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Identification of root morphology mutants in barley

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Abstract

In this study, a forward-genetics analysis was performed on a portion of TILLMore, a chemically mutagenized population of barley cv. 'Morex' (http://www.distagenomics.unibo.it/TILLMore/), to identify root morphology alterations by comparison with 'Morex' wild-type. For this purpose, a simple paper-roll approach was performed to identify phenotypic variants at the seedling stage. The analysis of *c*. 1000 M_4 families allowed us to identify *c*. 70 lines with altered root morphology. A more accurate phenotypic characterization of a portion of the mutant lines has been performed using stereomicroscopy and a scanning electron microscopy approach.

Keywords: forward-genetics; *Hordeum vulgare*; root hairs; root morphology; TILLING

Introduction

In agriculture, water is usually the predominant factor limiting plant growth. If plants do not receive adequate rainfall or irrigation, the resulting drought stress can reduce the growth more than all other environmental stresses combined. In this context, crops characterized by a more effective use of water will be at an advantage. Among the morpho-physiological traits that have been shown to influence drought resistance, root morphology (e.g. depth, angle, size, number of root-hairs, etc.) plays a crucial role (Richards, 2008; Shao *et al*., 2008; Hodge *et al*., 2009). In monocots, a systematic genetic analysis of root formation has been carried out mainly in maize and rice (de Dorlodot *et al*., 2007; Taramino *et al*., 2007; Hochholdinger and Tuberosa, 2009; Coudert *et al*., 2010). This notwithstanding, the information on the genetic control of root morphology and functions in barley (*Hordeum vulgare* L.) is rather poor, with only one notable exception (Kwasniewski and Szarejko, 2006). Additional evidence shows the complexity of root growth and how it is continually challenged by a wide spectrum of stimuli such as distribution of nutrients, obstacles, heat, light and oxygen availability (Eapen *et al*., 2003; Massa and Gilroy, 2003).

At DiSTA, a mutagenized population of barley (*H. vulgare* L.) cv. 'Morex' has been produced following chemical treatment with sodium-azide (Talamè *et al*., 2008, 2009). This Targeting Induced Local Lesions IN Genomes (TILLING; McCallum *et al*., 2000) resource, named TILLMore (http://www.distagenomics.unibo.it/TILLMore/), is available for both forward- and reverse-genetics applications in barley. We report the results of a forward-genetics analysis to identify mutants for root architecture.

Materials and methods

The phenotypic analysis for root-related traits was carried out on a subset of *c*. 1000 M_4 families from the TILLMore collection. The analysis was performed using a paper-roll approach as described by Woll *et al*. (2005). The method is based on seedlings grown in rollers made of a wound sandwich of filter papers and kept in a growth chamber under controlled conditions (16/8 h photoperiod and 24/22°C day/night temperature). The analysis was performed on *c*. 12 seeds/family per paper-roll. At the stage of 8-d-old seedling, the morphology of the seminal

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*Corresponding author. E-mail: maria.sanguineti@unibo.it roots was visually compared to that of the wild type cv. 'Morex'. The phenotypic evaluation was repeated (three reps of 12 seeds/paper-roll) only for the putative mutants identified during the preliminary screening.

For those mutants that presented an altered root-hair phenotype, further observations were made in aeroponic conditions. The germinated seeds were placed in sterile glass tubes covered with cotton bungs, one seed/tube. Each tube with seed was then connected with an empty tube and both parts were held together with parafilm. The plants were grown for 5 d under controlled conditions. Microscopic observations and image analyses of 5- to 7-d-old seedlings were performed with the use of Stemi 2000-C (Zeiss) stereomicroscope and AxioVision LE (Carl-Zeiss) program. On the same samples, the terminal root segments (*c.* 1 cm portion) were excised and viewed with a Tesla BS-340 scanning electron microscopy (SEM) microscope.

Results and discussion

The screening of 1000 TILLMore M_4 lines showed altered root morphology for 72 families. One group of 26 mutants showed fairly stable phenotype, while 47 lines showed unstable, indistinct or segregating phenotypes. The mutations identified affected different aspects of root development and were classified as belonging to nine main categories (Table 1). Some of the unstable phenotypes may reflect genetic segregation or responses to environmental conditions like gases (e.g. O_2, ethylene and CO_2), light and physical impediments (Engvild and Rasmussen, 2004). Furthermore, shortened roots could sometimes be due to a pleiotropic effect of other mutations such as dwarf or chlorophyll mutants. Almost all the detected mutants showed reduced length of seminal roots ranging from slightly short to extremely short. In various cases, short-root mutants developed other alterations such as curled morphology and thick appearance. In barley, an experiment similar to what is herein reported has been performed for the identification of short-root mutants (Nawrot *et al.*, 2005). In this case, nine mutants showing significant shortening of seminal roots were identified in a collection of dwarf and semi-dwarf lines obtained after mutagenic treatment of spring barley with *N*-nitroso-*N*-methylurea and sodium-azide (Nawrot *et al.*, 2005). The analysis on our population was carried out on lines randomly chosen without considering the phenotype of the visible portion of the plant. Thus, the majority of mutants (*c.* 70%) showed alterations in the root but not in other organs.

