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VARIABILITY OF COLEOPTILE LENGTH IN MUTANT/RECOMBINANT WHEAT DH (DOUBLED HAPLOID) LINES

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Abstract

Most of the modern wheat cultivars carry GA (gibberellic acid) insensitive height reducing gene, Rht-B1b, that reduces stem elongation but improves yield potential, lodging resistance and increased harvest index. Recent cultivars released in Romania are semi-dwarf, carrying the Rht-B1b allele which confers an advantage in excessive temperate environmental conditions. However, such cultivars are characterized as having short coleoptile that influences stand establishment and seedling vigor, especially in dry autumns. Genotypes carrying Rht gene-alleles can contribute to increasing coleoptile length, and having semi-dwarf characteristics can be used as sources in advanced breeding programs. A set of 86 mutant/recombinant wheat doubled haploid (DH) lines, was obtained through a complex protocol including two genotypes, two irradiation cycles with gamma rays (200Gy, 100Gy), direct and reciprocal hybridization, rapidly homozygosity attained by using Zea system. This study was aiming to explore the genetic variability for the coleoptile length and plant height, produced by mutagenesis and recombination, in the selected 86 mutant/recombinant DH lines, and compared to the parents. The analyses performed in this study, reveal that are lines which registered a longer coleoptile than parents, and also one line was highlighted for a semi-dwarf stature and a long coleoptile, that can be used in wheat breeding programs.

Key words: *coleoptile, mutant/recombinant, Rht-B1b, semi-dwarf;*

INTRODUCTION

The climate changes from the last period and the unpredictable weather conditions makes very difficult to maintain yield stability through good and acceptable levels of resistance against biotic and abiotic stress factors.

Drought stress has become a major factor that influences negatively the plant growth and the productivity in the last years. For this reason the breeders have manifested a real interest for obtaining a resistant material against abiotic stress factors.

Semi-dwarf cultivars, with longer coleoptile could ensure an early and good seedling emergence, a better ground coverage that confers an advantage in competition with seeds, and finally an efficient soil weather use by reducing evaporative water loss. If the coleoptile is short and sowing is deeper, the first leaf may suffer by pushing through the soil and emerge in a dark environment, being also exposed to soil crusting and diseases (Gulnaz et al., 2011). Consequently, the length of coleoptile is very important for crop establishment, allowing also a deeper sowing to utilize efficiently the soil moisture and uniform seed germination (Budak et al., 1995).

In wheat, more than 20 genes were described for reducing plant height (Mc. Intosh et al., 2013), from which, the *Rht-B1b* was used prevalently in breeding program at NARDI Fundulea. The most widely used genes in wheat breeding programs all over the world are *Rht-B1b, Rht-D1b, Rht-D1c* and *Rht8* (Korzun et al., 1988; Worland et al., 1998; Li et el., 2012). The *Rht- B1b* and *Rht-D1b* insensitive alleles at gibberellic acid (GA) induce lower sensitivity of vegetative tissue to endogenous gibberellin, and reduce cell elongation (Rebetzke et al., 2001).

The *Rht-B1b* and *Rht-D1b* alleles can increase yield by 6.1 and 14.1%, respectively; *Rht-B1* can also produce more productive tillers and higher yield per plant (Sial et al., 2002).

Rht8 is quoted as having contradictory effects on yielding in different environments (Sial et al., 2002; Kovalski et al., 2016).

Serban (2012) identified in a set of mutant/recombinant DH lines, with large variability for plant height and for coleoptile length, some semi-dwarf genotypes with longer coleoptile, that constitute an important breeding material. In Romania, the modern cultivars carry *Rht-B1b* gene, several yield tests revealing real advantages of *Rht-B1b* carriers in most environment (Saulescu, 2001, Serban, 2012). In some comparative tests performed in different wheat growing areas with isogenic lines carrying either *Rht-B1b* or *Rht8* showed that *Rht-B1b* carriers were superior for yielding capacity (Mustatea et al., 2000).

