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Chickpea root DNA tool to identify chickpea root distribution

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Key findings

- A new, advantageous DNA-based chickpea root measurement method was developed as an alternative to other root quantification methods that allows large numbers of soil cores to be analysed over a short time period.
- Field results showed the DNA method could be used to determine chickpea root depth distribution.
- The results also showed that differences in root dry matter distribution and soil water extraction exist among chickpea varieties and breeding lines.
- Comparing genotypes using the DNA method is complex due to differences in DNA copy number between genotypes; currently the method is most readily used to determine factors affecting root distribution in a single chickpea genotype.

Introduction

Currently there is a lack of knowledge about the basic root traits in Australian chickpea varieties under field conditions. This means it is difficult to select varieties with shallow or deep root distributions that might provide production advantages to growers. Traditional root sampling methods are both laborious and time consuming, which limits the ability to sample comprehensive field experiments. Alternative methods such as the mini-rhizotron have been developed, but still require extensive analysis of images and do not allow for opportunistic sampling outside the viewing fixed point access tube.

The soil DNA was evaluated through extraction and the qPCR method, which is used to quantify chickpea root DNA concentrations and was developed by SARDI. Importantly this method allows hundreds of samples to be processed per day and requires no root separation from soil before analysis. Root dry weight to root DNA values in both glasshouse and field studies were compared. This paper presents the key findings of the field experiment and identifies the key advantages and limitations of the DNA method.

Site details

Location	Tamworth – Tamworth Agricultural Institute.
Rainfall	A total of 635 mm of rain was recorded at the experiment site during 2017. The growing season rainfall was 148 mm, with the majority occurring in October (90 mm). The long-term average rainfall for Tamworth is 671.5 mm.
Experiment design	Randomised complete block design with four replicates.
Sowing date	11 May 2016.
Fertiliser	50 kg/ha Granulock Z (nitrogen:phosphorus:sulfur:zinc; 11:21.8:4:1) placed in furrow with seed.
Plant population	Target 32 plants/m^2.

Weed management	Post plant pre-emergent herbicide: 1 kg/ha Terbyne® 750 WG (750 g/kg terbuthylazine) plus 80 g/ha Balance® 750 WG (750 g/kg isoxaflutole) applied on 12 May. Grass weed management: 100 mL/ha Verdict™ 520 (520 g/L haloxyfop) applied on 5 June and 27 June.
Insect management	Targeting *Helicoverpa* spp: Dupont™ Steward® EC 300mL/ha (150 g/L indoxacarb) applied on 21 September, 11 and 24 October.
Disease management	Targeting ascochyta blight: 2 L/ha Unite 720 (720 g/L chlorothalonil) applied on 18 July, 1 August, 1 September and 6 October.

Treatments and sampling regime

Varieties (5)

04067-81-2-1-1(B) C, Kyabra(Φ), PBA HatTrick(Φ), PBA Seamer(Φ), and Sonali.

Soil sampling

Hydraulic coring (core diameter 45 mm) was used to collect soil cores. The root DNA levels were assessed in the five genotypes at four depths (0–15, 15–30, 30–60 and 60–90 cm) and three time points (vegetative [one core/plot], flowering [one core/plot] and physiological maturity [two cores/plot]). Soil cores were sent to SARDI for chickpea root DNA analysis. For the cores taken at flowering one set of cores had the chickpea roots extracted by washing the soil samples over sieves and the dry weight (DW) of roots recorded after drying the samples at 65 °C for 48h. A separate core was also collected at each time point from each plot to determine the gravimetric soil moisture content at each sample depth, including at 90–120 cm.

Results

Establishment

Post-emergence counts showed some variation in populations between genotypes, although the differences were not significant. The average plant density was 30 plants/m^2, with a range of 25–32 plants/m^2.

Root DNA and dry weight results

Chickpea DNA concentration analysis showed significant effects from depth at each of the three time points, with lower DNA concentrations as depth increased (Table 1). Root DW at flowering, was also significantly higher in the 0–15 cm soil layer than the three deeper depths, but there was no statistical difference between the three deeper depths. It was noted that the DNA results were less variable, as indicated by the % the coefficient of variation, as the season progressed (data not presented).

Table 1 Sampling depth effects on chickpea DNA concentrations at flowering.

Sample depth	Root DNA concentration (Log kilo copies/g soil/cm core depth)			Root dry weight (DW, mg/cm core depth)
	Vegetative [A]	Flowering [A]	Physical maturity [B]	Flowering [A]
0–15 cm	5.75	6.53	6.88	1.840
15–30 cm	2.89	5.71	5.54	0.294
30–60 cm	0.36	4.30	4.76	0.249
60–90 cm	-2.55	2.36	3.87	0.122
l.s.d. (P<0.001)	1.252	0.798	0.301	0.2987

[A] Results for one core analysed per plot; [B] Average results of 2 cores analysed per plot.

