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IDENTIFICATION OF LONGER COLEOPTILE MUTANTS IN AN *Rht-B1b* SEMIDWARF WHEAT POPULATION

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ABSTRACT

Drought stress is a major abiotic stress limiting wheat plant growth and productivity in Romania. Stand establishment, especially in dry autumns, is much influenced by coleoptile length, which is smaller in genotypes carrying GA insensitive height reducing genes, such as *Rht-B1b,* which is present in most modern Romanian wheat cultivars. Additionally, longer coleoptiles are major determinants of increased vigour, which can reduce evaporative water loss by contributing to an early canopy closure. This study was aimed at exploring the genetic diversity for height and coleoptile length in a set of doubled haploid lines obtained after two cycles of mutagenic treatments with gamma rays applied first on two *Rht-B1b* semidwarf winter wheat cultivars and second on the hybrid population from crossing the M1 parents. Although parents had similar height and coleoptile length, both being *Rht-B1b* carriers, most lines had lower plant height and shorter coleoptiles, but about 20% of the lines had coleoptiles longer than the parents. Plant height and coleoptile length were not significantly correlated and coleoptiles significantly longer than in parents were identified in lines with plant height similar or shorter than the parents.

Key words: wheat, drought, stand establishment, mutagenic protocol, coleoptile length, plant height, mutant, doubled haploids lines (DH).

INTRODUCTION

Poor seedling emergence has become particularly important for wheat in recent decades because of the global adoption of semidwarf cultivars, with reduced height principally due to the presence of gibberelic acid (GA) insensitive alleles, *Rht-B1b* and *Rht-D1b* (Keytes et al., 1989). These dwarfing genes induce lower sensitivity of vegetative tissue to endogenous gibberellin, to reduce cell and subsequent stem elongation (Rebetzke et al., 2001). As a consequence, these wheat dwarfing genes increase harvest index and grain yield potential, but reduce stand establishment (especially in dry autumns and at deep sowing), and seedling vigour.

Many studies have demonstrated the importance of good seedling emergence in achieving high wheat yields (Rebetzke et al., 2007). On the other hand, better seedling vigour proved to be important for reducing evaporative water loss, by contributing to an earlier canopy closure (Spielmeyer et al., 2007).

This has lead to attempts to replace the *Rht-B1* gene with other dwarfing genes, despite its contribution to higher lodging resistance and grain yield demonstrated in many environments. Recent results showed that along with the negative pleiotropic effect of decreasing coleoptile length, the *Rht-B1b* allele tended to increase root biomass, which perhaps partially alleviates the negative effect of reduced coleoptile length (Li et al., 2011).

Several GA responsive dwarfing genes, such as *Rht8* (Rebetzke et al., 2007) or *Rht13*, *Rht4*, *Rht12* etc. (Rebetzke et al., 2012) are being used in wheat breeding programs around the world for increasing lodging resistance, grain number and yield, without compromising aerial biomass or coleoptile length in wheat. Other genes that can contribute to increasing coleoptile length have also been identified (Spielmeyer et al., 2007).

Chandler (2010) obtained by mutagenesis in an *Rht3* genotype new dwarfing alleles producing longer coleoptile length and having semidwarf characteristics.

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In Romania, most modern wheat cultivars are semidwarfs carrying the *Rht-B1b* allele, and results of yield tests demonstrated a clear advantage of *Rht-B1b* carriers in most environments (Săulescu, 2001). On the other hand, several studies demonstrated that, despite the longer coleoptiles and better stand establishment, near isogenic lines carrying the *Rht 8* gene were lower yielding than the *Rht-B1b* carriers derived from the same cross (Mustățea et al., 2000).

Here we report the first results of our search for longer coleoptile in a population showing high genetic variability, based on recurrent mutagenesis and recombination, using a specific mutagenic protocol in which DH (doubled haploids) technology was included.

MATERIAL AND METHODS

We studied the variability of coleoptile length and plant height in a set of 172 mutated/recombinant DH lines.

