Root Hair Mutants of Barley

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Abstract
Almost sixty mutants with shortened or reduced root hairs or without root hairs at all have been isolated among 2000 M_1 plant progenies (20.000 M_2 seeds) of the spring barley cultivar "Lux" after sodium azide mutagenesis. One group of 25 mutants show fairly stable phenotype while 33 lines have unstable, indistinct or segregating phenotypes. The mutants were selected after 3 to 4 days growth in tap water on black filter paper. Root hair mutants were quite common, although not as common as chlorophyll mutants. Some of the unstable phenotypes may reflect responses to gases like ethylene or CO_2. Shortened root hairs were sometimes a pleiotropic effect of other mutations, such as dwarf or chlorophyll mutants.

Introduction
Root hairs are small, tubular, 1-2 mm long extensions of single epidermal cells of the root surface. Their primary function seems to be to extend the surface of the root, thereby improving access to strongly bound nutrients like phosphorus, iron, zink, silicon and other micronutrients (Peterson and Farquhar 1996, Bibikova and Gilroy 2003). Root hair variation was first investigated in clover by Caradus (1979). There is one tomato mutant "cottony root" with longer root hairs than normal (Hochmuth et al. 1985). Root hair mutants have been investigated extensively in *Arabidopsis*, where a large collection of mutants is available (Grierson et al. 2001, Schiefelbein and Somerville 1990, Schiefelbein 2000). Root hair mutants have been studied in barley by Gahoonia et al. (2001) and by Szarejko et al. (2003). Root hairs are not absolutely necessary for plant growth, because root hairless mutants of barley, rice and maize grow quite well, if nutrients are readily available (Wen and Schnable 1996, Ma et al. 2001, Gahoonia et al 2001).At present about 40 genes are known to be involved in Arabidopsis root hair formation (Grierson et al. 2001, Parker et al. 2001).

Methods
Seed of "Lux" barley (Sejet Plant Breeding, Denmark) were soaked overnight in tap water, then treated with 1.5 millimolar sodium azide in 0.1 molar sodium phosphate buffer, pH 3, for 2.5 hours according to the IAEA manual on mutation breeding (2nd. Ed). After rinsing in tap water and air drying, the M_1 seeds were sown in the field the same day. Spikes, 4-6 per M_1 plant, were harvested.

9 or 16 seeds were germinated on 7 × 11 cm black filter paper in transparent polystyrene boxes 8 × 12 × 3 cm with almost tight-fitting lids. The germination took place in 5 ml of tap water at room temperature for 3 or 4 days. 0.5 mg Thiram decreased but did not eliminate fungal contamination. Scoring for root hair mutants was done directly through the transparent lid under a stereo microscope.

Results and discussion

The agar growth technique of isolating *Arabidopsis* root hair mutants did not work well for barley in our hands as there were many problems with infection. The chlorophyll mutation frequency of the mutagenized "Lux" material was 7 % of the M_1 progeny and 0.9 % of the M_2 seedlings, comparable to that obtained with other barley
cultivars from which low-phytate mutants were isolated (Rasmussen and Hatzack 1998).

The results show that root hair mutants can also be easily isolated in other plants than *Arabidopsis*. Most of the mutants are viable and some of them can undoubtedly be used as genetic markers, or in investigations of root hair physiology, root architecture, nutrient uptake, and the function and importance of mycorrhiza . Some of the mutants show unstable, variable or indistinct phenotypes. Some of these instabilities may be caused by lack of control of gases such as ethylene or CO_2 which influence root hair formation (Pitts et al. 1998, Ohashi et al. 2003, Müller and Schmidt 2004). The large majority of mutants are recessive, judged by the segregation ratios of mutant/wild type among the M_2 seeds. No crossing experiments have been done.

Due to other commitments the work on the root hair mutants has been discontinued at an early stage. Therefore the mutants have not been grown further than to M_3 (M_4 seeds). They need further purification of other unwanted mutations and more examination of phenotype stability. An important group of mutants without characterisation are the completely sterile root hair less which could presumably have defects in the tip growth mechanism common to pollen tube and root hair.