It is desirable to use as genitors *Rht-B1b* carriers and, if it is possible, with a long coleoptile. Improving stand establishment in dry autumns and at deep sowing by obtaining new genotypes with superior value of coleoptile length, without increasing plant height, is the main concern in the wheat breeding program at NARDI Fundulea.

The aim of this paper is to present the results obtained for plant height and coleoptile length for a selected set of 86 mutant/recombinant wheat DH lines, released at NARDI Fundulea (Giura, 2011), comparative with the two parents and identification of valuable semi-dwarf genotypes with short coleoptile.

MATERIAL AND METHODS

A number of 86 mutant/recombinant DH lines, derived from a complex protocol that included two modern genotypes, two irradiation cycles (100 Gy and 200Gy) and *Zea* system application for material homozygosis (Giura, 2011), were analyzed in laboratory and field conditions and compared with the two parents.

The 86 genotypes are part of a set with over 550 mutant and mutant/recombinant DH lines. A first set with 172 lines were analyzed in 2012 and the results proved that mutagenesis could generate extensive variability affecting not only the plant height, but also coleoptile length (Serban, 2012). It was noted the line *BiII 294* with approximately 2.5 mm longer coleoptile than both parental forms. This line has already been extensively used in NARDI Fundulea wheat breeding program.

The parents of this set of lines combines short stature with good winter hardiness and very good filling grain, due to a higher resistance to drought and heat.

F0628G1-34 parent - has in genealogy a line of triticale which conferred tolerance to rust, a greater length of coleoptile and good resistance to frost.

Izvor parent- variety with superior behavior under water stress conditions due to high osmotic adjustment capacity.

The 86 mutant/recombinant DH lines were planted in the autumn of 2014, in the field in plots of 1 m length, 20 cm between rows and 50 cm among genotypes for plant height determination. Plant height measurements were made, in June 2015, at complete heading, when plant growth ceased.

To determine the coleoptile length, seeds with uniform appearance, without any diseases and pest traces, were planted at uniform depth (10 mm) in pots with commercial soil (Flora Sol) to germinate, watered to field capacity, in December 2015. After a 3-days period, at 1°C, for obtaining uniform seed imbibition, trays were introduced in a growth chamber at 20°C in dark conditions (Serban, 2012). When coleoptile growth ceased and the first leaf appeared (figure 1), coleoptile length was measured with a ruler (figure 2). A number of 21 mutant/recombinant DH lines, best for coleoptile length measured at 20°C germination were retested after the same protocol, but germinated at 17°C.

Figure 1. Plantlets developed in dark conditions at 20°

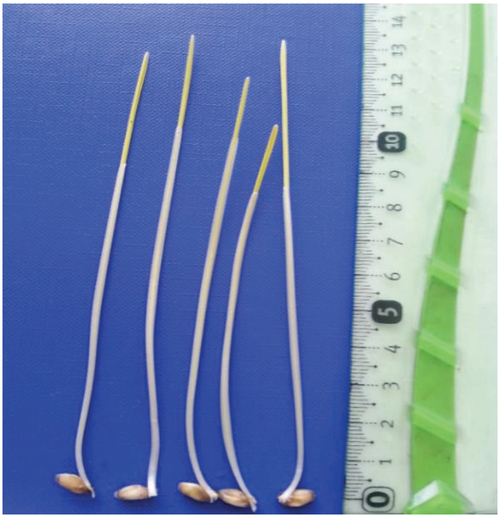

Figure 2. Coleoptile length measuring

RESULTS AND DISCUSSIONS:

Coleoptile length and plant height analysis for 86 lines included in study, revealed a large variability for both characters; thus, plant height varied between 85-113 cm (28 cm amplitude) and coleoptile length was in the range 4.23-6.6 cm (Table 1).