The material subjected to mutagenic treatments and recombination was based on cultivar Izvor and the advanced breeding line F00628G-34, both carrying GA insensitive height reducing gene *Rht-B1b* and each having valuable but contrasting agronomic traits. Mutagenic treatments with gamma rays consisted of two irradiation cycles were performed by IAEA Vienna. For the first cycle, seed of each parental genotype was treated with 200 gamma rays (Gy). In the second cycle, the treatments were done on the hybrid seeds resulted from direct (100 Gy) and reciprocal (200 Gy) crosses of M1 plants derived from the first cycle. Plants from the M1 generation of the hybrid, together with parental plants were then crossed with maize under greenhouse conditions to obtain DH plants (Giura, 2011). Combining both mutagenic and recombination events, using a specific protocol in which doubled haploids technology was included, supposedly increased the probability of obtaining new genetic variability.

To determine coleoptile length, seeds selected for uniformity, from the DH lines, were planted at uniform depth (10 mm) in trays filled with a fertile potting mix watered to field capacity. After a 3 days period, at 1°C, for obtaining uniform seed imbibitions, trays were introduced in a growth chamber at 20°C, where light was excluded. The coleoptile length was measured with a ruler, when coleoptile growth ceased and the first leaf appeared.

Plant height was determined in the field, on primary tillers at maturity.

RESULTS AND DISCUSSION

Parents had similar height, both being *Rht1* carriers. Most DH lines had lower plant height than both parents, and only less than 1% of the lines were significantly taller than both parents (Figure 1).

A negative effect of mutagenesis on plant height in wheat has been often reported (Irfaq and Nawab, 2001; Pandini et al., 1997, etc.).

Coleoptile length of parent F628G34 (P2) was a little longer than that of parent Izvor (P1), but the difference was not significant. Most lines had shorter coleoptiles, but about 20% of the lines had longer coleoptiles (Figure 2). Best lines had coleoptiles up to 2 cm. longer than the parents, which offers perspectives for a breeding program directed towards increasing coleoptile length. However, lines having much shorter coleoptiles, less than 5 cm, were also obtained.

An unexpected result was the lack of correlation between plant height and coleoptile length (r=-0.02 n.s.). The longest coleoptile, about 2 cm longer than that of the parents, was found in a line of plant height similar with the parents (Figure 3). The next longest coleoptiles were found in lines that were even shorter than the parents.

Although the longest coleoptiles in each plant height class showed a trend of positive correlation, coleoptiles longer than in parents were found in all plant height classes, except the shortest (below 65 cm). On the other hand, all plant height classes included lines with very short coleoptiles (less than 5 cm), at least 1 cm shorter than in parents.

The short lines with longer coleoptiles might offer new possibilities in improving stand establishment and seedling vigour of semidwarf wheat. Further studies are planned to verify the potential value of these lines and to unravel the mechanisms behind their characteristics.

