TOLERANCE TO PRE-HARVEST SPROUTING IN LITHUANIAN WINTER WHEAT ADVANCED LINES

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Tolerance to pre-harvest sprouting of winter wheat advanced breeding lines was evaluated at the Lithuanian Institute of Agriculture in 2005 and 2006. The tests were conducted with intact ears germinated in plastic boxes on wet filter paper in a plant growth chamber. A total of 131 breeding lines were tested during the experimental period. The experiment revealed that sprouting cumulative index (SCI) characterises resistance of lines to pre-harvest sprouting more precisely than sprouting final score (SFS). The SFS ranged from 4.3 to 9.0 in 2005 and from 5.4 to 9.0 in 2006. The SCI value was 0.14–1.0 in both years. Lines in 2005 were more susceptible to pre-harvest sprouting than those in 2006. The most resistant were found to be the lines Flair/Asketis, Torfrida/Beaver//Tarso, Širvinta1/LIA3480, while the most susceptible ones were Elena/Flair, Mermaid/Alidos, Flair/Lut.96-3 in 2005. In 2006, the most resistant lines were Pegassos/Dream, Belisar/Briz, Lars/Lut.96-3 and the most susceptible were Rostovchanka/Lut.96-3, Rector/Briz, Rostovchanka/Flair. The SFS of resistant lines was up to score 6 and the SCI value reached 0.3 in both years. The SFS of susceptible lines was 9.0 and the SCI value more than 0.9 in both years.

Key words: *wheat, advanced lines, pre-harvest sprouting.*

INTRODUCTION

Pre-harvest sprouting (PHS) is common in cereals, when maturing grains are exposed to rainfall or high moisture conditions, especially when in genotypes lacking grain dormancy. Environmental conditions such as drought and high temperatures during grain filling have a great effect on the expression of sprouting tolerance (Biddulph *et al.*, 2005).

Sprout damage is common in wheat producing areas, every three or four years of ten. The areas commonly affected by sprout damage include a part of the Pacific Northwest, the upper Midwest, and Northeast USA, Australia, the EC and the central provinces of Canada. In addition, many countries in central South America and Southern Africa are prone to sprout damage (Derera, 1989). PHS in wheat results in large economic losses throughout the wheat growing regions in the world. Economic losses are unpredictable due to the frequency of PHS, which is weather related and the severity of sprout damage is influenced by the degree of grain ripening and amount of rainfall before harvest. Sprouted kernels are generally bleached and mealy in appearance, and have lower test weight than sound kernels. PHS wheat is reduced in grade and value, depending on the quantity of sprouted kernels present in a sample. In commercial milling indus-

tries, a sample of wheat is often discounted at the time of sale when falling number, an indirect measure of sprout damage, is < 200–250 due to high α-amylase activity (Meredith and Pomeranz, 1985; Mares, 1993). Several factors can contribute to increased tolerance to PHS: reduced levels of α-amylase activity in the grains, the presence of inhibitors of germination, reduced water absorption by the grains and altered responses to hormones, among others (Roy *et al.*, 1999; Zanetti *et al.*, 2000).

PHS tolerance is a combination of quantitative and qualitative traits influenced by many environmental factors and controlled by several grain dormancy related genes (Bailey *et al.*, 1999). However, the influence of quantitative traits dominates. A number of genes and quantitative trait loci (QTL) involved in PHS tolerance have been found and mapped in wheat. One potential complication for analysing the PHS phenotype is that it involves genes expressed in maternal plant as well as tissues belonging to the next generations; the embryo and endosperm. As a result, the breeding of tolerant genotypes is a difficult process.

The objective of this work was to evaluate the tolerance of Lithuanian winter wheat advanced lines to pre-harvest sprouting in intact ears.

