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RELATIONSHIP OF COLEOPTILE LENGTH AND PLANT HEIGHT IN WINTER WHEAT ACCESSIONS

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Abstract

Climate change prognoses suggest more unpredictable and less favourable conditions for cereal crop establishment in Europe. The development of varieties possessing long coleoptiles could improve winter wheat environmental adaptability. A total of 564 winter wheat control varieties and breeding lines developed basically using European winter wheat germplasm were screened for coleoptile length and plant height during 2004-2009. The filter paper towel method showed significant differences among the accessions. Most of the accessions (50.8%) possessed a coleoptile length ranging between 3.00 and 6.00 cm. The correlation between coleoptile length and plant height was weak in 2005 and 2006 (respectively, r=0.485* & 0.468*, $p \leq 0.01$), but medium (respectively, r=0.578* - 0.625*) in 2004, 2007-2009. Accessions screening extent could be reduced selecting accessions with acceptable plant height (85-100 cm). Accessions percentage that fitted to this group ranged from 23.4% in 2006 to 67.9% in 2009 and average of 6 years was 40.3%. Among the 564 tested accessions, 36 breeding lines or 6.4% possessed coleoptiles longer than 8 cm but none of them exceeded 10 cm. Plant heights of these accessions ranged from 94 to 118 cm. The 13 accessions possessed acceptable plant height. The lines possessing in pedigree varieties Flair and Pegassos (each in 7 lines) were the most frequent. This data shows that the new winter wheat breeding lines possessing long coleoptiles in combination with acceptable plant height can be developed using European winter wheat varieties.

Introduction

Coleoptile length is an important winter wheat varietal trait as it determines the maximum depth at which seeds can be sown. It is a primary trait for varieties grown in dry areas where seeds are placed deeper than optimal to reach wet soil. Most of the landraces grown in such areas possess a coleoptile length of about 10 cm (Yu & Bai, 2010). Currently, semi-dwarf varieties prevail in the world due to intensive use of dwarfing genes *Rht-D1* and *Rht-B1* which shorten both plant height and coleoptile length. Less widespread gene *Rht8* also conditions wheat dwarfing but has no negative impact on coleoptile length (Bai *et al*., 2004). Many varieties are semi-dwarf in Europe. Previous research on dwarfing genes on 870 wheat varieties showed that *Rht8* occurred in most of the screened Southern and Middle European varieties, whereas genes *Rht-D1* and *Rht-B1* dominated in the varieties from the rest of Europe (Worland *et al*., 2001). A recent study on 95 German winter wheat varieties showed that 52 of them had no *Rht* gene, 37 possessed *Rht-D1b* and 6 *Rht-B1*, and none of the varieties possessed *Rht8* (Knopf *et al*., 2008).

Winter wheat receives enough precipitation for shallow sowing and high yield in many parts of Europe. However, general climate change forecasts in wide and narrow regional perspective (Meehl & Tebaldi, 2004; Lavalle *et al*., 2009) suggest the necessity to elaborate programmes for the development of varieties adapted to wider environments. At present, varieties possessing long coleoptiles have no exceptional advantage for European countries over a wide area. However, such varieties can perform better in some situations. Longer coleoptiles improve stand establishment where stubble retention is practiced (Rebetzke *et al*., 2005). This trait for European varieties can become desirable, as soil non-plough techniques have become more widespread in Europe (Anonymous, 2010). Longer coleoptiles can increase seedling emergence when small seeds are used (Cornish & Hindmarsh, 1988). Deeper sowing results in higher survival of seeds because it prevents damage done by animals (Pascual *et al*., 1999; Brown *et al*., 2003). Longer coleoptiles provide greater seedling early vigour, competition with weeds, crop establishment, more efficient soil water use, and better penetration through soil crust (Causens *et al*., 2003; Condon *et al*., 2004; Rebetzke *et al*., 2007). Genotypes possessing longer coleoptiles show better seedling survival rate after dehydration and rehydration (Guedira *et al*., 1997) and this could be even improved by use of specific amino acid as a pre-sowing seed treatment (Tahir *et al*., 2009).