Four lines showed an altered root-hair formation. Such mutations affected two stages of root-hair development and were classified as root hairless (*rhl*) or short root-hair (*srh*). A more detailed phenotyping of the root-hair mutants was carried out growing the mutant lines in aeroponics and root observation was performed using a stereomicroscope and a SEM microscope. The images showed a noticeable difference between the hairless mutants, with a perfectly smooth root surface, and the short root-hair mutants, where the elongation of the root hairs is blocked in the early stages of development (Fig. 1). Recently, a similar survey was conducted at the University of Silesia in Poland with a population of barley mutants screened in aeroponic conditions (Szarejko *et al.*, 2005). This analysis allowed for the identification of 17 mutants with changes in root-hair development and the cloning of an expansin gene related to root-hair formation in barley (Kwasniewski and Szarejko, 2006). Except for this outcome, until now, very little information is available on the molecular control of root-hair growth in barley and, in general, in almost all the monocots.

Remarkably, two TILLMore root mutant categories presented alterations that, to our best knowledge, so far have not yet been described in barley. The most interesting is a mutant category where the root does not appear and is replaced by a leaf (root-to-leaf). Further studies on these materials may provide important information for a better understanding of the signalling networks that lead to root/leaf differentiation. The second category of mutants presented a highly geotropic growth. Similar mutants of *Arabidopsis*, presenting an increased response

Table 1. List of barley mutant categories with altered root phenotype[a]

Mutant category[b]	Root-mutants (*n*)	No. of root-mutant families/total no. of root-mutant families (%)
Short root[c]	25	34.7
Very short root[d]	12	16.7
Short and thick root	16	22.2
Short and curly root	8	11.1
Curly root	3	4.2
Short root-hair	3	4.2
Highly geotropic	2	2.8
Root-to-leaf	2	2.8
Hairless	1	1.4
Total	72	100.0

[a] Pictures of these mutants are available at http://www.dista.unibo.it/TILLMore/check.php. The images can be viewed by typing the mutant code number (e.g. highly-geotropic: 194, 3580; hairless: 5545; short root-hair: 5387, 2588, 1568; root-to-leaves: 2956, 3109) or search for the mutant category. [b] For each mutant category, we report the total number of mutants identified and their percentage among the root-mutant families. [c] Roots *c.* 50–60% shorter compared with wild-type 'Morex'. [d] Roots *c.* 70–90% shorter compared with wild-type 'Morex'.

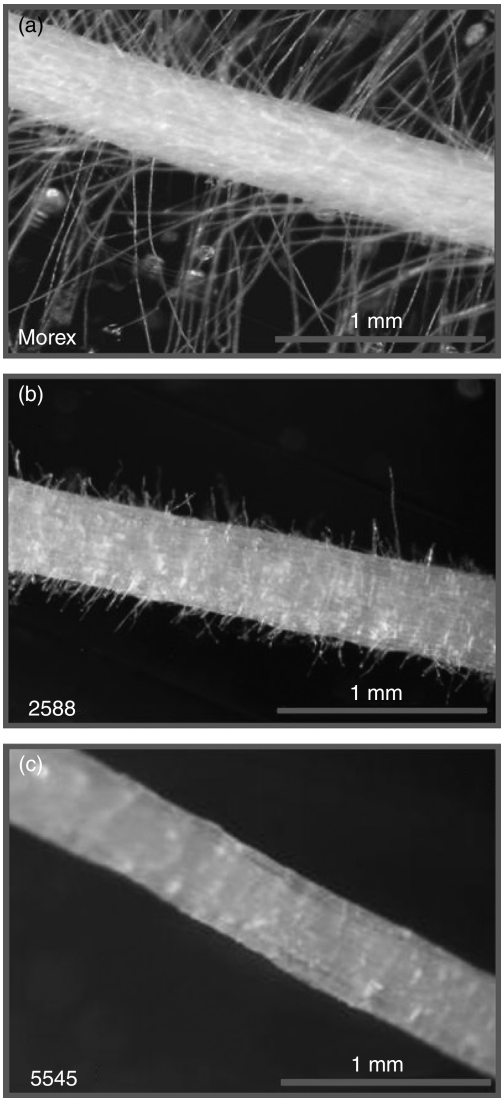

Fig. 1. Two root-hair mutants compared with Morex wild-type (a) used as reference. The picture (b) shows a short root-hair mutant (cod. 2588), where the hairs are strongly shortened. The image (c) shows an hairless mutant with a smooth root (cod. 5545). All the pictures were obtained with a Stemi 2000-C (Zeiss) stereomicroscope at 50× of magnification.

to gravitropism, lack the capacity to perceive a moisture gradient that is thought to be important for controlling root orientation (Kobayashi *et al*., 2007). Consequently, the two barley highly geotropic mutants may actually be hydrotropic mutants, unable to perceive water gradient in the paper-roll system. Further studies are required to validate this hypothesis.