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MATERIAL AND METHODS

Tolerance of winter wheat advanced breeding lines to PHS was evaluated at the Lithuanian Institute of Agriculture in 2005 and 2006. During the investigation period a total of 131 breeding lines were tested. Plot size was 17 m^2, replicated four times. Soil of the experimental site is loam (*Endocalcari-Epihypogleyic Cambisol* (*sicco*) (CMg-p-w-can)) with a clay content of 24–27%, pH 6.5–7.0; percentage of organic mater 2.5–2.7; P_2O_5 /190–240; K_2O 185–264 mg·kg^{-1} soil. $N_{90}P_{60}K_{60}$ was applied annually. Phosphorus and potassium fertilizers were applied before sowing and nitrogen in spring after resumption of vegetative growth. Plots were sprayed with herbicides in spring when weeds started to grow intensively. Other pesticides and additional fertilizing were not applied.

At Zadoks' Growth Stage 92 (Zadoks *et al.,* 1974), 50 upper canopy spikes per advanced breeding line were harvested per line. The spikes were dried and stored at 16–18 ^{o}C for 6 months. Before sprouting resistant test spikes were immersed in tap water at room temperature for two hours. Afterwards the spikes were placed in plastic boxes on moistened filter paper and covered. Four replications with five ears per replication were used. Each replication was placed in a separate box with ten ears per growth chamber. Photoperiod was 12 h light and 12 h dark. Ears in the chamber were maintained at 20 ^{o}C in the light and 16 ^{o}C in the dark. The evaluation of ears was continued until 10 per cent of lines were heavily sprouted. This period lasted for six days in 2005, and eight days in 2006, due to the differences in weather conditions during the plant growing period. All ears per replication were evaluated as one unity. Sprouting was evaluated on a scale of 1 to 9, where 1 – no visible sprouts, 2 – ≤ 4%, 3 – 5–15%, 4 – 16–25%, 5 – 26–45%, 6 – 46–65%, 7 – 66–85, 8 – 86–95, 9 – > 95%. Sprouting final score (SFS) was sprouting score on the last evaluation day. Cumulative sprouting index (CSI) was calculated as the sum of sprouting percent of all evaluation days divided by the sum of line with maximum average of sprouting percent: CSI = Σ(GD1,%+GD2,%+…+GDn,%)/maxΣ.

"GD" is sprouting severity (%) on germination day (n).

The weather in July of 2005 was very hot and dry. In the next year the summer was dry and hot. In both years there was no rainfall within the period of two weeks before head harvesting.