Winter wheat germplasm possessing long coleoptiles is determined and available (Bai *et al*., 2004; Yu & Bai, 2010). However, involvement of exotic germplasm with a complex of undesirable agronomic traits can impede breeding. The best way to deal with this problem might be to select regionally adapted germplasm as it does not complicate breeding due to introduction of undesirable traits. Most of the recent European semi-dwarf winter wheat varieties possess genes *Rht-D1* and *Rht-B1* and possibly short coleoptiles (Rebetzke *et al*., 1999; Pereira *et al*., 2002; Addisu *et al*., 2009). On the other hand, there are some tall varieties without *Rht* genes (Knopf *et al*., 2008).

The aim of this research was to screen advanced Lithuanian winter wheat breeding lines and some check varieties for their coleoptile length and plant height.

Materials and Methods

In total, 564 winter wheat breeding lines and check varieties were screened (Fig. 1). Parental material used for development of breeding lines basically originated from Germany, Denmark, Sweden, Russia, Ukraine and at lower frequency form other European countries. The investigation of plant material for plant length was done at the Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry (55º23/N, 23º51/E) situated in the centre of Lithuania during 2004-2009 growing seasons under the same growing technology. The accessions tested were sown at a rate of 4.5 million viable; pesticide-treated seeds per ha at a depth of about 4 cm, with a 15 cm row spacing. The crop was planted in the first half of September. The accessions were grown in 17 m^2 plots in four replications without growth retardants. Fertilizer rate N_{30}-P_{60}-K_{60} was broadcast applied before sowing and additionally N_{90} in spring. Weeds were controlled by herbicides in autumn. The other pesticides during plants vegetation were not applied. Plant height from soil surface to tip of the ears was measured at milk stage. Grain was harvested at full seed ripe. The weather conditions during the growing seasons 2004, 2007, 2009 were characterized as favourable for formation of maximal plant height, whereas seasons 2005 and 2006 and were characterized as heavily plant stressing due to dry vegetation period. The year 2008 was characterized as slightly plant height stressing.

