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Genetic analysis of several root hair mutants of barley

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Introduction

Root hairs are specialized epidermal cells that play an important role in uptake of water and nutrients from rhizosphere. Each hair is a long, tubular, non-dividing cell characterized by tip growth, i.e. by deposition of new membrane and cell wall precursors at a cell-growing tip. Several root hair mutants have been isolated and genetically characterized in *Arabidopsis thaliana* (Parker, 2000, Shiefelbein et al., 2000) however, besides a few reports in maize (Wen and Schnable, 1994), there is a lack of information about genetic basis of root hair development in monocotyledons. Recently, Engvild and Rasmussen (2004) described some barley mutants with changes in root hair phenotype, but for now without information on their genetic basis. Nevertheless, the growing resources of such barley germplasm can help to reveal the mechanisms underlying the process of root hair morphogenesis in monocotyledon species.

Material and Methods

The mutagenic treatment of barley varieties 'Rudzik', 'Dema', 'Krona', 'Diva', 'Karat', and 'Delisa' was performed with the use of N-methylo-N-nitroso-urea (MNU) and sodium azide. To generate high frequency of gene mutations, the combined treatment with both mutagenes (Szarejko and Maluszynski, 1999) or double treatment of MNU was applied. The doses for treatment of Rudzik and Dema varieties were as follows: 1.5 mM NaN_3/3h – inter-incubation germination/6h – 0.75 mM MNU/3h and for variety Krona: 1.75 mM NaN_3/3h – inter-incubation germination/6h – 0.9 mM MNU/3h. For the varieties Diva, Karat and Delisa double treatment of MNU was used: 0.7 mM MNH/3h. - inter-incubation germination/6h - 0.7 mM MNH/3h. The selection of root hair mutants was performed in the M_2 or M_3 generations. Subsequent generations (M_4-M_6) were used to perform homozygosity test and multiply seeds of homozygous lines with mutated root hair phenotype. The observations of root hair zone were done for plants growing in aeroponic conditions (Fig.1). The inheritance of the root hair phenotypes was analyzed in the F_1 and F_2 generations from the crosses between mutants and their parent varieties. Allelism test between mutants was performed.

Results

Seventeen mutants with changes in the root hair development and morphology have been identified after mutangenic treatments of barley varieties Rudzik, Dema, Krona, Diva, Karat, and Delisa. The mutations affected different stages of root hair development and were classified as belonging to one of four main root hair phenotypes (Table 1):

- root hairless mutants, designated rhl,
- mutants with development of root hairs blocked at the initial stage, forming only small root hair primordia, seen as 'bulges', designed rhp,
- mutants with very short root hairs, designed rhs,
- mutants with irregular pattern of root hairs, designed rhi.

The genetic analysis revealed that each of the characters in the analyzed mutants was controlled by single, recessive gene. According to the results of allelism tests, the locus number

and letter symbol of allele was added to the three-letter code of the mutant name. The three root hairless mutants derived from variety Karat proved to be allelic. Similarly, one gene controlled the phenotype of four mutants developing root hairs blocked at the primordium stage. The rhp1.a – rhp1.d mutants showed slightly elongated root hair primordia, indicating that root hair development has been stopped at the stage of transition to tip growth. All three mutants with very short root hairs represented three different loci. The root hair length in these mutants has not reached 10% of the parent variety. Additionally, the mutant rhs1.a was characterized by the short seminal roots and short spike (reaching about 50% of the length of parent variety). These characters were controlled by separated genes linked with the gene controlling the root hair length. The other mutant with short root hairs, 522DK, not yet characterized according to the allelism with other forms, showed about 40% reduction of root length and 30% reduction of the shoot length in comparison to the parental form (Nawrot, 2005).

The remaining group of four mutants with irregular root hairs was representing two loci. In mutants rhi2.a and rhi2.c, the length and density of root hairs varied between roots and different parts of the same root, but never reached the length and density of root hairs characteristic for the parent variety. The other two mutants: rhi1.a and rhi2.b develop only a few, very sparsely located root hairs of differentially reduced length. The root hair density was lower than in two mutants described above. Another mutant, 800Q with irregular root hairs develops very short root hairs, similar in length to the rhs1.a mutant, but spread on the root in a very irregular pattern.

The analysis of genetic relationships between genes of study was performed for four loci: *rhl1* (mutants 834Q and 931Q), *rhp1* (mutants DM 209.1.3.1 and DM 204.1.8.5), *rhs1* (mutant 225DV) and *rhi1* (mutant DM 204.1.8.12). Gene *rhl1*, responsible for the lack of root hairs was epistatic to three other genes involved in further steps of root hair formation, i.e., formation of root hair primordia (gene *rhp1*), location of root hair bearing cells (gene *rhi1*) and root hair elongation (gene *rhs1*). Similarly, genes: *rhp1* and *rhi1* were epistaic to the *rhs1* locus. The genes: *rhp1* and *rhi1*, however, give hairless phenotype in a double recombinant. Further analysis will be performed to describe relationships between the remaining loci.

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Fig. 1. Aeroponic culture of barley seedlings

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Table. 1. List of barley mutants with changes in root hair morphology

No. of mutant line	Parent variety	Phenotypic class	Symbol of locus and allele*
834Q	Karat	root hairless	*rhl1.a*
931Q	Karat	root hairless	*rhl1.b*
934Q	Karat	root hairless	*rhl1.c*
DM 209.1.3.1	Dema	root hair primordia	*rhp1.a*
DM 204.1.8.5	Dema	root hair primordia	*rhp1.b*
DM 201.11.3	Dema	root hair primordia	*rhp1.c*
RD 103.1.1	Rudzik	root hair primordia	*rhp1.d*
225 DV	Diva	root hair short	*rhs1.a*
DM 55.1	Dema	root hair short	*rhs2.a*
740Q	Karat	root hair short	*rhs3.a*
DM 204.1.8.12	Dema	root hair irregular	*rhi1.a*
RD 100.1	Rudzik	root hair irregular	*rhi2.a*
RD 101.2.7	Rudzik	root hair irregular	*rhi2.b*
DM 208.1.6	Dema	root hair irregular	*rhi2.c*
522DK	Delisa	root hair short	n/a
800Q	Karat	root hair irregular	n/a
KR 36.6	Krona	root hair irregular	n/a

* - symbols designated according to the genes symbolization in barley (Franckowiak and Lundquist, 2004)

n/a – not available, mutants not yet characterized according to the locus and allele number