RESULTS

The resistance of winter wheat in 2005 to PHS was estimated as SFS (Fig. 1A) and SCI (Fig. 1B). PHS resistance varied widely among wheat genotypes. SFS ranged from
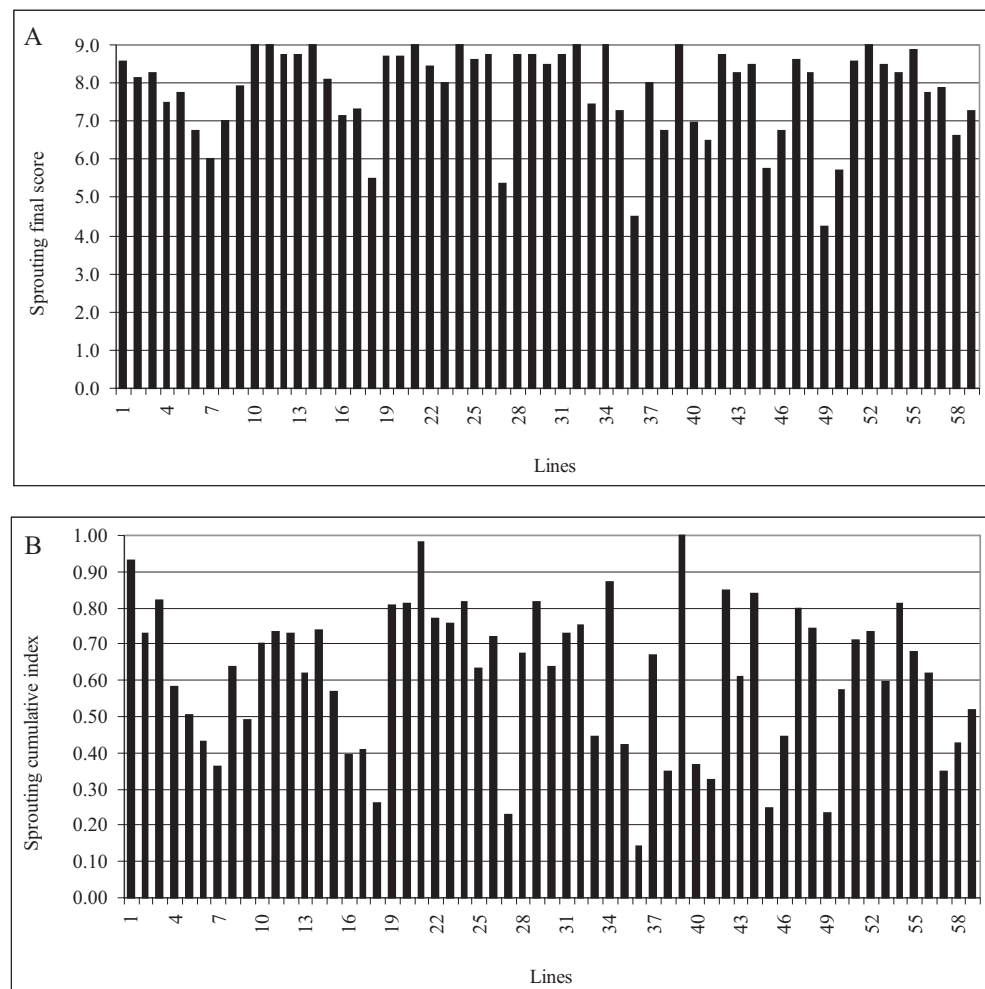

Fig. 1. Resistance to pre-harvest sprouting of winter wheat advanced breeding lines in 2005. **A**, sprouting final score; **B**, sprouting cumulative index.
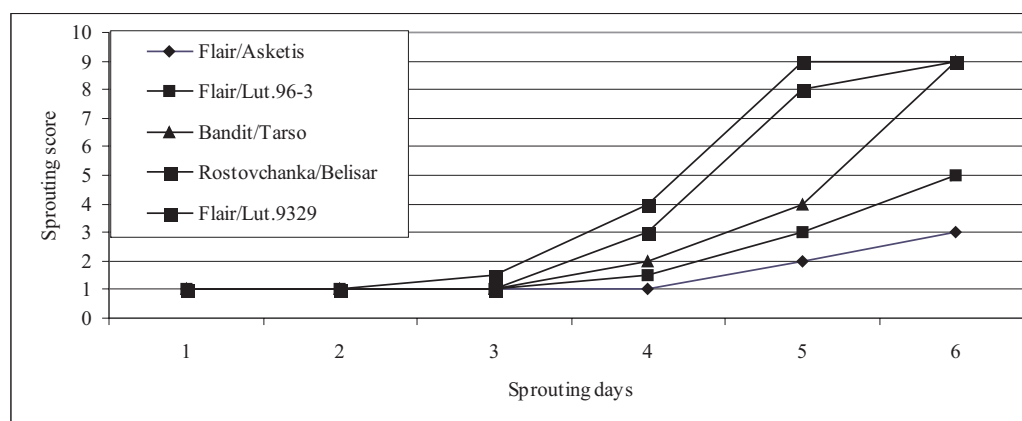
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Fig. 2. Cumulative sprouting of lines with different resistance in 2005.