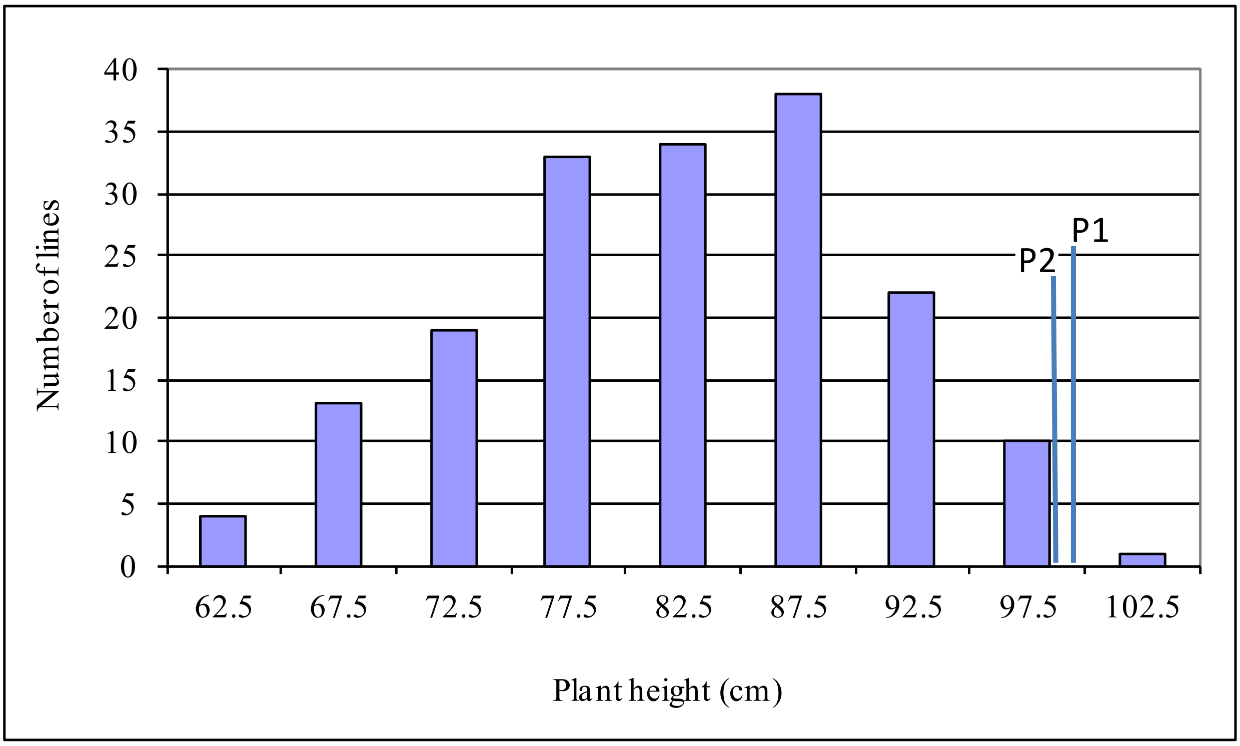

Figure 1. The distribution of plant height in DH lines from mutated/recombinant population

