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Genetic variation in specific root length in Scandinavian wheat and barley accessions

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Summary

Specific root length (SRL, m root g^{-1} root dry matter) was studied in a broad selection of old and current accessions of spring wheat and barley from Norway and Sweden, at sub-optimal phosphorus conditions in nutrient solution and soil. The results indicated that genotype did not have a significant effect on SRL. A close relationship between root length (RL) and root weight (RW) was found, and more than 70% of the variation in root length was explained by root weight of representative and homogenous root samples. In nutrient solution, the relationship between RL and RW was described by the regression equations RL = 0.32 RW – 0.19 ($R^2 = 0.74$) for wheat and RL = 0.20RW + 0.73 ($R^2 = 0.56$) for barley. In the soil experiment, the relationships between RL and RW were described by the equations RL = 0.15RW + 0.95 ($R^2 = 0.67$) for barley and RL = 0.16 RW + 0.50 ($R^2 = 0.77$) for wheat. Hence, in screenings of a large number of cereal genotypes, the root length may be estimated with good accuracy by records of root weight and an appropriate regression equation.

Abbreviations: CV: coefficient of variance (%), dpi: dots per inch, DM: dry matter, RL: root length, RW: root weight, SRL: specific root length (m root g^{-1} root dry matter)

Introduction

The total root surface of a plant is important for capturing water and nutrients. At sub-optimal nutrient concentrations in soil a large root surface is of advantage, especially for absorbing less mobile nutrients such as phosphorus (P). A large root surface is achieved by a combination of reduced mean root diameter and elongation of the relatively thinnest roots (Fitter, 2002). The SRL integrates root length and fineness. From a given amount of root DM, a plant with fine roots produces a relatively larger root system and achieves a high SRL. SRL has been suggested as a useful trait in breeding of P-efficient varieties (Clark, 1983; Sattelmacher et al., 1994). Root characteristics such as total RL (Römer et al., 1988), RL density (Egle et al., 1999) and RL per plant DM (Nielsen & Schjørring, 1983) have been shown to vary considerably between cereal genotypes. For further references on genotypic variation in these root characteristics, see O'Toole and Bland (1987) or Manske and Vlek (2002). However, with respect to the SRL, information on the genetic variation in cereal species is rudimentary, often based on studies with few lines or varieties. Significant variation was found between eight barley varieties in nutrient solution (Schjørring and Nielsen, 1982), but for six winter wheat varieties grown in the field, the differences were smaller and not consistent from one sampling to another (Welbank et al., 1974). Therefore, the value of SRL in breeding programmes is still difficult to assess. Errors in sampling and measuring RL, even with modern image analysis systems, add to the reasons for the discrepancies. Hence, simple ways of comparing the size of root systems of large number of

genotypes are desired. RL is measured by line intersection (Newman, 1966) or scanning methods (Richner et al., 2000), which are both time-consuming.

We studied the SRL of old and recent Scandinavian spring wheat and barley accessions grown at suboptimal P conditions in nutrient solution and soil. This paper aims to discuss the influence of cereal genotype on SRL, and to test the possibility whether less laborious root weight determination can be a good indicator of the root length of cereal genotypes.

Material and methods

SRL was studied in a selection comprised of 17 accessions of spring wheat and 35 of spring barley, ranging from old land races collected around 1900 to modern lines not yet released. The pedigree of the accessions is shown in Table 1. For all accessions, SRL was measured in nutrient solution (low P; 25 μM), and for a subset of 15 accessions, SRL was additionally measured in a pot experiment with low-P soil.

Nutrient solution experiment

Seeds were germinated on filter paper and the seedlings were grown for 3 weeks in a circulating well-aerated nutrient solution in a climate chamber (light intensity 130 μE s^{-1} m^{-2}, light/dark period 16/8 h, temperature 18 °C day, 15 °C night and 75% relative humidity). There were three replicates; each composed of five single plants fixed in a strip of foamed plastic. The initial complete basic nutrient solution was according to Gahoonia et al. (1999), except that a lower P concentration (25 μM as compared to 50 μM) was used. The nitrate concentration was 5 mM; no ammonium was added. To adjust pH close to 5.5 and to keep the electrical conductivity at 0.67 mS m^{-1}, a complete maintenance solution as described by Gahoonia et al. (1999) was added. Alternatively, only ammonium nitrate solution was added when pH increased without change in electric conductivity. Although no visual signs of P deficiency were observed, the value of the N/P ratios in plant DM at harvest indicated a moderate P stress during growth, according to Gorshkova (1978). The plants grew rapidly and were extending, DC32 (Zadoks et al., 1974), at harvest for root measurements.

