Rooting patterns in double-haploids lines of wheat for reduced-tillering and their relationship with early N uptake

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## Abstract

Early uptake of N by wheat crops is low in the deep sandy soils of the Mediterranean-type climate of southern Australia, and as a result large quantities of  $NO_3^{-}$  are leached rapidly below the rooting zone before stem elongation. Regardless of the availability of  $NO_3^{-1}$  in the soil profile the  $NO_3^{-1}$  demand by the crop is low due mainly to slow growth of the root system. Improving the ability of wheat roots to grow early and fast is a practical strategy being sought to enhance NO<sub>3</sub><sup>-</sup> capture and minimising early looses. Using a new glass-windowed root growth box technique and 4 double haploids lines of wheat for reducing tillering, measurements of root length, root biomass and distribution within the top 1 m soil profile were made destructively at stem elongation, together with shoot biomass, tiller production, leaf area and N uptake. The vigorous line Vigor 18 and the cultivar Janz were used as the check genotypes against which the double haploids lines were evaluated. There was evidence for differences among double haploids lines for reduced-tillering in the early rooting patterns and N uptake. Extreme restricted-tillering lines such as A35 and F25 had rooting patterns and low N uptake as similar to Janz, and less restricted-tillering lines as B25 and F20 had rooting patterns and high N uptake as similar to Vigor 18. There was no correlation between tiller number and root biomass, root length or root number, suggesting that expansion or enlargement of the root system may be a direct hormonal response associated with the reduced-tillering gene (tin) rather than an allometric assimilate trade response.

# **Key Words**

Doubled haploids wheat, reduced-tillering, vigour wheat, rooting patterns, nitrogen capture

### Introduction

Wheat (*Triticum aestivum* L.) is the most important crop in the Mediterranean climatic region of Western Australia with over 5 million ha sown each year. Nitrogen (N) impacts directly on wheat yield and quality, but it is an expensive input, representing one third of the costs of wheat production in many areas of the wheatbelt of Western Australia (Anderson). Wheat crops in the Western Region are inefficient users of available profile nitrogen at the break of the season as well as the N applied as fertiliser (Fillery and McIness 1992, Fillery, 2001). A poor synchronisation between the availability of NO<sub>3</sub><sup>-</sup> in the soil profile and the NO<sub>3</sub><sup>-</sup> demand by the wheat crop is responsible for these inefficiencies (Angus 2001) and for losses of N via deep drainage particularly in the winter months when crop growth and N uptake are slow due to low temperatures and low solar radiation (Turner and Nicolas 1988). Losses of N by leaching reduce crop yields and profitability and contribute to soil acidification causing offsite pollution of ground waters.

Improving the ability of root systems to recover soil  $NO_3^-$  by earlier and faster uptake is an efficient strategy to improve the synchronisation between the availability of  $NO_3^-$  in the soil profile and the  $NO_3^-$  demand by the wheat crop (Liao *et al.* 2004). This strategy dictates that roots grow fast, proliferate early and profusely to intercept and capture the  $NO_3^-$  before it moves below the rooting depth of wheat crops. Genotypic differences in the early uptake of N have been shown in wheat genotypes which differ in vigour and have been associated with differences in early root biomass, root branching and root length (Liao *et al.* 2004; 2006). Reduced-tillering lines putatively have a surplus of assimilates beyond the shoot demands that is invested in expanding the root system into the soil, which presumably improves the capture of N. However, little is known about the early rooting characteristics and rooting patterns of

reduced-tillering lines of wheat and their role in capturing the early available N. We have commenced studies to examine the rooting patterns of reduced-tillering wheat lines and in this paper we present a general analysis of root growth, proliferation and early N uptake by doubled haploids lines selected for reduced-tillering by Richard Richards and Greg Rebetzke at CSIRO Plant Industry

## Methods

A glasshouse study was conducted between August 2004 and January 2005 in Perth, Western Australia to evaluate the rooting patterns and the early N uptake of double haploids wheat lines for reduced tillering. The study was conducted in two phases. In the first phase the double haploids lines B25 and A35 were compared with those for the cultivar Janz and the vigour breeding line Vigor 18. In the second phase the double haploids lines F20 and F25 were compared against Janz and Vigor 18. In each phase, the 4 genotypes were grown in glass-walled boxes (Liao *et al.* 2004) filled to a depth of 1.0 m with soil. The soil was yellow sand (Typic, Xeric Psamment; Uc5.22; siliceous sand, obtained from two field sites. The soil was packed to a bulk density of approximately 1.54 g cm<sup>-3</sup>. At sowing, the equivalent of 5g N m<sup>-2</sup> as urea and 1.8 g P m<sup>-2</sup> as amended super phosphate (Cu, Mo, Zn) was mixed into top 0.1 m of soil in each box. There were 4 seedlings per box (equivalent to150 plants/m<sup>2</sup>). Each genotype was replicated 4 times with boxes arranged randomly 0.05 m apart. The glass wall of each box was covered with a black PVC sheet to avoid any exposure to light. The plants were grown in a naturally-lit greenhouse with day/night temperatures of 20/10 ?C, and natural photoperiod from about 10 to 13 hours. The plants were watered daily by hand to maintain soil water content close to field capacity. Every 48 hours from plant first leaf stage until stem elongation, root growth and proliferation were followed by tracing visible new roots on transparent plastic film.