The genotype did not have a significant effect on DNA concentrations at the vegetative or flowering growth stages (Table 2). However, at physiological maturity, the variety Sonali showed significantly higher DNA concentrations than the other entries.

Although not the primary focus of this field experiment, results showed that there are significant differences in the root DW distribution in chickpea genotypes. For example, there was a significant genotype effect on root DW values in samples collected at flowering (Table 2). Kyabra$^{\Phi}$, PBA HatTrick$^{\Phi}$ and PBA Seamer$^{\Phi}$ all had higher DW values than either 04067-81-2-1-1(B) or Sonali.

These results included a significant genotype-by-depth interaction whereby at the 0–15 cm soil depth both PBA Seamer$^{\Phi}$ and Kyabra$^{\Phi}$ had higher values than the three other genotypes. PBA HatTrick$^{\Phi}$ had an intermediate DW value that was also significantly higher than either 04067-81-2-1-1(B) or Sonali (data not presented). Differences were also found between genotypes at physiological maturity in November. For a separate set of 0–10 cm samples, PBA Seamer$^{\Phi}$ had a higher fine root DW value than 04067-81-2-1-1(B) (data not presented).

Table 2 Genotype effects on chickpea root DNA concentration at flowering.

Genotype	Root DNA concentration (Log kilo copies/g soil/cm core depth)			Root dry weight (DW, mg/cm core depth)
	Vegetative [A]	Flowering [A]	Phys. maturity [B]	Flowering [A]
04067-81-2-1-1(B) [C]	2.05	4.82	5.039	0.456
Kyabra	2.33	4.59	5.226	0.754
PBA HatTrick	1.56	4.74	4.999	0.758
PBA Seamer	0.91	4.75	5.232	0.801
Sonali	1.20	4.74	5.823	0.362
P value	0.241	0.990	<0.001	0.010
l.s.d.	1.40	0.892	0.3364	0.2672

[A] Results for one core analysed per plot, [B] Average results of 2 cores analysed per plot; [C] this line is a cross between chickpea (*C. arietinum*) and a wild *Cicer* species

The gravimetric soil moisture results from the field experiment showed there are significant differences in the extraction of soil water among genotypes, particularly at depth (>30 cm) where 04067-81-2-1-1(B) and Sonali left more soil water than other genotypes, and Kyabra$^{\Phi}$ had superior water use at the 60–90 cm depth (Table 3).

Table 3 Field experiment gravimetric soil moisture content (%) genotype by depth interaction. l.s.d. = 2.06.

Genotype	Depth, cm			
	0–15	15–30	30–60	60–90
04067-81-2-1-1(B) [A]	18.8	24.9	24.2	19.1
Kyabra	20.2	25.7	23.6	16.6
PBA HatTrick	19.9	24.1	23.5	20.0
PBA Seamer	19.4	25.2	22.4	18.9
Sonali	20.8	25.6	25.0	22.4

[A] this line is a cross between chickpea (*C. arietinum*) and a wild *Cicer* species

Conclusions

The findings in the field that Australian varieties and breeding lines differ in root dry weight values are supported in both controlled environment and overseas studies. Significant variation exists in root traits, including root depth distribution, between chickpea genotypes. Further, both the DW and gravimetric soil moisture data reinforces the need for high throughput methods for studying chickpea root distribution. This will enable varieties and breeding lines to be identified that have superior traits. This knowledge can then be used to support improved production in specific environments.

The DNA results showed significant differences in root distribution across soil depths at each sampling time, but genotype DNA results only differed at the final sample time at physiological maturity. In contrast, there were significant differences in root DW among genotypes at flowering. Further, due to differences in root DNA concentrations among genotypes, high DNA results did not correlate with root DW results for some genotypes, such as Sonali.

Due to genotype DNA concentrations not reflecting root DW values for some genotypes such as Sonali, currently this method is most readily used to determine factors affecting root distribution in a single chickpea genotype rather than for comparing genotypes.

Through careful implementation, the DNA chickpea root method, including adequate sampling regimes, could be used by industry (land managers, agronomists, breeders, research scientists) to determine the vertical and lateral root distribution of chickpea roots in soil. This will enable varieties to be selected with either shallow or deep root distributions, where such traits will provide agronomic advantages in specific growing environments. Further, the method can be used to identify sites where soil constraints, such as sodicity and acidity, are affecting vertical or lateral chickpea root distribution. Plant pathologists and breeders might be able to use the method to identify which genotypes have superior compensation abilities (root replacement) in the presence of different abiotic or biotic constraints. The DNA test could also be used to simultaneously co-quantify root pathogen populations (e.g. PRR) and chickpea roots, which might facilitate identification of disease management practices or chickpea genetics with superior resistance or tolerance to chickpea root diseases.

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