

Induced Mutations Affecting Root Architecture and Mineral Acquisition in Barley

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Abstract

Root architecture influences the acquisition of mineral elements required by plants. In general, plants with a greater root/shoot biomass quotient and a more extensive root system acquire mineral elements most effectively. In barley (*Hordeum vulgare* L.) induced mutation has produced commercial cultivars with greater root system size, and genotypes with greater root spread, longer roots and roots with denser root hairs. Work is in progress investigating whether these phenotypes improve the acquisition of mineral elements and, thereby plant growth and grain yield.

Root architectural requirements for mineral acquisition by plants

Plants require at least 14 mineral elements to complete their life cycles. These include six macronutrients (N, K, P, Mg, Ca, S), which are present in relatively large concentrations in plant tissues (g kg⁻¹ dry weight) and several micronutrients (Fe, Zn, Mn, Co, Cu, B, Cl, Mo), which are present in smaller amounts (mg kg⁻¹ dry weight). Tissue concentrations of these elements must be maintained within a certain range, since any deficiency can limit plant growth, and an excess can be toxic. In most areas of the world, agricultural production requires the application of fertilizers to supply a crop's requirement for essential mineral elements. However, commercially viable sources of most mineral elements are diminishing, and the unbalanced or excessive application of fertilizers can lead to environmental problems. Therefore, it is important to develop management strategies and crops that utilize our mineral resources most efficiently.

Mineral elements are acquired from the soil solution by the plant root system, and the acquisition of each mineral element has its own challenges. Root architecture influences most the acquisition of mineral elements that are required by plants more rapidly than they arrive at the root's surface, and for which the root system must forage in the soil [1], [2]. These mineral elements include P, K, Fe, Zn, Mn and Cu [3]. The ability of the root system to proliferate rapidly throughout the soil, especially in areas where these elements are locally abundant, is an advantage, as are the release of exudates and enzymes that solubilize essential mineral elements and the fostering of beneficial associations with symbiotic fungi and microbes [1], [4], [5], [6].

Barley (*Hordeum vulgare* L.) is a high yielding cereal crop that is cultivated worldwide for animal feed, human food, malting, brewing, and distillation. Its root system is comprised of between three to eight seminal roots, which arise from the embryo, and a greater number of adventitious (nodal) roots that originate from the base of the main stem and tillers during development [7]. It is thought that the ability of a barley plant to acquire mineral elements might be improved by it having a greater root/shoot biomass quotient [8], [9], a more extensive root system [1], [8], longer, thinner roots with more root hairs [10], [11], [12], [13], a greater number and even spread of seminal roots [14],

[15] and the ability to proliferate lateral roots in mineral-rich patches [16]. It has been demonstrated that there is considerable variation in these parameters between barley genotypes. For example, wild barley has fewer seminal roots with a narrower spread [15], and invests less biomass in its root system [17] than cultivated genotypes. These observations suggest that root traits have been selected, albeit inadvertently, for particular environmental conditions during the domestication and improvement of the barley crop.

Root traits associated with the *sdw1* and *ari-e.GP* mutations

Induced mutation increases the genetic variation within a species and this technique has a long history of producing barley genotypes with agronomically beneficial traits. The FAO/IAEA Mutant Varieties Database lists 303 barley cultivars that have been produced through induced mutation (<http://www-mvd.iaea.org/MVD/default.htm>, accessed 21st May 2008). Among these cultivars are the semi-dwarf genotypes Diamant (*sdw1* = *denso*, chromosome 3H), which was generated from the Czech cultivar Valticky using X-ray irradiation and released in 1965 [18], and Golden Promise (*ari-e.GP* = *GPert*, chromosome 5H), which was generated from the Maythorpe cultivar using Gamma-ray irradiation and released in 1966 [19], [20]. Other important cultivars bearing mutations in these genes include Triumph (*sdw1*), Prisma (*sdw1*), Derkado (*sdw1*), Optic (*sdw1*), Tocada (*sdw1*), Westminster (*sdw1*) and B83-12/21/5 (*ari-e.GP*).