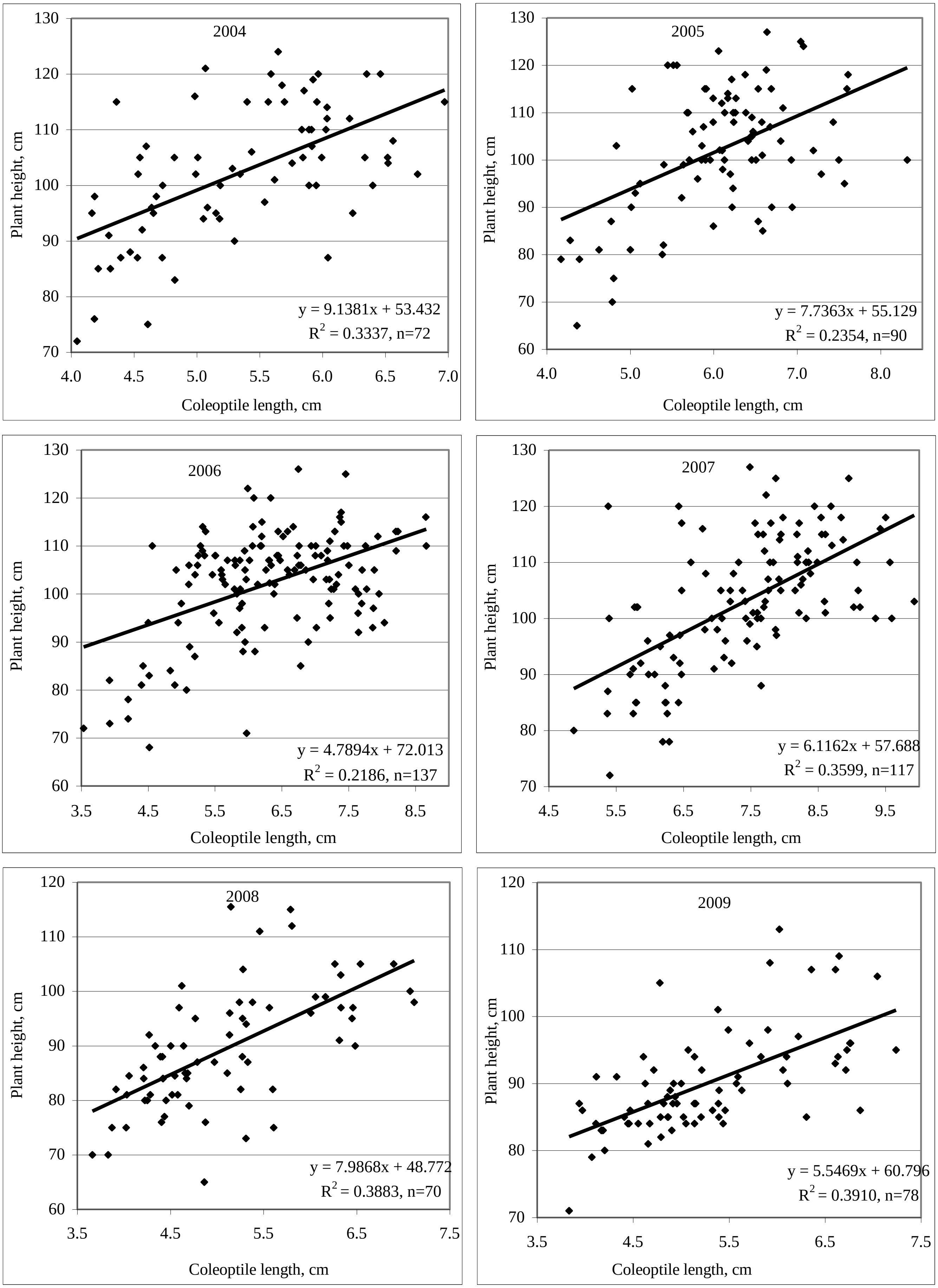

Fig. 1. Relation of coleoptile length and plant height of winter wheat breeding lines during 2004-2009.

The coleoptile length evaluation experiment was started after 6 months after harvesting to ensure higher seed germination rate. The seed was surface sterilized in 1.0% hypochlorite solution for 30 s and then rinsed in sterile water. Fifteen uniform seeds per accession were spaced 1 cm apart and 5 cm from the bottom of a germination towel made from filter paper. Total height of the towel was 20 cm. Each seed was placed on the germination towel with its embryo down. The towels were rolled loosely and fastened with a rubber band. The wrapped towels were moistened and randomly arranged vertically in closed plastic containers. The samples were placed in a growth chamber at 4ºC for 2 d to interrupt dormancy. Later, the samples were incubated at the same humidity at 15ºC for 7 days, followed by 6 days at 20^{O}C. The constant darkness and 100% relative humidity was maintained throughout all experiment. This procedure was replicated four and repeated two times for all accessions. Coleoptile length was recorded as the distance from the seed to where the first leaf broke through the coleoptile sheath (Bai *et al*., 2004).

The Duncan multiple range test, least significant difference (LSD) and correlation-regression analysis was done with a significance level p≤0.01.

Results

The correlation between coleoptile length and plant height was weak in 2005 and 2006 (respectively, r=0.485* & 0.468*), but medium (respectively, r=0.578* - 0.625*) in 2004, 2007-2009 (Fig. 1). Weak correlation in 2005-2006 was mainly due to shorter plants as weather was dry. However, relatively stable correlation of plant height and coleoptile length of hundreds of accessions suggests moderate environmental influence and satisfactorily repeatability of results. The distribution of the winter wheat accessions was similar in all years; however the considerable differences among genotypes were determined every year. The coleoptile length and plant height of the most accessions was fairly highly related, but correlations were lower due to some accessions characterized as more divergent from the main trend in every year.