Soil experiment

Five genotypes of wheat and 10 of barley (five two-row, five six-row), producing contrasting plant DM in the nutrient solution, were chosen and grown in low-P soil in the same climate chamber at the same conditions as mentioned above. Only distilled water was given to the plants during the experiment. The soil was topsoil (0–30 cm, 15% clay, 18% silt, 65% sand, 1.5% total C) taken from a field where no P was added since 1966, and 60 kg N, 60 kg potassium (K) $ha^{-1}y^{-1}$ had been added as mineral fertilisers to cereals. The concentration of Olsen-P (Olsen & Sommers, 1982) was 4.5 mg P kg^{-1} and exchangeable K (Knudsen et al., 1982) was 60 mg kg^{-1} dry soil. Other soil chemical characteristics were pH (H_2O) 6.0, pH ($CaCl_2$) 4.9, and soil mineral N 26 mg nitrate and 11 mg ammonia kg^{-1} dry soil (Bremner & Keeney, 1966). After sieving through 5 mm-mesh, 800 g soil (water content 12%) was filled into plastic pots and one vigorous seedling planted in each pot. There were five to seven replicates (pots) per genotype and four of these were sampled for RL measurement. Each accession was harvested in the sequence as plant growth diminished and nutrient deficiency symptoms had developed, at three to five leaves stage (DC 13–15, Zadoks et al., 1974). Two-row barley was harvested on days 15–19, six-row barley on days 18–20 and wheat genotypes on days 19–22 from planting. The correlation between SRL and harvest day was negligible ($R^2 = 0.09$).

Root sampling and length measurement

The roots from nutrient solution grown plants were stored moist in dark at 4 °C until length measurements, when two or three roots, 10–30 cm long, were randomly taken from the root mass of each genotype replicate. The roots from the soil experiment were carefully washed, and immersed in water. A representative sample was cut off, gently pulled out and stored dark at 4 °C in 15% alcohol solution.

For length measurement, the root samples were spread in distilled water in a glass tray that was placed on flat bed scanner (ScanJet IIcx), and the scanned images were stored. The roots from nutrient solution were scanned at a resolution of 300 dpi, but as we realised that 150 dpi gave a sufficient quality, this resolution level was used for the roots from soil. The root samples were collected after scanning, dried at 70 °C and weighed to obtain RW values. The digital image files were analysed by Dt-Scan Software (Delta-T Devices, Cambridge, England) to measure RL. In Dt-Scan, the standard analysis for RL measurement (Newman-Head) divides the total area of the image by the mean

Table 1. Pedigree or geographical origin, year of approval or collection, and specific root length (SRL) values for spring barley and wheat accessions arranged by increasing SRL value in low-P nutrient solution, and for a subset of 15 accessions additionally in low-P soil[a]