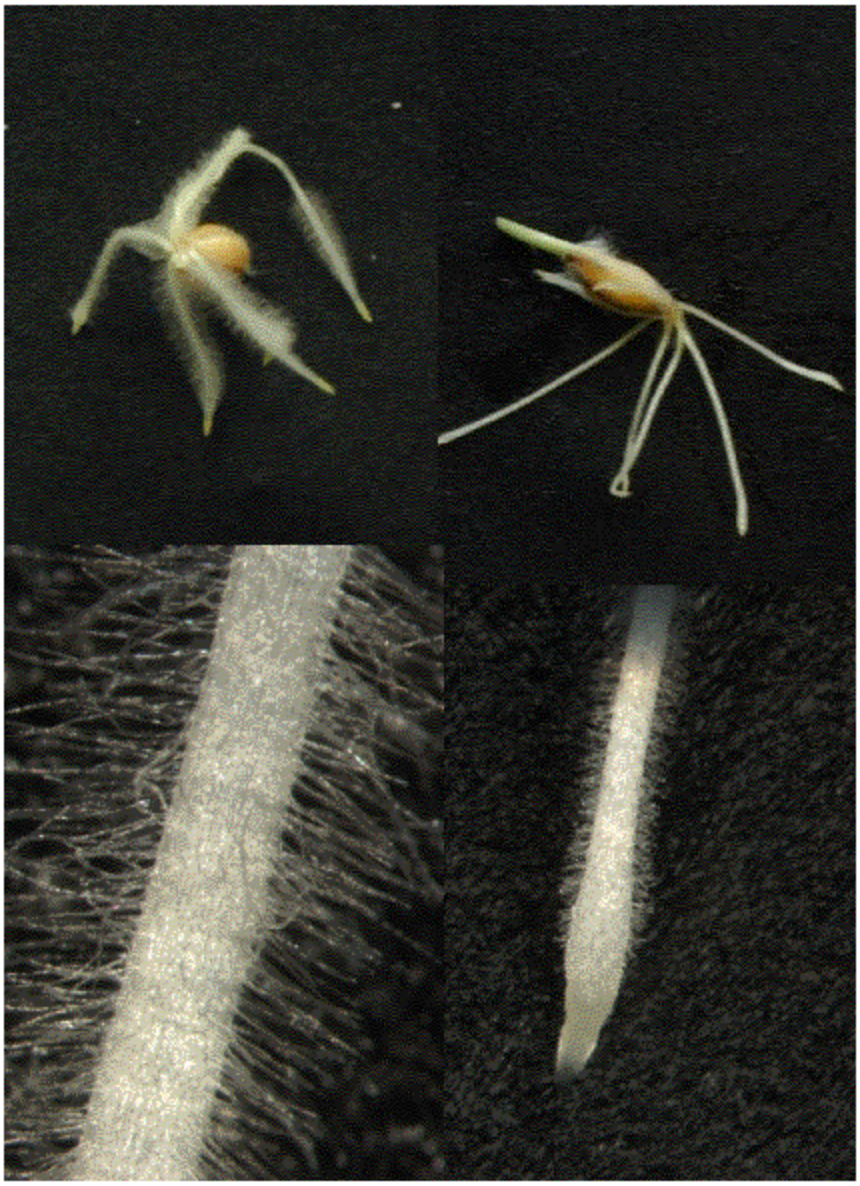

Figure 1. Wild type “Lux” barley to the left, two mutants with very short root hairs to the right

Table 1. Root hair mutants isolated among 2000 M_1 progenies		
"Lux" Progeny No	Phenotype	Remarks
76	Few root hairs	
460	Short	
465	Minus root hairs	
534	Minus root hairs	
681	Short	
754	Short	
837	Short	
846	Short, few	
919	Minus root hairs	
931	Short	
991	Short	Swollen root tip
1033	Minus root hairs	
1119	Short	
1205	Minus root hairs	
1209	Minus root hairs	
1210	Short	
1223	Short	
1339	Very short	
1400	Very short	
1533	Very short	
1537	Very Short	
1544	Very short	
1667	Minus root hairs	
1807	Short, few	
1920	Short	

Table 2. Putative mutants that are not "well behaved", either unstable, indistinct or segregating phenotypes		
"Lux" progeny No	Phenotype	Remarks
112	Short	Poor germination
160	Short	Segregating tuft?
194	Short	Segregating?
219	Short	Segregating?
226	Short, few	Segregating?
234	Tuft	Indistinct
244	Tuft	Short root?
250	Short	Segregating?
272	Short	Indistinct
337	Short	Poor plants
362	Short	Segregating
369	Short	Infected
430	Tuft	Segregating?
488	Short	Indistinct
522	Short	Indistinct
577	Minus root hairs	Segregating short?
635	Minus root hairs	Segregating
706	Minus root hairs	Segregating
741	Short	Almost normal
778	Short	Sterility
813	Short	Poor plants
840	Short	
923	Short, tuft	Poor plants
1261	Tuft	Indistinct
1263	Long?	Indistinct
1278	Short, few	Poor plants
1374	Short	Segregating
1450	Tuft?	Indistinct
1467	Tuft?	Indistinct
1505	Short	Segregating
1656	Short	Segregating
1707	Tuft	Segregating
1712	Tuft	Indistinct
1840	Short, tuft	Indistinct
1919	Short	Segregating?

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