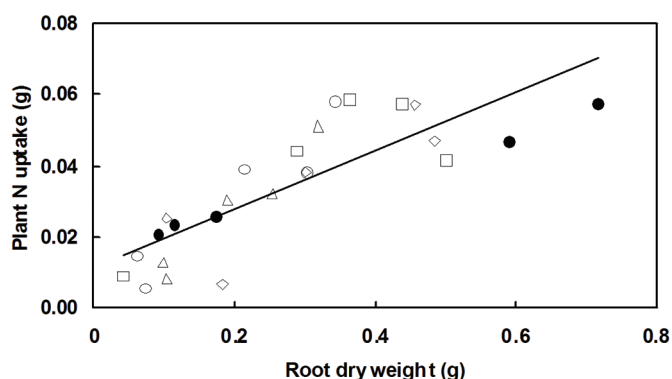


Figure 1 The significant positive relationships ($R^2=0.685$, $P<0.0001$, $n=25$ individual plants) between plant N uptake and root system size at grain maturity among five barley cultivars grown in the glasshouse at SCRI in 1m long tubes filled with a grit-sand-gravel mixture and irrigated daily with a water flush followed by a standard liquid nutrient application. Genotypes included a tall cultivar (Kenia, filled circles), and cultivars bearing the dwarfing gene *sdw1* (Derkado and Westminster, open circles and triangles, respectively) or *ari-e.GP* (Golden Promise and B83-12/21/5, open squares and diamonds, respectively). In this experiment, grain yield was positively correlated with both plant N uptake and root dry weight (A.J. Karley, data not shown).

The *sdw1* and *ari-e.GP* mutations were originally selected because they reduce plant height and increase grain yield, but they also affect root traits such as root length, root weight and nitrogen isotope dis-

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crimination [21], although the manifestation of their effects on root traits is influenced greatly by the environment. It has been observed that genotypes bearing the *sdw1* or *ari-e.GP* mutations often have larger root systems than non-dwarf genotypes when grown in the field [17] and that both mutations are present in chromosomal regions (QTL) affecting root system size among genotypes of a Derkado x B83-12/21/5 double haploid mapping population [17], [22]. The *sdw1* mutation was associated with increased size of both seminal and adventitious root systems in the Derkado x B83-12/21/5 population, particularly during the early stages of plant development [17], [22]. However the *ari-e.GP* mutation was associated with fewer roots, shorter roots and lesser root spread in 10-day-old seedlings [22], lower root mass of six-week-old seedlings, and reduced size of the root system of field grown plants at grain filling [17]. Other chromosomal regions associated with total root length, root number and root spread have been identified in the Derkado x B83-12/21/5 mapping population [17], [22] and in a collection of mutants backcrossed into the Bowman cultivar [14].

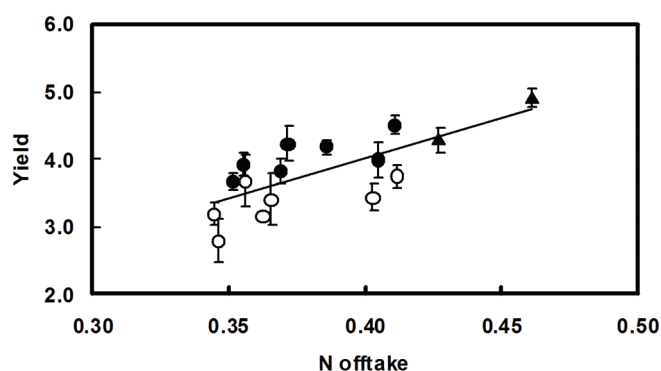


Figure 2 The significant positive relationship ($R^2=0.549$, $P=0.001$, $n=16$ genotypes) between grain yield and N offtake (kg kg^{-1} supplied) among 16 barley cultivars grown in the field at SCRI in 2006. Genotypes included tall cultivars (open circles) and cultivars bearing the dwarfing gene *sdw1* (filled circles) or *ari-e.GP* (filled triangles). Yield data are shown as means \pm standard error of four replicates.

In glasshouse (Fig. 1) and field experiments (Fig. 2, [17]), N uptake and grain yield have been positively correlated with root system size. When grown in field plots, and supplied with ample N fertilizer, cultivars bearing the *sdw1* or *ari-e.GP* mutations generally have higher grain yields than taller genotypes with a comparable N offtake, and cultivars bearing *ari-e.GP* mutation often have higher offtakes of N and other mineral elements (Fig. 2). However, cultivars bearing the *sdw1* or *ari-e.GP* mutations can have lower concentrations of mineral elements in their grain, despite their greater offtake of mineral elements, which is possibly a consequence of yield dilution ([21] and I.J. Bingham and P.J. White, unpublished data). The *ari-e.GP* mutation has also been found to decrease shoot Na concentrations and increase salt tolerance [19], [23].