Genotype	Pedigree or origin, year of approval or collection	Nutrient solution SRL (m g^{-1} DM)	Low-P soil SRL (m g^{-1} DM)
Barley			
Fager	HN355-93/Thule, 2000	186	
Tore	Lise/Clermont, 1986	199	
Fløya	Purified line of land race Ørnes, N Norway, 1918	200	
Lise	(Asplund x Ds295) x Varde, 1960	200	
Jadar	Land race, SW Norway, 1900–1910	208	
HDw021, Dw	Lise x Ashdon /x Tore / xTore	209	
Asplund, S	Purified line from two-row barley, 1900–1910	210	
Thule	Ensenada/Bamse//H313-248, 1993	215	
Finnebygg	Land race, central Norway, 1900–1910	216	
Skjåk	Land race, central Norway, 1900–1910	218	224
Varde	Asplund x Maskin (1924), 1939	219	
Maskin	Purified line of land race Bjørneby, 1910	222	
Olsok	Bode/Agneta, 1994	224	
Jarle	Jadar x (Asplund x Maskin)(1932), 1952	228	
Mari, 2r	Mutation in Sv. Bonus (1963), 1977	228	
Herse	Asplund x Maskin, 1939	234	182
Tyra, 2r	Sold/Sv71164, 1988	236	
NK95036	Tyra/P-13, n.r.	236	
NK94682	Arve//HS72-8/MØ75-278/3/PH107, n.r.	239	
SWE018, S, 2r	Meltan x Svani, Sweden, n.r.	240	
Domen, 2r	Maskin x Opal B, 1949	240	
Gunilla, 2r	Birgitta x Sv Å 56888, 1973	257	223
Gaute	SvN82114/V13647-77, 2000	258	216
SWE9306, S, 2r	Derkado x Sv84580, n.r.	259	
Refsum, 2r	Land race, S Norway 1900–1910	263	229
SWE9319, S, 2r	Meltan x Svani, Sweden, n.r.	265	
Tunga	Fræg x (Juli x Rigel), 1975	268	233
Arve	Agneta//Otra/Vigdis, 1990	273	
Dønnes	Land race from N Norway, <1900	275	205
SWE013, S, 2r	Goldie x Svani, Sweden, n.r.	280	243
Olve, 2r	Gunilla/Lilly, 1994	283	243
Møyar, 2r	Domen x Herta, 1964	295	
SWE019, S, 2r	Meltan x Svani, Sweden, n.r.	295	
Scotch Bere	Adapted to soil pH (H_2O) 4.5, land race from Scotland	303	
Herta, 2r	Kenia x Isaria, 1941	329	235
LSD barley		75	49
Wheat			
Diamant, S (or Diamant II)	Kolben x Steninge, 1928 (Diamant x Ekstra Kolben, 1938)	255	193
NK97520	SvB87293/Bastian, n.r.	279	
Brakar	T8058/T8073//T8080/Bastion, 1995	288	173

(*Continued on next page*)

Table 1. (*Continued*)

Genotype	Pedigree or origin, year of approval or collection	Nutrient solution (SRL m g^{-1} DM)	Low-P soil (SRL m g^{-1} DM)
Bastian	Baijo/Runar/3/Yactana//Norin10/Brevor/5, 1989	289	
Møystad	Mö043-40 x KärnII, 1966	292	
Østby	Land race, S Norway, 1900–1910	304	
Reno	Els x (Tammi x KärnII), 1975	304	197
Børsum	Land race, S Norway, 1900–1910	312	197
Norrøna	FramII x Sopu, 1952	313	
NK97535	Reno/Genesis//Drabant /Hanno, n.r.	316	
Rollo	KärnII x Norrøna, 1963	318	
Sibirian	Land race, 1900–1910	320	
Ås	Purified line from land race, 1900–1910	323	
NK98602	Brakar/Rida x T2038, n.r.	331	
SnøggII	(Jo3 x Sibirisk) x Ås1927, 1940	334	
NK0058, Dw	Brakar/T1022	339	187
NK97537	T4023/WW27328, n.r.	340	
LSD wheat		98	39

[a]S: Swedish (all other accessions are of Norwegian origin), 2r: two-row barley (others are six-row), n.r.: not released at the time of the study, Dw: dwarf line for breeding purpose only. LSD: least significant difference, 5%.

diameter. Such RL values were considerably higher than those measured by hand (Table 2). This is due to shade and root debris particles, which were interpreted as image area. System limitation did not allow setting a limit to particle size for excluding this bias. The analysis "Object scan" measures the perimeter of objects, and here a lower limit of particle size could be set. These values came close to the values measured manually (Table 2) when the limit was set to 0.03 mm^2, and for all SRL values presented here, the RL values were obtained by this procedure. SRL values were achieved by dividing the RL of the sample with the RW of the sample.

Table 2. Values of root length (RL) obtained by hand measurement as compared to two digital analyses of scanned root images[a]

Sample	Hand measured (mm)	Object scan (mm)	Newman-Head (mm)
1	1401	1404	1828
2	1559	1663	2500
3	1616	1623	2568
4	1902	1950	2372
5	2235	2726	3585
6	2576	2381	4022

[a]"Object scan" gave RL values close to hand-measured values, whereas "Root length, Newman-Head" greatly exaggerated the root length values.

Statistical analysis

The effect of accession and root type on SRL was analysed by variance analysis (Anova or GLM, SAS Institute, 1989). Tukey's *t*-test was used to analyse if accessions were significantly different. Levels of significance are shown in the text as *P* values. The CV (standard deviation/average value × 100%) provides information on the variability of the data.

