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Barley mutants with short roots

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Abstract

Nine mutants showing significant shortening of seminal roots have been identified in the collection of dwarf and semi-dwarf forms obtained after mutagenic treatments of spring barley varieties with N-nitroso-N-methylurea and sodium azide. Genetic analysis, performed at the seedling and the spike-emergence stage of plant development, indicated that a single recessive gene was responsible for root shortening in each of analyzed mutants. One short-root mutant developed also very short root hairs. Short roots and short root hairs were controlled by separate genes. The reciprocal crosses of four mutants revealed that they were non allelic.

Introduction

Roots play a decisive role in plant growth and development. The mutational analysis of root system provides a useful tool for revealing the genetic basis of root characters, such as root apical organization, root cell proliferation, secondary root formation, root hair development, and root elongation. The most advanced results on genetics of root system development and morphology were achieved in a model plant species *Arabidopsis thaliana*, where several root mutants have been described (Baskin et al., 1992; Benfey et al., 1993; Aeschbacher et al, 1994; Baskin et al, 1995; Hauser et al., 1995; Di Laurenzo et al., 1996). In agronomically important crop plants, only a few root system mutants are known (Yao et al., 2003; 2002; Inukai et al., 2001; Tsyganov, 2000; Tsuchiya, 1974). In the presented paper we report on barley mutants characterized by seminal roots significantly shorter than roots of parent varieties during whole vegetation period.

Material and methods

The studies included nine dwarf and semi-dwarf (sd) spring barley mutants developing shorter seminal roots than parent varieties. The mutants, obtained after mutagenic treatment with N-nitroso-N-methylurea (MNH=MNU), were identified in the collection of dwarf and semi-dwarf forms of the Department of Genetics, University of Silesia, Poland. Additionally, one genotype (225DV from cv. 'Diva') developed also very short root hairs.

Root length at the early developmental stage (8-day old seedling) was evaluated using a paper roller method. The method based on growing seedlings in rollers made of filter paper wrapped tightly around a glass tube (ϕ 2.5 cm). The plastic-coated wires were enclosed between each layer of paper, and the surface of rollers was covered with black foil. Sterilized and pre-germinated seeds (with coleorhiza emerged by 1-2 mm) were placed embryo down, one beside each of two sides of a wire. The rollers were placed in containers with equal level of distilled water and kept in a growth chamber under controlled conditions: illumination 180 $\mu Em^{-2}s^{-1}$, 16/8h photoperiod and temperature 24/22^{o}C day and night, respectively. At the

stage of 8-day old seedling, the length of the longest seminal root and the length of the first leaf were measured. Three replications of each mutant and its parent variety (10 seedlings per replication) were included in each analysis.

The second analysis of root growth was performed at 6-week stage. Plants were grown in the PVC tubes, 125 cm long and 7.5 cm in diameter, filled with sand. To facilitate the extraction of intact roots, plastic foil was used to line the inside of each tube. Pre-germinated seeds were sown into the tubes, and covered with 2-3 cm layer of soil. The experiments were conducted in a glasshouse, under semi-controlled conditions: illumination 200 $\mu Em^{-2}s^{-1}$, 16/8h photoperiod and temperature 25/15^{o}C day and night, respectively. Every two days, plants were nourished with 100 ml of ½ MS mineral medium (Murashige and Skoog, 1962). The excess of solution flew out freely from the tubes. Plants were gently sprinkled with tap water throughout to prevent desiccation. The experiments were performed in three replications with 3-5 plants per replication. Plants were harvested and washed after 6 weeks and the length of the longest seminal root, and the number of roots was measured.

The analysis of F_1 and F_2 generation of the crosses between mutants and their parent varieties as well as among selected mutants was performed at the seedling stage. Ten to 20 F_1 plants and 100-200 F_2 plants were examined for the longest root and the first leaf length.

Results

The selected sd mutants developed significantly shorter seminal roots at both analyzed stages of plant development (Table 1). Mutant 225DV from variety 'Diva' presented the highest level of root and shoot reduction, with root length reaching only about 40% of parent variety at the seedling and 50% at the spike-emergence stage. The root length reduction observed in mutants from variety 'Aramir' ranged from 25% - 48% at both analyzed stages. The results obtained for four mutants from variety 'Delisa' indicated similar level of root length reduction. In most mutants, the shoot length was slightly more reduced at the seedling stage than at maturity.