Table 1. The results registered for plant height and coleoptile length

No.	Genotype	Plant height (cm)	Coleoptile length (cm) at 20^{o}
1	AiII - 214	100	5.44
2	AiII - 215	95	5.45
3	AiII - 216	95	5.62
4	AiII - 217	100	5.40
5	AiII - 218	95	5.75
6	AiII - 219	105	4.85
7	AiII - 220	100	5.35
8	AiII - 221	102	5.51
9	AiII - 222	105	5.09
10	AiII - 223	105	5.83
11	AiII - 224	97	5.70
12	AiII - 225	100	5.50
13	AiII - 226	93	5.18
14	AiII - 227	94	5.60
15	AiII - 228	102	5.05
16	AiII - 229	109	5.73
17	AiII - 230	110	5.50
18	AiII - 231	105	5.68
19	AiII - 232	101	4.76
20	AiII - 233	95	5.45
21	AiII - 234	100	5.11
22	AiII - 235	100	5.49
23	AiII - 236	97	6.09
24	AiII - 237	95	5.68
25	AiII - 238	97	4.95
26	AiII - 239	91	5.58
27	AiII - 240	95	6.51
28	AiII - 241	100	5.24
29	AiII - 242	87	4.90
30	AiII - 243	92	5.94
31	AiII - 244	105	5.61
32	AiII - 245	110	5.64
33	AiII - 246	110	5.27
34	AiII - 247	101	5.78
35	AiII - 248	86	5.48
36	AiII - 249	103	6.59
37	AiII - 250	101	6.36
38	AiII - 251	104	6.11
39	AiII - 252	100	5.73
40	AiII - 253	95	5.63
41	AiII - 254	90	5.89
42	AiII - 255	106	6.57
43	AiII - 256	91	6.28
44	AiII - 257	95	6.47
45	AiII - 258	102	6.27
46	AiII - 259	94	5.65
47	AiII - 260	115	5.79
48	AiII - 261	100	5.79
49	AiII - 262	105	5.26
50	AiII - 263	90	6.28
51	AiII - 264	102	5.30
52	AiII - 265	108	5.63
53	AiII - 266	97	4.33
54	AiII - 267	105	5.51
55	AiII - 268	103	6.22
56	AiII - 269	104	5.34
57	AiII - 270	85	4.23
58	AiII - 271	92	4.96
59	AiII - 272	93	4.93
60	AiII - 273	97	5.16
61	AiII - 274	100	5.80
62	AiII - 275	91	4.96
63	AiII - 276	95	4.23
64	AiII - 277	112	5.18
65	AiII - 278	100	5.89
66	BiII - 127	113	5.76
67	BiII - 128	95	5.93
68	BiII - 129	100	6.29
69	BiII - 130	107	5.44
70	BiII - 131	105	5.71
71	BiII - 132	110	6.12
72	BiII - 133	103	5.87
73	BiII - 134	91	4.99
74	BiII - 135	90	4.88
75	BiII - 136	101	6.41
76	BiII - 137	100	5.97
77	BiII - 138	102	5.00
78	BiII - 139	102	6.02
79	BiII - 140	93	5.16
80	BiII - 141	93	5.91
81	BiII - 142	94	5.98
82	BiII - 143	88	6.51
83	BiII - 144	88	5.34
84	BiII - 145	97	6.22
85	BiII - 146	113	6.23
86	BiII - 147	85	6.60
87	IZVOR (Check)	99	5.92
88	F0628G1-34 (Check)	97	6.30

Plant height is one of the main morphological characteristics that can be modified by irradiation and can be easily detected in field experiments. In previously field experiment, with 338 mutant/recombinant wheat DH lines, the amplitude for plant height was 50 cm (between 60 cm, the shortest lines, to the 110 cm for the tallest ones) (Giura, 2011).
Results of the 21 mutant/recombinant wheat DH lines with registered higher values for coleoptile length than parents and selected for a new test, following the same protocol, but germinated at 17°C, are presented in Table 2.
The correlation coefficient between coleoptile length measured at 20°C and at 17°C, was significant for P=0.01 for variability (r= 0.49*). Higher values for coleoptile length compared with the superior parent (F0628G1-34) for both temperatures were registered for the lines BiII 143, AiII 268, AiII 240, AiII 255 and BiII 147 (Figure 3).