The medium values of coleoptile length and plant height ranged from 5.1 to 89.4 cm in 2008 and from 7.4 to 103.1 cm in 2007. The least coleoptile length range occurred in 2004 (4.05-6.97 cm) and the highest range was found in 2006 (3.54-8.66 cm). The least difference of accessions by plant height was determined in 2009 (71-113 cm) and the highest range was found in 2005 (65-127 cm). The mean coleoptile length increased from 2004 to 2007 (respectively, from 5.4 to 7.4 cm), whereas the mean plant height was very similar (from 101.8 cm in 2005 to 103.1 cm in 2007). The data of these years did not show relation between coleoptile length and plant height. However, this relation was obvious in 2008-2009 as plant height and coleoptile length decreased mutually and these values were 89.4, 90.0 cm and 5.1, 5.3 cm.

An acceptable winter wheat plant height in many European countries ranges from about 70 to 100 cm, some accessions can be slightly taller than 100 cm (BSA, 2009). Regarding this trend, accessions possessing the similar plant height should be selected from breeding material as conversely national varieties would be out-competed by foreigners ones. Therefore, accessions with long coleoptiles should be selected from the same plant height group. It is obvious that only accessions in every year possessing plant height ≥85 cm could be selected for coleoptile length screening as they possessed promising coleoptile length among accessions of one year, whereas the shorter accessions did not posses longer coleoptiles at all. However, selection of accessions possessing plant height 85-90 cm suggested only a low probability of success as in average over all years 7.8% of accessions in this plant height group had promising coleoptile length. Analysis of 10% accessions possessing the longest coleoptiles in every year showed that slightly over 5% of accessions possessed acceptable plant height about 100 cm during 2004-2007, whereas the similar plant height possessed 10.0% of accessions in 2008 and 7.9% of accessions in 2009. Coleoptile length screening extent could be reduced by selecting accessions with acceptable plant height (85-100 cm). Accessions percentage that fitted to this group ranged from 23.4% in 2006 to 67.9% in 2009 and average of 6 years was 40.3%. However, such screening narrowing would be unfavourable for more detailed pedigree analysis. Also, considerable time and supply saving would not be achieved due to relatively low screening amount decrease.

Accessions possessing different plant height and coleoptile length are presented in Table 1. These show high stability of above mentioned traits at probability p≤0.01 over 6 year's period. The main difference of coleoptile length from mean occurred in 2007. The line Biscay/Pegassos was less stable as coleoptile length varied from 5.81 to 7.62 cm. Also plant height of this line differed significantly from mean in one year, whereas the plant height of rest lines significantly did not differ from mean.

Among the 564 tested accessions, 36 accessions (not including standards) or 6.4% possessed coleoptiles longer than 8 cm but none of them exceeded 10 cm (Table 2). High variability of coleoptile length was found for the majority of the accessions. The greatest variation in coleoptile length was determined for lines Dream/Flair (1.5–12.0 cm) and Rostovchanka/Ebi (3.0–13.4 cm), whereas the least variation was found for Miron.61/BUL63131 (6.0–10.5 cm) and Astron/Tarso//Mobil (5.3–10.5 cm). The differences of coleoptile length were not considerable among accessions possessing shorter or longer coleoptiles. The correlation analysis between coleoptile length and plant height of all 36 accessions revealed no correlation (r = 0.002, data not shown).

Table 1. Coleoptile length and plant height changes of contrast winter wheat genotypes during 2004-2009.