Results and discussion

Across all accessions grown in nutrient solution, the mean SRL for wheat, 309 m g^{-1} root DM, was significantly higher ($P = 0.0001$) than for barley, 242. Comparable results were found for the subset of accessions that were used in the soil experiment, where SRL was 300 for wheat and 263 for barley. Within the barley accessions, the mean SRL values ranged from 186 for cv. "Fager" to 329 for cv. "Herta" (Table 1). These

were the only two accessions among the 35, which differed statistically ($P = 0.02$) in SRL. This is because there was large variation in SRL values among the replicates. Similarly, in case of wheat there was no statistically valid differences in SRL in nutrient solution ($P = 0.95$), despite that the lowest mean value was for cv. "Diamant", 255 and the highest for NK97537, 340. Römer and Schenk (1998) reported a similarly large variation, where the LSD values for total RL were 39–48% of the average for 24 barley genotypes. One of the reasons for the large data variability in the present study may be the small sample size and only three replicates in nutrient solution, where the CV was 19%. With four replicates and a double average sample size in the soil experiment (see below), the CV decreased, to 14–15%. However, increasing the number of replicates will increase the number of root samples to be processed for length measurement, making the measurement even more time-consuming. This endorses the difficulties related to root sampling and length measurements for detection of variation in root characteristics of cereal varieties and emphasises the need to explore simpler ways of comparing root systems.

No significant variation in SRL was found between the accessions of wheat ($P = 0.64$) or barley ($P = 0.31$) grown in soil. Across all accessions in the soil experiment, the mean SRL for wheat, 189 was significantly lower ($P = 0.0003$) than for barley, 223. This result was opposite to what was found in nutrient solution, and may indicate an interaction between plant species and root environment with respect to root fineness.

The range of variation in SRL in soil was from 182 to 243 in barley and from 173 to 197 in wheat. The root diameters in soil were obviously not larger than in nutrient solution (Figure 1). A reason for the lower SRL values in soil may be that these roots were relatively heavier due to clay particles that had become an integrated part of the root surface, and hence could not be removed despite a thorough cleaning process.

With regard to the scanned root samples, there was a significant correlation between root weight and root length ($P < 0.001$) in each of the two experiments. In nutrient solution (Figure 2), the relationships were given by the regression equations RL = 0.32RW – 0.19 for wheat ($R^2 = 0.74$) and RL = 0.20RW + 0.73 ($R^2 = 0.56$) for barley. In the soil experiment (Figure 3), the relationships were RL = 0.16RW + 0.50

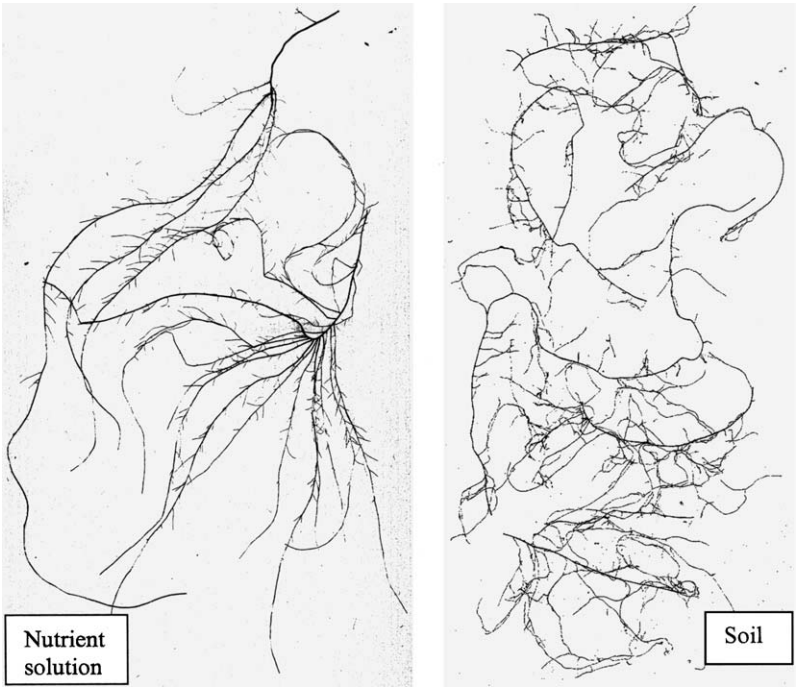

Figure 1. Roots from wheat cv. Brakar grown in nutrient solution (left) and soil (right).