Table 1. Analysis of root and shoot length in mutants and their parent varieties at the seedling and spike-emergence stage.

Genotype	Seedling stage				Spike emergence stage		Maturity	
	Root length (cm) $\bar{x} \pm SD$	Reduction (%)	First leaf length (cm) $\bar{x} \pm SD$	Reduction (%)	Root length (cm) $\bar{x} \pm SD$	Reduction (%)	Shoot length (cm) $\bar{x} \pm SD$	Reduction (%)
Aramir	32.0±0.7^{A*}		15.1±0.3^{A}		132.6±3.2^{A}		80.4±4.5^{A}	
014AR	19.8±0.2^{B}	38.1	12.9±0.4^{B}	14.5	98.9±5.3^{B}	25.4	56.6±0.4^{BC}	29.6
035AR	23.9±1.4^{B}	25.3	10.1±0.4^{B}	33.1	92.9±0.4^{B}	29.9	45.3±3.1^{D}	43.6
037AR	16.7±1.6^{B}	47.8	8.7±0.4^{C}	42.4	80.8±4.2^{C}	39.1	46.8±0.6^{D}	41.8
090AR	20.2±0.8^{B}	36.9	12.9±0.5^{B}	14.6	99.8±4.8^{B}	24.7	61.4±1.4^{B}	23.6
Diva	31.9±3.7^{A}		14.7±1.0^{A}		136.0±3.3^{A}		85.5±1.3^{A}	
225DV	11.8±0.5^{B}	63.0	5.6±0.4^{B}	61.9	66.6±2.2^{B}	51.0	41.0±1.1^{B}	52.0
Delisa	27.0±2.2^{A}		13.3±0.2^{A}		128.2±2.3^{A}		86.3±5.4^{A}	
522DK	15.5±0.1^{B}	42.6	7.9±0.4^{B}	59.4	80.6±5.2^{B}	37.1	58.9±2.0^{C}	31.8
538DK	16.9±0.6^{B}	37.4	7.9±0.3^{B}	59.4	88.6±2.7^{B}	30.9	66.3±2.1^{B}	23.1
587DK	16.1±1.3^{B}	40.4	7.0±0.9^{B}	47.4	87.1±2.5^{B}	32.0	65.5±3.4^{B}	24.1
588DK	15.9±1.3^{B}	41.1	7.0±0.4^{B}	47.4	95.6±2.3^{B}	25.4	66.6±0.7B	22.8

* - the same letter for the group of the mutant and parent variety indicates not significant difference for P=0.05.

Analysis of seminal root length in the F_1 generation of the crosses 'mutant x parent variety' revealed the recessive character of root phenotypes in all examined mutants. The segregation of F_2 progeny indicated that short seminal roots were recessive and monogenically inherited (Tab. 2). The allelism test performed up to now for 4 mutants crossed to each other revealed four different loci responsible for the seminal root shortening. The allelism analysis of other mutants is in progress. Short roots and short root hairs of mutant 225DV from variety Diva were controlled by two separate but linked genes (Tab. 3).

Table 2. Analysis of F_1 and F_2 generation of the crosses 'mutant x parent variety and 'mutant x mutant'.