Genotype	Trait	Years							
		2004	2005	2006	2007	2008	2009	Mean	LSD$_{01}$
Dirigent/ Cortez	CL[1]	3.92	3.78	3.54	4.98	3.82	3.84	3.98	0.51
	PH[2]	72	70	72	72	70	71	71.2	1.98
Flair/ Lut.9-329	CL	4.51	4.63	4.90	4.89	4.48	4.50	4.65	0.46
	PH	83	81	81	80	85	86	82.7	3.42
Zentos	CL	5.73	5.68	6.32	7.61	5.82	6.40	6.26	0.73
	PH	115	110	107	115	112	114	111.0	5.63
Biscay/ Pegassos	CL	5.81	5.90	6.73	7.62	6.95	6.73	6.62	0.68
	PH	102	100	95	101	98	100	99.3	3.41

[1] CL – coleoptile length, cm; [2] PH – plant height, cm

Table 2. Pedigree, coleoptile length and plant height of breeding lines possessing coleoptile length over 8 cm.

Breeding line	CL[1] mean, cm	SD[2]	Range, cm	PH[3] mean, cm
Pegassos/Aspirant	8.04a[4]	0.41	4.1-10.2	94a[4]
Flair/Lut.9-329	8.20b	0.50	2.1-11.7	105c
Zentos/Lut .97-5	8.21b	0.39	3.2-11.2	110d
Miron.61/BUL63131	8.22b	0.26	6.0-10.5	113de
Zentos/Lut.97-4	8.22b	0.36	4.5-10.0	109d
LIA5018/Aspirant	8.25b	0.47	3.0-11.5	101b
Dream/Pesma	8.26b	0.31	4.9-11.2	117ef
Astron/Tarso//Mobil	8.27b	0.36	5.3-10.5	113de
Lone/Inna//Lut.96-6	8.28bc	0.46	4.5-12.5	106c
Biscay/Flair	8.30bc	0.63	2.4-11.4	107cd
Pegassos/Residence	8.31bc	0.46	3.8-10.4	100b
STH1096/Bussard	8.32bc	0.36	4.3-11.5	110d
STH1096/Bussard	8.34c	0.36	4.3-11.5	111d
LIA5183/Kris	8.35c	0.56	1.3-11.5	100b
Olivin/Aspirant	8.37c	0.37	3.1-10.1	112de
Dream/Bill	8.40c	0.54	1.5-11.0	110d
STH1096/96-101	8.43cd	0.41	3.5-13.1	108cd
Dream/Flair	8.51d	0.47	4.5-12.2	110d
Dream/Flair	8.54d	0.64	1.5-12.0	118f
Residence/Flair	8.60de	0.42	4.0-12.5	103bc
Elena/Flair	8.63de	0.41	5.5-13.0	101b
Rostovchanka/Sj965210	8.66e	0.43	3.4-12.1	116ef
Pegassos/BUL661.2	8.66e	0.37	4.0-11.5	110d
Tarso/Bussard	8.72e	0.46	3.7-12.1	113de
Rostovchanka/Ebi	8.83ef	0.44	3.0-13.4	118f
Pegassos/Belisar	8.91ef	0.46	3.2-12.1	114de
Bill/Dream	9.02ef	0.45	3.0-13.1	102bc
Tarso/Bussard	9.10f	0.31	3.7-11.9	110d
Pobeda/Lut.9-321	9.14f	0.42	2.9-12.2	105c
Dream/Asketis	9.17f	0.28	3.8-12.3	102bc
Pegassos/Residence	9.40g	0.40	4.0-12.5	100b
Rostovchanka/Ebi	9.45gh	0.27	3.7-12.8	116ef
MV0695/Winni	9.54h	0.33	3.1-12.6	118f
Biscay/Flair	9.63i	0.36	4.0-12.4	110d
Pegassos/Residence	9.67i	0.30	5.8-12.9	100b
Pegassos/Lasta	9.91j	0.46	4.3-13.1	103bc

[1]CL – coleoptile length, [2]SD – standard deviation, [3]PH – plant height, [4]Duncan multiple range test, $p \leq 0.01$.