Further details on root mutants and an informative set of pictures are available in an on-line database at http://www.distagenomics.unibo.it/TILLMore/. This database is publicly accessible and seed for mutants of interest is available on request.

For some of the TILLMore mutants with clear changes in root architecture, an appropriate series of backcrosses and outcrosses are in progress prior to their mapping in order to fix the mutations and then map and clone new genes involved in root formation and growth. From a physiological standpoint, these mutants may also help to better comprehend the role of roots in water and nutrient uptake (Gahoonia *et al*., 2001), association with symbiotic organisms (Chen *et al.,* 2005) and root–soil interaction, hence providing valuable information for agronomic applications.

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References

Chen B, Roos P, Borggaard OK, Zhu YG and Jakobsen I (2005) Mycorrhiza and root hairs in barley enhance acquisition of phosphorus and uranium from phosphate rock but mycorrhiza decreases root to shoot uranium transfer. *Plant Phytology* 165: 591–598.

Coudert Y, Périn C, Courtois B, Khong NG and Gantet P (2010) Genetic control of root development in rice, the model cereal. *Trends in Plant Science* 15: 219–226.

de Dorlodot S, Forster B, Pagès L, Price A, Tuberosa R and Draye X (2007) Root system architecture: opportunities and constraints for genetic improvement of crops. *Trends in Plant Science* 12: 474–481.

Eapen D, Barroso ML, Campos ME, Ponce G, Corkidi G, Dubrovsky JG and Cassab GI (2003) A no hydrotropic response root mutant that responds positively to gravitropism in *Arabidopsis*. *Plant Physiology* 131: 536–546.

Engvild KC and Rasmussen SK (2004) Root hair mutants of barley. *Barley Genetics Newsletter* 34: 13–15.

Gahoonia TS, Nielsen NE, Joshi PA and Jahoor A (2001) A root hairless barley mutant for elucidating genetic of root hairs and phosphorus uptake. *Plant and Soil* 235: 211–219.

Hochholdinger F and Tuberosa R (2009) Genetic and genomic dissection of maize root development and architecture. *Current Opinion in Plant Biology* 12: 172–177.

Hodge A, Berta G, Doussan C, Merchan F and Crespi M (2009) Plant root growth, architecture and function. *Plant and Soil* 321: 153–187.

Kobayashi A, Takahashi A, Kakimoto Y, Miyazawa Y, Fujii N, Higashitani A and Takahashi H (2007) A gene essential for hydrotropism in roots. *Proceedings of the National Academy of Science USA* 104: 4724–4729.

Kwasniewski M and Szarejko I (2006) Molecular cloning and characterization of beta-expansin gene related to root hair formation in barley. *Plant Physiology* 141: 1149–1158.

Massa GD and Gilroy S (2003) Touch modulates gravity sensing to regulate the growth of primary roots of *Arabidopsis thaliana*. *Plant Journal* 33: 435–445.

McCallum CM, Comai L, Greene EA and Henikoff S (2000) Targeting Induced Local Lesion IN Genomes (TILLING) for plant functional genomics. *Plant Physiology* 123: 439–442.

Nawrot M, Szarejko I and Maluszynski M (2005) Barley mutants with short roots. *Barley Genetics Newsletter* 35: 3–8.

Richards RA (2008) Genetic opportunities to improve cereal root system for dryland agriculture. *Plant Production Science* 11: 12–16.

Shao HB, Chu LY, Jaleel CA and Zhao CX (2008) Water-deficit stress-induced anatomical changes in higher plants. *Comptes Rendus Biologies* 331: 215–225.

Szarejko I, Janiak A, Chmielewska B and Nawrot M (2005) Genetic analysis of several root hair mutants of barley. *Barley Genetics Newsletter* 35: 36–38.

Talamè V, Bovina R, Sanguineti MC, Tuberosa R, Lundqvist U and Salvi S (2008) TILLMore, a resource for the discovery of chemically induced mutants in barley. *Plant Biotechnology Journal* 6: 477–485.

Talamè V, Bovina R, Salvi S, Sanguineti MC, Piffanelli P, Lundquist U and Tuberosa R (2009) TILLING with TILLMore. In: Shu QY (ed.) *Induced Plant Mutations in the Genomics Era*. Joint FAO/IAEA Programme. Rome: Food and Agriculture Organization of the United Nations, pp. 240–242.

Taramino G, Sauer M, Stauffer JL Jr, Multani D, Niu X, Sakai H and Hochholdinger F (2007) The maize (*Zea mays* L.) *RTCS* gene encodes a LOB domain protein that is a key regulator of embryonic seminal and post-embryonic shoot-borne root initiation. *Plant Journal* 50: 649–659.

Woll K, Borsuk LA, Stransky H, Nettleton D, Schnable PS and Hochholdinger F (2005) Isolation, characterization, and pericycle-specific transcriptome analyses of the novel maize lateral and seminal root initiation mutant rum1. *Plant Physiology* 139: 1255–1267.