Genotype	No. of analyzed plants	Seminal root length (cm) $\bar{x} \pm SD$	No. of F_2 plants with the phenotype of		$\chi^2_{3:1}$
			parent variety	mutant	
Aramir	30	24.5±2.0^{A*}			
014AR	30	18.7±1.8^{B}			
F_1 014AR x Aramir	17	23.0±0.7^{A}			
F_2 014AR x Aramir	97		69	28	0.76
Aramir	30	28.9±0.1^{A}			
035AR	30	18.9±0.5^{B}			
F_1 035AR x Aramir	28	27.7±1.3^{A}			
F_2 035AR x Aramir	109		109	36	0.002
Aramir	30	24.5±2.0^{A}			
037AR	30	12.9±0.5^{B}			
F_1 037AR x Aramir	11	23.3±1.7^{A}			
F_2 037AR x Aramir	90		65	25	0.36
Aramir	30	24.5±2.0^{A}			
090AR	30	13.9±0.7^{B}			
F_1 090AR x Aramir	13	24.9±1.2^{A}			
F_2 090AR x Aramir	85		63	22	0.01
Diva	30	27.9±0.7^{A}			
225DV	30	7.0±0.8^{B}			
F_1 225DV x Diva	27	26.5±0.9^{A}			
F_2 225DV x Diva	187		134	53	1.11
Delisa	30	19.6±0.7^{A}			
522DK	30	11.8±0.2^{B}			
F_1 522DK x Delisa	11	21.8±1.1^{A}			
F_2 522DK x Delisa	122		85	37	1.84
Delisa	30	19.6±0.7^{A}			
538DK	30	14.2±1.0^{B}			
F_1 538DK x Delisa	14	19.6±0.5^{A}			
F_2 538DK x Delisa	150		113	37	0.01
Delisa	30	19.6±0.7^{A}			
587DK	30	13.1±1.0^{B}			
F_1 587DK x Delisa	12	21.9±1.1^{A}			
F_2 587DK x Delisa	103		71	32	2.03
Delisa	30	19.6±0.7^{a}			
588DK	30	12.1±1.2^{b}			
F_1 588DK x Delisa	9	21.8±2.0^{a}			
F_2 588DK x Delisa	68		48	20	0.18
Diva	30	28.1±1.8^{A}			
225DV	30	6.4±0.9^{C}			
Aramir	30	30.4±1.4^{A}			
014AR	30	15.5±1.5^{B}			
F_1 225DV x 014AR	8	28.9±1.7^{A}			

Table 2 (continued) . Analysis of F_1 and F_2 generation of the crosses 'mutant x parent variety and 'mutant x mutant'.					
Genotype	No. of analyzed plants	Seminal root length (cm)	No. of F_2 plants with the phenotype of		$\chi^2_{3:1}$
		$\bar{x} \pm SD$	parent variety	mutant	
Diva	30	28.1 ± 1.8^A			
225DV	30	6.4 ± 0.9^C			
Aramir	30	30.4 ± 1.4^A			
035AR	30	17.6 ± 1.2^B			
F_1 225DV x 035AR	9	29.4 ± 2.7^A			
Diva	30	28.1 ± 1.8^A			
225DV	30	6.4 ± 0.9^C			
Aramir	30	30.4 ± 1.4^A			
090AR	30	14.2 ± 1.3^B			
F_1 225DV x 090AR	10	28.6 ± 1.0^A			
Aramir	30	30.4 ± 1.4^A			
014AR	30	15.5 ± 1.5^B			
035AR	30	17.6 ± 1.2^B			
F_1 014AR x 035AR	9	29.8 ± 1.3^A			
Aramir	30	30.4 ± 1.4^A			
035AR	30	17.6 ± 1.2^B			
090AR	30	14.2 ± 1.3^B			
F_1 035AR x 090AR	10	28.7 ± 2.1^A			
Aramir	30	30.4 ± 1.4^A			
014AR	30	15.5 ± 1.5^B			
090AR	30	14.2 ± 1.3^B			
F_1 014AR x 090AR	8	28.5 ± 1.0^A			

* - the same letter in each individual cross indicates non significant difference for P=0.05

Table 3. Analysis of seminal root length and root hair morphology in the F_2 generation of the cross 'mutant 225DV x Diva'

Number of seedlings with a phenotype				$\chi^2_{3:1A,a}$	$\chi^2_{3:1B,b}$	χ^2_L
A.B.	A.bb	aaB.	aabb			
129	5	7	46	1.11	0.52	152.72*

*χ^2_L>3.84, P=0.05

A.B. – parental phenotype (long seminal root, normal root hair), A.bb – recombinant phenotype (long seminal root, short root hairs), aaB. – recombinant phenotype (short seminal root, long root hairs), aabb – mutant phenotype (short seminal root, short root hairs).

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