Plant height of these accessions ranged from 94 to 118 cm. The 13 accessions possessed acceptable for Lithuanian conditions plant height up to 105 cm (Tarakanovas & Ruzgas, 2007). The lines possessing in pedigree varieties Flair and Pegassos (each in 7 lines) were the most frequent. Also, varieties Dream and Bussard were among the most frequent lines (respectively, 6 & 4 lines).

Discussion

Distribution of accessions' coleoptile length was similar to that found in many other experiments (Rebetzke *et al.*, 1999; Bai *et al.*, 2004; Addisu *et al.*, 2009). Most of the accessions differed significantly in coleoptile length. Very short (≤5.0 cm) coleoptiles were characteristic of 22.3% and some longer (>5.0-6.0 cm) of 28.5% (the mean of 6 years) of accessions. These finding show that deep sowing for most of the lines is not appropriate. The data on the European varieties' coleoptile length is very limited indicating that deep sowing is not used or experiments are only in progress. Also, European varieties possessing genes *Rht-B1b* and *Rht-D1b* were characterized as possessing reduced seed viability and lower early resource capture in combination with lower competitiveness with weeds (Addisu *et al.*, 2009). However, some negative traits are outweighed by highly desirable traits of yield potential and convenient growing technology (Jamali & Ali, 2008; Addisu *et al.*, 2009). Lithuanian winter wheat breeding lines possessing these genes should posses the same negative characteristics. Varieties possessing longer coleoptiles generally have faster, more even emergence and improved early vigour (Rebetzke *et al.*, 2005). This trait is not essential for varieties grown under technologies of intensive pesticide input. However, it is a very important trait considerably improving weed competitiveness of winter wheat grown in organic conditions (Bertholdsson, 2005). Conventional growers can get some benefits since more competitive varieties should require lower herbicide doses and/or less sophisticated herbicides. Also, variety competitiveness due to soil covering trait complex is very important trait under areas with low yield potential where herbicide application is not profitable or available due to technological peculiarities. On the other hand, crop competition's can be achieved by manipulation of sowing rates (Causens *et al.*, 2003; Khan *et al.*, 2008).

During the past decade winter wheat establishment due to dryer seed bed was insufficient in some experimental plots in the central part of Lithuania (Feizienė *et al.*, 2007) as well as in the rest of Europe (Lavalle *et al.*, 2009). This problem could be solved applying deeper sowing, but growing technologies are oriented to shallow seed bed. Also, varieties and breeding lines are not screened under field conditions as well as soil preparation technologies for deep sowing in Europe. Increasing use of non-plough tillage in Europe (Anonymous, 2010) also does not favour better seedling establishment under dry soil conditions. The trends of climate change suggest greater variability and unpredictability of weather events constraining sustainable agriculture (Meehl & Tebaldi, 2004; Ferranti & Viterbo, 2006; Stanevičius *et al*., 2008; Lavalle *et al*., 2009). Also, we can find various examples of changes in winter wheat breeding tasks in Europe over the last decades. Several decades ago winter wheat frost resistance was a mandatory trait for a variety in Northern and Western Europe. At present it has become a questionable trait due to the climate warming. In the near future the development of new winter wheat type possessing long coleoptiles can be a new challenge for European breeders.