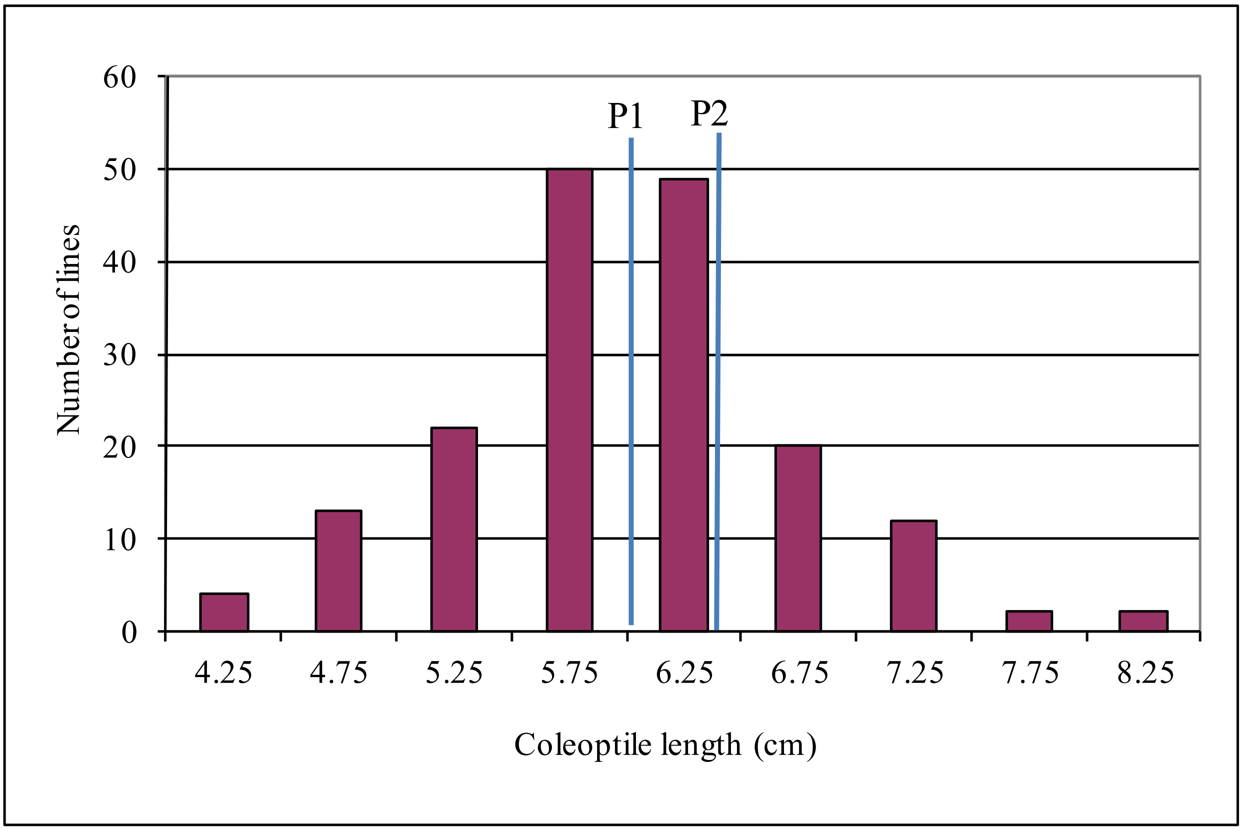

*Figure 2.*The distribution of coleoptile length in DH lines from mutated/recombinant population

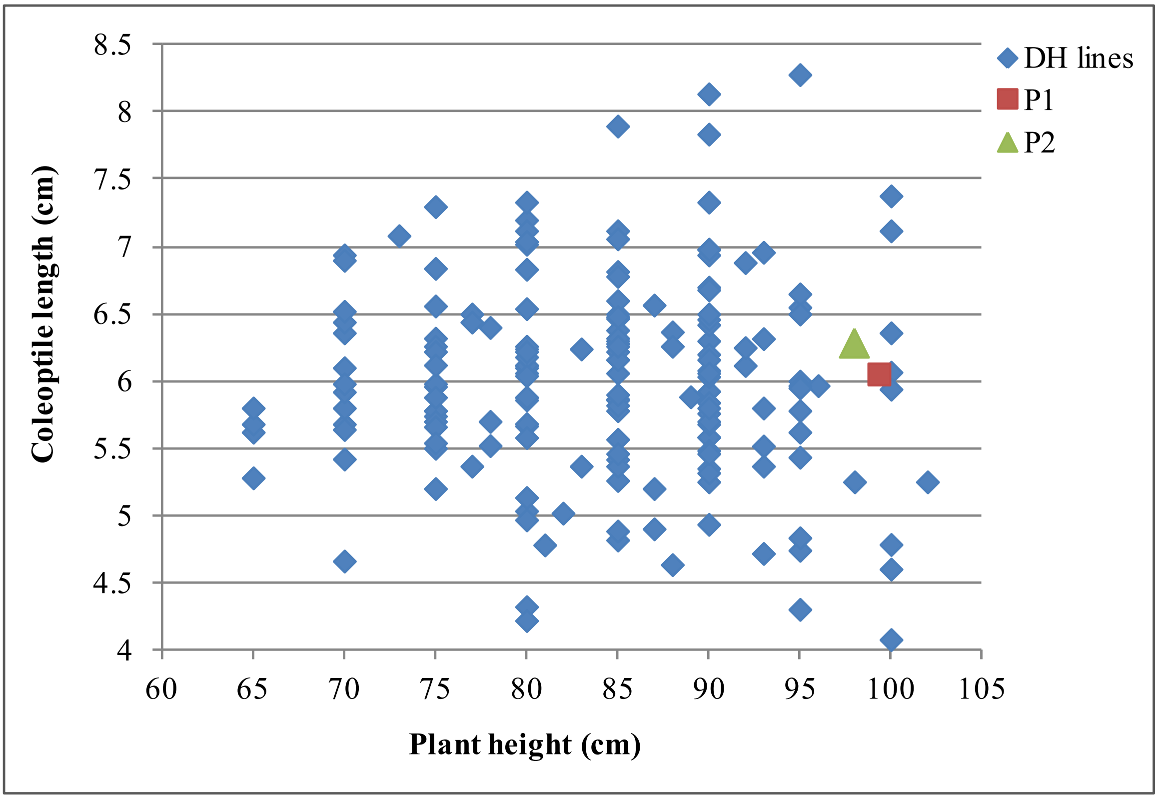

Figure 3. The relationship between plant height and coleoptile length in DH lines from mutated/recombinant population

CONCLUSIONS

Our results suggest that recurrent mutagenesis in a hybrid population, combined with the DH technology, produced a large variability in height and coleoptile length, which allowed the identification of completely homozygous semidwarf genotypes with longer coleoptiles. These may open additional possibilities in breeding for improved stand establishment and early vigour without increasing plant height.

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