Table 2 Coleoptile length measured at 20°C and 17°C

No.	Genotype	Coleoptile length at 20°C (cm)	Coleoptile length at 17°C (cm)
1	BiII - 147	6.6	7.6
2	AiII - 249	6.6	7.1
3	AiII - 255	6.6	7.5
4	BiII - 143	6.5	8.2
5	AiII - 240	6.5	7.6
6	AiII - 257	6.5	6.7
7	BiII - 136	6.4	7.2
8	AiII - 250	6.4	7.0
9	F00628G-34 (Check)	6.3	7.3
10	BiII - 129	6.3	7.0
11	AiII - 256	6.3	6.9
12	AiII - 263	6.3	6.9
13	AiII - 258	6.3	6.6
14	BiII - 145	6.2	6.3
15	AiII - 268	6.2	7.9
16	AiII - 251	6.1	7.0
17	AiII - 236	6.1	6.4
18	BiII - 139	6.0	7.2
19	BiII - 142	6.0	6.6
20	BiII - 137	6.0	6.6
21	AiII - 243	5.9	5.9
22	BiII - 128	5.9	7.2
23	IZVOR (Check)	5.8	6.9

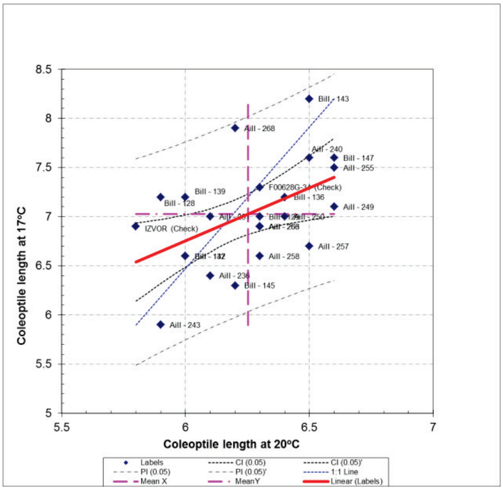

Figure 3 The correlation between the values registered for coleoptile length at 20°C and 17°C

These results are in contrast with those reported by Trethowan et al (2001) who observed higher values for coleoptile length at 20°C than at 10°C or 30°C.
The relationship between plant height and coleoptile length for the analyzed set is non-significant (Figure 4). For favorable stand establishment in unfavorable conditions during seedling, precise selection for coleoptile length could be made, without a significant decrease of plant height.

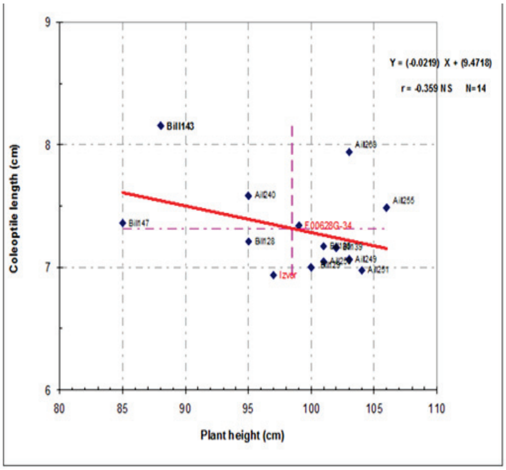

Figure 4. The relationship between plant height and coleoptile length

Among the analyzed lines, the line BiII143 was highlighted to be semi-dwarf and having the longer coleoptile, surpassing the both parents.

CONCLUSIONS

The line BII143 represents a valuable genotype for breeding programs, recording the best results in our study, a semi-dwarf stature and a long coleoptile.

Even if the differences between the superior parent for coleoptile length and the best mutant/recombinant DH lines are non-significant (maximum 0.55cm), the material represent a valuable quantitative accumulation for wheat breeding programs.

Lines that revealed long coleoptile and semi-dwarf plants will be studied further for a more accurate determination of their potential, either for direct use as varieties or as genitors in the breading programs for the creation of new varieties adapted to the conditions of Romania.

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