Different methods are used for coleoptile length measurement. However, the main differences are germinating medium (paper towels or soil substrates) and temperature regime. Coleoptile length determined by a paper towel method should be lower than that obtained by soil substrates method (Takahashi *et al*., 2008). The temperature regime used in our experiment did not warrant obtaining the maximal coleoptile length. However, the majority of investigations show that wheat genotypes retain their ranking across temperatures and lines producing long coleoptiles at higher temperatures should produce longer ones at cooler temperatures (Rebetzke 1999; Pereira *et al*., 2002). On the other hand, some quantitative trait loci (QTLs) influencing coleoptile length can perform better at higher than at lower temperatures (Rebetzke *et al*., 2001). It was also determined that seed source for coleoptile measurements has little effect on coleoptile length (Hakizimana *et al*., 2000). Such relationship allows more flexible use of the evaluation methods. Among the 564 tested accessions only 36 or 6.4% possessed coleoptiles longer than 8 cm and none of them exceeded 10 cm. Such a low frequency of accessions with long coleoptiles shows that high accuracy could hardly improve experimental data, because hundreds if not thousands of accessions should be screened during the initial screening stages to find the several ones possessing long coleoptiles in combination with desirable traits for each specific area. On the other hand, high correlation between sites and years (Pinthus & Abraham, 1996; Hakizimana *et al*., 2000; Yu & Bai, 2010) could help screening of many accessions in certain institutions using seed samples received from other geographical regions or previous yield years without spending time and resources for reproduction of these accessions at the same institution.

Close to normal distribution of coleoptile length as well as plant height shows genetic variability based on combinations of mono and polygenes (Rebetzke *et al*., 2007). The genetic mechanisms regulating coleoptile length is not comprehensively studied. One of the constraints is masking effect of *Rht* genes. Recent researches showed many QTLs regulating coleoptile length with major and minor effects. QTLs were identified on a range of chromosomes (Rebetzke *et al*., 2001, 2007; Wang *et al*., 2009; Yu & Bai, 2010). At the same time, some of these QTLs showed pleiotropic effects on plant height, but others did not. QTL's, increasing coleoptile length but not associated with plant height, may be useful for improving long coleoptiles with decreased plant height.

Plant height of accessions possessing the coleoptile length over 8 cm did not correlate (r=0.002) with coleoptile length. It indicates that these accessions possess various plant height and coleoptile length regulating QTLs. For example, the tallest lines Dream/Pesma, Dream/Flair (117 & 118 cm) did not possess the longest coleoptiles (8.26 & 8.54 cm), whereas lines Pegassos/Residence, Pegassos/Lasta possessing the longest coleoptiles (9.67 & 9.91 cm) had about 15% shorter plant height (100 & 103 cm). Positive correlations between plant height and coleoptile length were determined in our study, but the relation level was dependent on accession selection for analysis. These results agree with various studies on wheat coleoptiles (Rebetzke *et al*., 1999, 2007; Yu & Bai, 2010). Almost all lines possessed in pedigree varieties without *Rht* genes (Knopf *et al*., 2008). This suggests higher possibility to select accessions with longer coleoptiles when accessions are pre-screened by *Rht* genes. The coinciding plant height, but not coleoptile length data shows a possibility to develop new varieties with acceptable height in combination with long coleoptiles and other desirable agronomic traits. According to BSA (2009) some varieties without *Rht* genes were cultivated in Germany during their intensive use period under the area that was twice as high as that of some varieties possessing *Rht-B1b* and *Rht-D1b*. This suggests that plant height control without *Rht* genes was successfully fulfilled in breeding and agricultural praxis.

Coleoptile length depends on polygenic inheritance, many QTLs were determined (Rebetzke *et al*., 2001, 2007; Wang *et al*., 2009; Yu & Bai, 2010), but much fewer QTLs exerted major effect. Such relation shows relatively simple wheat breeding for long coleoptile exploiting European wheat pool as the few major QTLs ensure sufficiently high heritability (Rebetzke *et al*., 1999) and possibility to use marker assisted selection (Wang *et al*., 2009). Involvement of exotic germplasm could facilitate development of new European wheat varieties with very long coleoptiles and accelerate breeding for this trait. However, the problem for deep sowing is not particularly relevant in Europe at the moment. The transfer of a new trait relies on the advantages of the newly developed varieties with new traits over existing ones. Therefore, the most promising option for the development of varieties with improved coleoptile length and desirable plant height is to choose European winter wheat germplasm.

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