4.3 to 9.0, SCI value was 0.14–1.0 in 2005. SFS range minimum and maximum differed by 2.1 times, and SCI by 7.1 times. Two lines of 59 had SFS up to 5, four lines up to 6 and seven lines up to 7. The remaining lines (77.9%) were more susceptible and were evaluated from score 7 to 9. Nineteen lines (32%) were evaluated with SCI up to 0.5, the others (68%) were more susceptible and not valuable for selection for resistant to pre-harvest sprouting. Figure 2 shows sprouting progress of lines with different tolerance. The data presented in this figure are based on the most distinct replications of the tested lines. On the third evaluation day sprouting was visible (1%) in most susceptible lines. On the 4th day, the most resistant lines were without visible sprouting, whereas the most susceptible showed sprouting up to 20%. The most tolerant lines sprouted up to 10% on the 6th day while the most susceptible ones over 95% on the 5th day.

Lines showed in Table 1 represent winter wheat genotypes most susceptible and most resistant to PHS in 2005. The most resistant were the lines Flair/Asketis, Torfrida/Beaver//Tarso, Širvinta1/LIA3480. These lines were characterised by SFS scores 5.4–5.8, and SCI values 0.23–0.26. Whereas, the most susceptible lines Elena/Flair, Mermaid/Alidos, Flair/Lut.96-3 had SFS scores 8.6–9.0 and SCI values 0.93–1.00.

Similar relationships were observed in 2006 (Fig. 3 A, B; Table 2). SFS ranged from 5.4 to 9.0, SCI value was 0.14–1.0. Genotypes in 2006 differed in SFS score by 1.7 times and by SCI value 7.1 times. Average SFS for all lines was higher in 2006 (scores 8.2) than in 2005 (score 7.8). However, SCI value was lower in 2006 (0.51) than in 2005 (0.61). Sprouting in four lines (5.5%) was rated up to score 6, 4.1% up to score 7, 19.4 % up to score 8, the rest lines (71.0 %) were sprouted up to score 9. Approximately half of the lines (47%) had SCI up to 0.5. However, the distribution of SCI showed a tendency that in 2006 the lines were more tolerant to PHS than in 2005.

Figure 4 shows the advantage of resistant versus susceptible to PHS lines. Sprouting was visible (1%) in the most resistant line on the 6th day, whereas sprouting in the most susceptible line on the same day was over 95%. The most resistant lines were Pegassos/Dream, Belisar/Briz and

Table 1

MOST RESISTANT AND SUSCEPTIBLE WINTER WHEAT LINES TO SPROUTING IN 2005

Lines	Sprouting Final Score ±SD	Sprouting Cumulative Index ±SD
Flair/Asketis	4.5±1.00	0.14±0.04
Torfrida/Breaver//Tarso	5.4±0.50	0.23±0.11
Zentos/Lut.9371	5.8±0.96	0.25±0.12
Širvinta1/LIA3480	5.5±0.91	0.26±0.17
Flair/Lut.96-3	6.5±0.58	0.33±0.05
Flair/Lut.9329	7.9±0.63	0.35±0.11
Alidos//Haven/Astron	7.1±1.03	0.40±0.07
Širvinta1/Batis	7.3±0.54	0.41±0.06
1905B/Alidos	6.8±0.50	0.43±0.07
Torfrida/Tarso	7.3±1.50	0.44±0.01
Flair/Lut.9365	8.3±0.96	0.81±0.10
Flair/Ukrainka Odesskaya	8.8±0.50	0.82±0.13
Rufa/Lut.96-10	9.0±0.00	0.82±0.13
Belisar/Rostovchanka	8.3±0.50	0.82±0.12
Strumok/Lut.9365	8.5±0.58	0.84±0.14
Flair/Lut.96-3	8.8±0.50	0.85±0.19
Flair/Lut.9329	9.0±0.00	0.87±0.10
Elena/Flair	8.6±0.57	0.93±0.15
Mermaid/Alidos	9.0±0.00	0.98±0.08
Flair/Lut.96-3	9.0±0.00	1.00±0.06
LSD$_{05}$	0.92	0.17

SD, standard deviation

Lars/Lut.96-3, characterised by SFS 5.3–5.8 and SCI value 0.13–0.17. In comparison, the most susceptible lines Rostovchanka/Lut.96-3, Rector/Briz, Rostovchanka/Flair had SFS 9.0 and SCI value 0.92–1.00.