Screening a collection of induced mutants in the Optic cultivar

A population of induced mutants was produced in the Optic cultivar by the Scottish Crop Research Institute using ethyl methane sulphonate [24], [25]. A structured mutation grid for exploiting TILLING (Targeted Induced Local Lesions IN Genomes) has been developed for this population, which consists of >20,000 M_3 families. This collection can be used in different ways to identify genotypes with increased efficiency to acquire mineral elements from the soil. One approach (forward genetics) is to screen these collections directly for an improved phenotype and, subsequently, to identify the genetic origin of the phenotype. The other approach (reverse genetics) is to use the techniques of molecular biology to identify accessions with mutations in target genes known to

influence the acquisition of mineral elements beneficially. This population has been screened for rooting phenotypes and their consequences for the acquisition of mineral elements have been investigated.

Early seedling root growth is an important agronomic characteristic, since it accounts for much of the N and P absorbed by a barley plant. The Optic collection was screened for differences in root traits from wildtype plants three days after germination (Fig. 3). The frequency of occurrence of mutant lines with altered root traits was high, but comparable to other collections [26], [27], [28]. The root phenotypes of several mutant lines were validated using a two dimensional root observation chamber specifically designed to measure rooting traits of young barley seedlings, such as root length, elongation rate, longest root, deepest root, seminal root number, and angular spread of roots (T.A. Valentine, unpublished data). These lines will be used for future physiological studies.

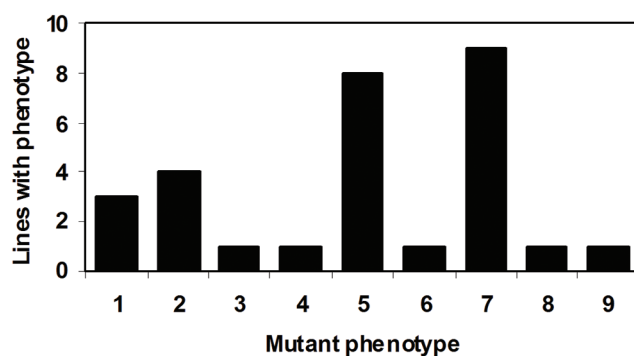


Figure 3 The frequency of specific root phenotypes among mutant lines of the Optic collection. The root phenotypes scored in comparison with the wild type were (1) long roots, (2) short roots, (3) reduced number of roots, (4) highly gravitropic roots, (5) agravitropic or curly roots, (6) more dense root hairs, (7) fewer or shorter root hairs, (8) presence of pigmentation, and (9) visible necrosis. Seeds were germinated on moistened filter paper and seminal roots were observed after three days. Thirty mutant lines with altered root characteristics were recorded from a screen of about 500 lines. (Data courtesy of W.T.B. Thomas, B.P. Forster and J. Lyons, SCRI)

Selected mutants from the Optic collection have also been screened in glasshouse and field trials. Accessions from the Optic collection with contrasting rooting traits (wild type, hairless, dense haired, long rooted and highly geotropic phenotypes) were grown to maturity in the glasshouse in 1 m long tubes filled with a grit-sand-gravel mixture and irrigated daily with a water flush followed by a standard liquid nutrient application. No significant differences in plant dry matter allocation were observed between these genotypes except for seed dry weight, which was significantly smaller in the hairless mutant compared with the wild type. However, the ability to acquire mineral elements was again correlated with the size of the root system. Root dry mass tended to be smaller in the mutant lines, although this was not significant.

Conclusions and Perspectives

The available evidence suggests that the acquisition of mineral elements by plants is related to the ability of their root systems to explore the soil. Mutants can be generated with root systems that exploit the soil better, acquire greater quantities of mineral elements, and produce greater yields on impoverished soils. In the coming years it is planned to screen the SCRI Optic mutant collection for multiple efficiencies in the acquisition and utilization of mineral elements and water. Current projects include screening for traits, and identifying genes, to improve the acquisition of N, P, Zn and Mn, improving the uptake and efficient use of water, and reducing the entry of toxic elements to the food chain. Knowledge of the genes impacting the acquisition of mineral elements can be used to develop genotypes of other common crops that can be

deployed in extreme environments: to increase their ability to grow on resource poor soils, to increase their accumulation of minerals required for animal nutrition and to reduce their accumulation of toxic elements. These outcomes should increase the sustainability of agriculture both at a subsistence and industrial level, and improve the health of populations by increasing the nutritional content and reducing the content of toxic elements in food consumed.

