (Matsunami et al., 2005). The results showing no reduction in the number and dry matter of root nodules in the kabuli cultivar Almaz might indicate that the effect of subsurface waterlogging on nitrogen fixation was less in this cultivar (Aslam et al., 2003; Carranca et al., 1999). However, it did not result in a grain yield improvement in the kabuli cultivar Almaz under waterlogging (Beck, 1992; Rupella and Saxena, 1987)

Genetic variation for waterlogging tolerance in chickpea has been shown (Bejiga and Anbessa, 1995; Larry et al., 2004). In cowpea (Takele and McDavid, 1994), soybean (Hartley et al., 1993) and faba bean (Solaiman et al., 2007) a low degree of root decay and the formation of adventitious roots with aerenchyma have been nominated as possible characteristics conferring tolerance to waterlogging (Bejiga and Anbessa, 1995). Root decay as a result of waterlogging was observed in this study rather than the formation of adventitious roots, suggesting poor tolerance to waterlogging. Production of new roots as opposed to regrowth in the existing roots after waterlogging was notable. If there is diversity for this characteristic then the restart of growth in existing roots could be an important trait for selecting chickpea with tolerance to waterlogging. We can only speculate on why it might be an important trait. It could provide an early and fast root growth recovery and will save carbon expenditure in new roots.

Root mapping through the glass-wall of the root boxes in this study allowed the dynamics of root growth to be measured down the soil profile (Liao et al., 2006; Palta et al., 2007). The data demonstrate the dramatic impact of subsurface waterlogging on the rooting pattern of chickpea, particularly in the top 0.2 m of the soil profile. Under well-drained conditions roots in the top 0.2 m of the soil profile grew at the same rate of 9.3 cm/day in both chickpea types, but with subsurface waterlogging the roots of the kabuli cultivar Almaz grew at a faster rate (2.5 cm/day, $P < 0.05$) than the roots of the desi cultivar Rupali (1.1 cm/day). Roots in the 0.2–0.4 m of the soil profile also grew slowly and the rates were faster in the kabuli cultivar Almaz than in the desi cultivar Rupali. The reduction in the rates of root growth in the top 0.4 m of the soil profile was responsible for changing the rooting patterns under subsurface waterlogging, because root growth below this soil layer was minimal, presumably due to lack of oxygen availability in the saturated soil. The reduction in root growth was apparent within 2 days after the commencement of the subsurface waterlogging and it was maintained through the remaining 10 days. Subsurface waterlogging inhibited root branching and the growth of the tap root, and the small increase in the length of root branches that did occur was restricted to the top 0.4 m of the soil profile. Release from the subsurface waterlogging did not immediately increase root growth. Root growth was only noticeable 11 days after waterlogging was removed. This was 2–3 days after the soil water content of the waterlogged root growth boxes was similar to that in the well-drained boxes. The root growth after waterlogging resulted mainly from the growth of newly-formed roots rather than from the regrowth of existing tap and adventitious roots. The newly-formed roots arise from the highest part of the tap root (the crown root) and were presumably produced as a consequence of the dead of the existing root tips (Palta, 2007). The newly-formed roots grew and proliferated in the top 0.3 m of the soil profile. Although both genotypes started producing new roots at the same time, the desi cultivar Rupali produced more new roots than the kabuli cultivar Almaz. The production of thick new roots rather than regrowth of the existing tap and adventitious roots presumably reflects the death and decay of existing roots (Barrett-Lennard et al., 1988; Malik et al., 2001; Trought and Drew, 1980) rather than a strategy for a rapid recovery of root growth. This will have important implications for the fluxes of carbon (C) in the plant which affects crop productivity (Gregory and Atwell, 1991; Palta and Gregory, 1997). Young plants of chickpea invest a large proportions of C in their root system, particularly when grown on soils with high clay content (Hooda et al., 1990) and the production of new roots instead of regrowth in the existing roots after waterlogging, represent not only losses of a previously-invested C, but also an investment in new C. Expenditure of C was not measured in this study, but the transient waterlogging had a marked effect on the production of leaf area and the accumulation of shoot dry matter when measured 14 days after the release of waterlogging. Moreover, the 12 days of waterlogging had a persistent effect on dry matter production and seed production through to maturity.

5. Conclusions

Transient subsurface waterlogging caused a severe reduction in root growth and proliferation in both the desi cultivar Rupali and kabuli cultivar Almaz. The vigorous early growth characteristic of the kabuli cultivar Alamaz was associated with faster rates of root growth than the desi cultivar Rupali under well-drained and subsurface-waterlogging conditions. However, this characteristic was not associated with a higher seed yield under transient waterlogging. The yield reduction in the kabuli cultivar Almaz from the transient waterlogging was greater than in the desi cultivar Rupali and resulted mainly from an increase in the number of empty pods rather than from a reduction in the number of pods. Subsurface waterlogging changed the rooting pattern of both chickpea types by inhibiting the production of root branches and the growth of the tap root, and existing adventitious roots. The recovery from the waterlogging depended on the production of new roots rather than the regrowth of the existing roots. This suggests that characteristics such as the re-establishment of growth in the existing roots, the early production and fast growth of new roots and the rapid recovery of root growth should be included when accessing genetic variation for waterlogging tolerance in chickpea germplasm collections.

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