SCI showed more distinction among the lines: SFS ranged from 1.7 to 2.1 times, while SCI ranged 7.1 times. SCI highlighted the genotypes most resistant to sprouting and characterised resistance of lines to pre-harvest sprouting more precisely than SFS.

The lines tested for two years are presented in Table 3. Correlation analysis showed medium strong (r = 0.60–0.65) relations between SFS and SCI in both years. These lines had the best agronomical traits of all tested lines. About one

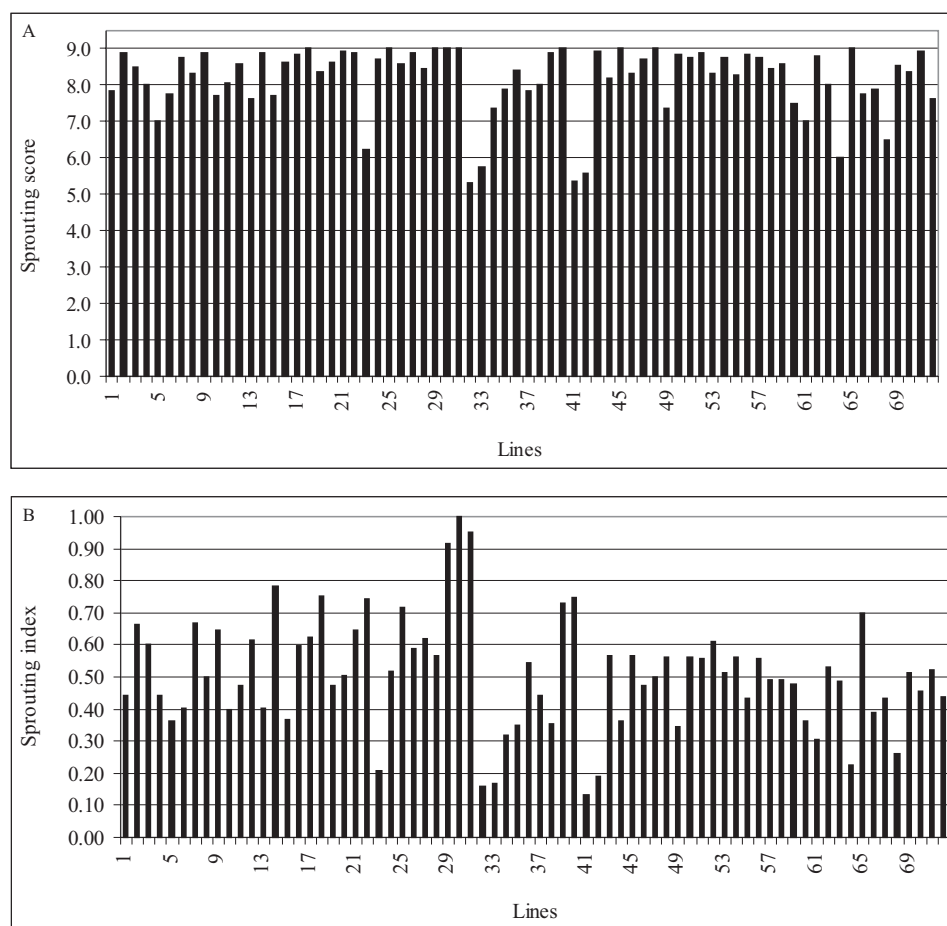*Proc. Latvian Acad. Sci., Section B*, Vol. **63** (2009), No. 1/2.

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Fig. 3. Resistance to pre-harvest sprouting of winter wheat advanced breeding lines in 2006. **A**, sprouting final score; **B**, sprouting cumulative index.