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BIBLIOGRAPHY

- White, P.J. *et al.* Proceedings of the International Fertilizer Society 568. Genetic Modifications to Improve Phosphorus Acquisition by Roots. *IFS*, York (2005).
- De Dordot, S. *et al.* Root system architecture: opportunities and constraints for genetic improvement of crops. *Trends Plant Sci.* **12**, 474-481 (2007).
- Barber, S.A. *Soil Nutrient Bioavailability: A Mechanistic Approach*. Wiley, New York (1995).
- Baligar, V.C. *et al.* Nutrient use efficiency in plants. *Commun. Soil Sci. Plant Anal.* **32**, 921-950 (2001).
- Gahoonia, T.S., Nielsen, N.E. Root traits as tools for creating phosphorus efficient crop varieties. *Plant Soil* **260**, 47-57 (2004).
- Rengel, Z., Marschner, P. Nutrient availability and management in the rhizosphere: exploiting genotypic differences. *New Phytol.* **168**, 305-312 (2005).
- Briggs, D.E. *Barley*. Chapman Hall, London (1978).
- Ozbas, M.O., Çağırğan, M.I. Variability and relationships of root traits in a subset of induced barley mutant collection. *Cereal Res. Commun.* **32**, 119-126 (2004).
- Hermans, C. *et al.* How do plants respond to nutrient shortage by biomass allocation? *Trends Plant Sci.* **11**, 610-617 (2006).
- Gahoonia, T.S., Nielsen, N.E. Variation in root hairs of barley cultivars doubled soil phosphorus uptake. *Euphytica* **98**, 177-182 (1997).
- Gahoonia, T.S., Nielsen, N.E. Phosphorus (P) uptake and growth of a root hairless barley mutant (*bald root barley, brb*) and wild type in low- and high-P soils. *Plant Cell Environ.* **26**, 1759-1766 (2003).
- Gahoonia, T.S., Nielsen, N.E. Barley genotypes with long root hairs sustain high grain yields in low-P field. *Plant Soil* **262**, 55-62 (2004).
- Loes, A.K., Gahoonia, T.S. Genetic variation in specific root length in Scandinavian wheat and barley accessions. *Euphytica* **137**, 243-249 (2004).
- Gordon, D.C. *et al.* A phenotypic screen for genetic analysis of barley seedling root traits. *Aspects Appl. Biol.* **73**, 81-84 (2005).
- Bengough, A.G. *et al.* Gel observation chamber for rapid screening of root traits in cereal seedlings. *Plant Soil* **262**, 63-70 (2004).
- Drew, M.C. Comparison of the effects of a localized supply of phosphate, nitrate ammonium and potassium on the growth of the seminal root system, and the shoot, in barley. *New Phytol.* **75**, 479-490 (1975).
- Chloupek, O. *et al.* The effect of semi-dwarf genes on root system size in field-grown barley. *Theor. Appl. Genet.* **112**, 779-786 (2006).
- Mlcochova, L. *et al.* Molecular analysis of the barley cv. 'Valticky' and its X-ray-derived semidwarf-mutant 'Diamant'. *Plant Breeding* **123**, 421-427 (2004).
- Forster, B.P. Mutation genetics of salt tolerance in barley: An assessment of Golden Promise and other semi-dwarf mutants. *Euphytica* **120**, 317-328 (2001).
- Walia, H. *et al.* Array-based genotyping and expression analysis of barley cv. Maythorpe and Golden Promise. *BMC Genomics* **8**, 87 (2007).
- Ellis, R.P. *et al.* Phenotype/genotype associations for yield and salt tolerance in a mapping population segregating for two dwarfing genes. *J. Exp. Bot.* **53**, 1163-1117 (2002).
- Forster, B.P. *et al.* Genetic controls of barley root systems and their associations with plant performance. *Aspects Appl. Biol.* **73**, 199-204 (2005).
- Pakniyat, H. *et al.* Comparison of salt tolerance of *GPert* and non-*GPert* barleys. *Plant Breeding* **116**, 189-191 (1997).
- Caldwell, D.G. *et al.* A structured mutant population for forward and reverse genetics in barley (*Hordeum vulgare* L.). *Plant J.* **40**, 143-150 (2004).
- Forster, B.P. *et al.* The barley phytomer. *Ann. Bot.* **100**, 725-733 (2007).
- Engvild, K.C., Rasmussen, S.K. Root hair mutants of barley. *BGN* **34**, 13-15 (2004).
- Nawrot, M. *et al.* Mutational analysis of root system characters related to agronomic performance in barley. In: *Mutational Analysis of Root Characters in Food Plants*, IAEA, Vienna, 23-38 (2006).
- Janiak, A., Szaejko, I. Molecular mapping of genes involved in root hair formation in barley. *Euphytica* **157**, 95-111 (2007).