Genotypes	Tillers/plant	Shoot biomass (g/plant)	Root biomass (g/plant)	Root length (cm)	Root number/ plant	Root mass/ root length (mg/cm)	N uptake (mg/plant)
B25	1.5	0.47	0.130	326.2	1191	0.36	19.4
A35	1.0	0.38	0.097	299.0	1109	0.32	16.5
Janz	2.8	0.37	0.104	288.1	1046	0.36	16.2
Vigor18	2.5	0.48	0.145	371.2	1376	0.39	21.4
l.s.d. ( <i>P=0.05</i> )	0.3	0.05	0.010	20.3	71	0.04	1.50

Table 1. Number of tillers, shoot and root biomass, root length and number and N uptake by the double haploids lines B25 and A35, the vigorous line Vigor 18 and the cultivar Janz grown in glass-walled growth boxes at stem elongation (Z31) in phase 1 of the study.

The visible new roots were also traced on the glass wall in order to identify the new root growth at the subsequent set of measurements. When the vertical roots reached the bottom of the box around stem elongation the plants in each box were harvested. Above-ground and below-ground biomass was measured in each box by separating the shoots from the roots before being dried at 70 ?C and weighed. The soil in each box was separated in 0.2 m sections, sampled and the roots in each section were recovered from the soil following the methodology described by Palta and Fillery (1993). Roots recovered from each section were stained for 30 minutes with 0.1% (w/v) methylene blue, scanned using a Scan

Jet, Hewlett Packard scanner connected to a computer. The images were analysed for the root length in each section using ROOTEDGE (Rootedge, 1999). Total nitrogen in shoot and root was determined using a VG-Micromass Sira 10 (V-G Isogas Ltd, Middlewich, England) connected to a Europa Roboprep C-N Analyzer (Europa Scientific Ltd, Crewe, England).

## Results

The number of tillers produced by the double haploids lines was lower than cv. Janz and the breeding line Vigor 18 (Table 1 and 2). Shoot and root biomass, root length and root number and the uptake of N by the double haploids line A35 were similar to Janz, but low compared with B25 and Vigor 18 (Table 1). Shoot biomass of the double haploids line B25 was similar to Vigor 18, but root biomass, root length and root number and the uptake of N was higher in Vigor 18 than in B25.

Shoot biomass of the double haploids lines F20 and F25 was greater than Janz, but lower than Vigor 18 (Table 2). F25 and Janz produced similar root biomass, but it was lower than that produced by F20 and Vigor 18. Root length, root number and N uptake were similarly higher in F20 and Vigor 18 than in F25 and Janz (Table 2).

Table 2. Number of tillers, shoot and root biomass, root length and number and N uptake by the double haploids lines F20 and F25, the vigorous line Vigor 18 and the cv. Janz grown in glass-walled growth boxes at stem elongation (Z31) in phase 2 of the study.

Genotypes	Tillers/ plant	Shoot biomass (g/plant)	Root biomass (g/plant)	Root length (cm)	Root number/ plant	Root mass/ root length (mg/cm)	N uptake (mg/plant)
20	1.9	1.64	0.20	428.9	2508	0.11	66.6
F25	1.4	1.28	0.18	324.0	1969	0.12	43.5
Janz	4.2	0.94	0.14	296.0	1564	0.13	38.4
Vigor18	2.8	1.85	0.29	451.4	2627	0.13	67.4
l.s.d. ( <i>P=0.05</i> )	0.7	0.12	0.09	24.1	125	0.04	3.5

No differences in the maximum rooting depth between the double haploids lines and Janz or Vigor 18 were detected up to stem elongation. While B25 had more roots than A35 and Janz in the 20-40 cm soil layer (Fig. 1a), F20 had more roots than F25 and Janz in the 20-100 cm layer of the soil profile (Fig. 1c). Root length in the double haploids lines B25 and A35 was similar to that of Janz and Vigor 18 in the top 40 cm, but down the soil profile their root length was lower than Vigor 18 (Fig. 1b). The double haploids lines F20 and F25 had root length similar to that of Janz and Vigor 18 in the top 20 cm of the soil profile, but down the soil profile the root length of F20 and Vigor 18 was similarly higher than that of F25 and Janz (Fig. 1d).



# Fig. 1. Number of roots (a and c) and root length (b and d) down the soil profile at stem elongation of 4 double haploids wheat lines for reduced-tillering and cv. Janz and the vigorous line Vigor18. The horizontal bars represent the l.s.d (P = 0.05).

### Discussion

Tillering of the double haploids lines ranged from 1 tiller per plant in A35 to 1.9 in F20. Compared with the tillering of Janz and Vigor 18, which ranged from 2.8 tiller per plant to 4.2, tillering in the double haploids lines was restricted. Assuming that reduced-tillering wheats have a limited capacity to invest assimilate reserves in the shoot to invest them in expanding the root system into the soil, it was expected that the extreme tillering restricted line A35 would have bigger root system than the other genotypes. However, root biomass, root length and number, and N uptake of A35 was similar to Janz but lower than B25 and Vigor 18. In addition, root mass per unit of root length, which is an indication of root thickness, was similar among genotypes in each phase of the study. However, less tillering restricted lines such as B25 and F20 had root biomass, root length and number, and N uptake as high as Vigor 18. This indicates that the effect of reducing tillering in wheat does not always result in heavier roots and expansion or enlargement of the root system. The lack of correlation between tiller number and root biomass, root length or root number (data not shown) suggest that expansion or enlargement of the root system in reduced-tillering wheats is a direct hormonal response associated with the reduced-tillering gene (*tin*) rather than an allometric assimilate trade response.

### Conclusions

There was indication of differences among the double haploids lines for reduced-tillering in the early rooting patterns and N uptake. Although extreme restricted-tillering lines had root length, root number and N uptake that were as low as in Janz, and less restricted-tillering ones had root length, root number and N uptake that were as high as in Vigor 18, there was not correlation between tiller number and root

biomass, root length or root number. More work is required to capitalize on the reduced tillering ability of some double haploids lines whose rooting patterns allow them to improve the early N uptake.

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