Table 2

MOST SUSCEPTIBLE AND THE RESISTANT WINTER WHEAT LINES TO SPROUTING IN 2006

Line	Sprouting Final Score ±SD	Sprouting Cumulative Index ±SD
Pegassos/Dream	5.4±0.63	0.13±0.05
Belisar/Briz	5.3±0.89	0.16±0.08
Lars/Lut.96-3	5.8±0.29	0.17±0.03
Pegassos/Aspirant	5.6±0.72	0.19±0.11
Astron/Tarso//Yacht	6.2±0.68	0.21±0.02
Pegasos/Pesma	6.0±0.33	0.23±0.05
Dream/Lut.9329	6.5±0.58	0.26±0.06
Pegassos/Biscay	7.0±0.41	0.31±0.05
Lut.96-3/Bold	7.4±0.48	0.32±0.06
Biscay/Pesma	7.4±0.64	0.35±0.09
Dream/Lut.9329	9.0±0.00	0.70±0.06
Hussar/Konsul A//Lut.96-6	9.0±0.00	0.72±0.06
Astron/Manef	8.9±0.12	0.73±0.03
Astron/Tarso//Mobil	8.9±0.12	0.74±0.06
Lone/Inna//Lut.96-2	9.0±0.00	0.75±0.07
Flair/Lut.9365	9.0±0.00	0.75±0.06
Flair/Lut.3-96	8.9±0.25	0.78±0.17
Rostovchanka/Lut.96-3	9.0±0.00	0.92±0.03
Rector/Briz	9.0±0.00	0.95±0.06
Rostovchanka/Flair	9.0±0.00	1.00±0.04
LSD$_{05}$	0.46	0.08

SD, standard deviation

fourth of lines in 2005 and about half of lines in 2006 had SCI up to 0.50. As a result, these lines can be characterised as satisfactorily tolerant to pre-harvest sprouting.

DISCUSSION

The winter wheat harvesting period from full ripening to completion in Lithuania lasts for 3–4 weeks and in some years longer. Long-term observations indicate that during the harvesting period rain occurs every third day in Lithuania. This means that the problem of pre-harvest sprouting is very relevant. The most complicated situation over the last few years occurred in 2005, when it rained for two weeks at the beginning of wheat harvesting period. According to quality standards, wheat grain was suitable only for feed, especially earlier ripening cultivars. Therefore, only genotypes with very high tolerance to PHS are desirable. One of the ways to solve the problem of pre-harvest sprouting is to develop resistant genotypes of winter wheat. On the other hand, it is necessary to find the most effective method for evaluation of pre-harvest sprouting resistance, because in dry years it is difficult to screen the susceptible lines. The lines were investigated for PHS after long storage, which highlighted the most resistant genotypes.

Pre-maturity alpha-amylase activity is an indicator of pre-harvest sprouting occurrence, is shown by falling number analyses. Sprouted wheat has low falling number. Pre-harvest prediction of falling number may be important for
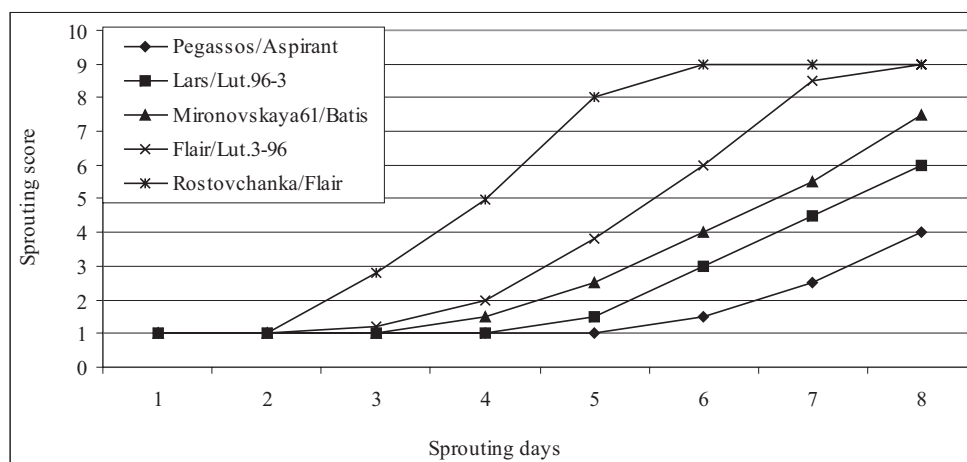
Fig. 4. Cumulative sprouting of lines with different resistance in 2006.