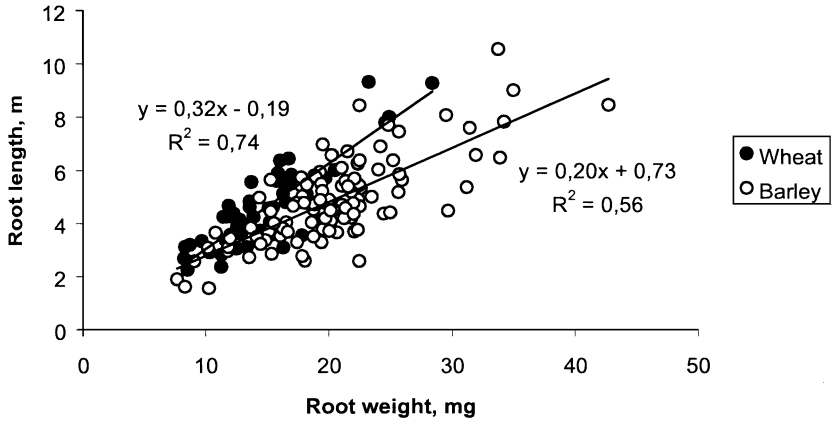

Figure 2. Relation between dry weight and length of scanned root samples of 17 wheat and 35 barley accessions grown in nutrient solution under moderate P stress.

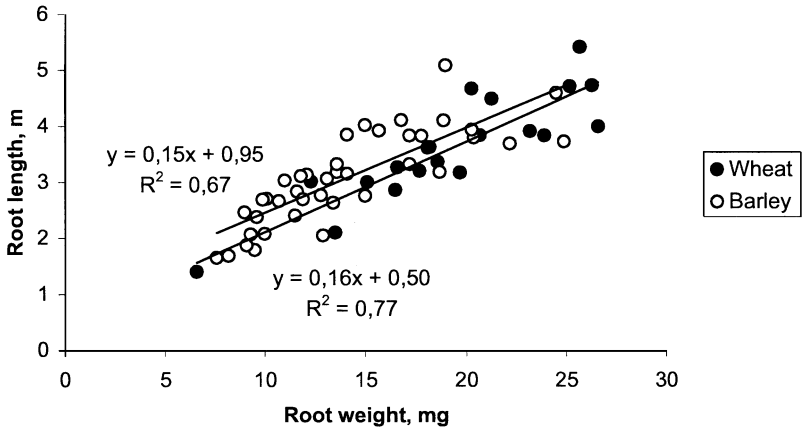

Figure 3. Relation between dry weight and length of scanned root samples of 5 wheat and 10 barley accessions grown in soil with low P availability.

(R^2 = 0.77) for wheat and RL = 0.15RW + 0.95 (R^2 = 0.67) for barley. A closer correlation between RW and RL in the soil experiment was probably due to that the soil root samples scanned for RL measurement comprised a larger part of the total root system, on average 9.1% of the root mass, as compared to 4.5% in nutrient solution. Further, there were more replicates for each accession.

The data suggested that there was a close relationship between root weight and root length both for wheat and barley. More than 70% of the variation in RL was then explained by the variation in RW. This is in accordance with Atkinson (2000), and with Heen (1980) who found a high coefficient (R^2 = 0.98) of determination for the relation between length and dry weight of hand-measured root pieces of barley. Hence, the root length may be estimated from the weight of homogeneous and representative root samples by using appropriate regression equations as mentioned above. This significantly simplifies the laboratory work in screening of genotypes for root size.

Conclusions

The influence of genotype on SRL within spring wheat and barley was not significant, suggesting uniform root fineness. Hence, in cereal genotype screenings where a large number of accessions are to be compared, the initial assessments of root size can be done with reasonable good accuracy simply by recording the root weight. Although such assessments may not entirely substitute measurement of actual RL for detailed

studies of nutrient uptake, they may significantly simplify the initial screening of cereals and perhaps also other grasses for their root systems. In this study, the relationships between RW and RL of wheat and barley, whose root fineness is fairly uniform as compared to woody roots, are presented. Therefore, the here reported close correlation between root length and weight may not be valid for woody roots.

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