SCI AND SFS OF WINTER WHEAT TESTED IN 2005 AND 2006

Line	SFS±SD	SCI±SD	SFS±SD	SCI±SD
Flair/Lut.3-96	7.5±0.58	0.33±0.05	8.6±0.48	0.60±0.08
Flair/Lut.3-96	6.8±0.56	0.35±0.14	7.6±1.03	0.40±0.15
Flair/Lut. 9329	7.9±0.63	0.35±0.11	8.7±0.44	0.51±0.07
Flair./Lut.3-96	7.0±0.42	0.37±0.16	7.7±0.82	0.37±0.14
Širvinta1/Batis	7.3±0.54	0.41±0.06	8.0±0.16	0.44±0.02
Flair/Lut.9329	7.8±0.76	0.62±0.13	8.4±0.25	0.47±0.06
Mironovskaya61/Batis	7.0±0.31	0.64±0.54	7.8±0.84	0.44±0.17
Flair/Lut.9365	8.8±0.50	0.67±0.07	8.1±1.06	0.47±0.14
Flair/Lut.96-2	9.0±0.00	0.70±0.15	8.9±0.25	0.66±0.04
Elena/Lut.956	8.8±0.50	0.72±0.05	7.7±0.48	0.40±0.07
Flair/Lut.96-2	9.0±0.00	0.73±0.16	8.5±0.41	0.60±0.09
Flair/Lut.9329	9.0±0.00	0.75±0.08	8.6±0.43	0.61±0.06
Rufa//Contra/ Astron	8.0±0.71	0.76±0.38	8.3±0.91	0.50±0.18
Rufa/Elena	8.5±0.97	0.77±0.33	8.8±0.50	0.67±0.05
Marabu/Flair	8.6±0.48	0.80±0.09	8.8±0.24	0.62±0.08
Širvinta1/LIA3480	8.7±0.60	0.81±0.25	7.8±1.20	0.40±0.14
Flair / Lut 9-365	8.3±0.96	0.81±0.10	9.0±0.00	0.75±0.06
Rufa/Lut.96-10	9.0±0.00	0.82±0.13	8.9±0.25	0.65±0.04
Flair/Lut.3-96	9.0±0.00	1.00±0.06	8.9±0.25	0.78±0.17
LSD_{05}	0.88	0.16	0.51	0.09

SCI, Sprouting Cumulative Index; SFS, Sprouting Final Score; SD, standard deviation

the producer to make right decisions on the grain costs and estimated benefit. Rajaram (1992) observed no significant correlation between pre-harvest sprouting and alpha-amylase activity. In contrast, DePauw and McCaig (1991) reported that sprouting score, percentage of sprouted kernels and alpha-amylase activity were highly correlated. Clarke *et al.* (1994) found a moderate correlation between mean spouting score and alpha-amylase activity. According to Upadhyay and Paulsen (1988), alpha-amylase is probably not an appropriate selection criterion for pre-harvest sprouting resistance because of relatively low correlation with visual sprouting after simulated rain. This shows that reliable determination of PHS resistance can be made by evaluation of sprouting in ears using various methods, but not using falling number analysis.

SFS and SCI were correlated (r = 0.60 – 0.65) in both years of investigations indicating that environmental factors had moderate influence. One of the reasons was rapid increase of temperature in combination with rainfall shortage during the milk development stage which lasted up to complete ripening in 2005. The next year's growing season was dry and hot. The impact of temperature fluctuation on grain dormancy was previously observed by Cochrane (1993) and Biddulph *et al.* (2005). The conditions of 2005 reduced tolerance to PHS and testing of lines lasted for six days, whereas lines of the year 2006 germinated within eight days. Average SFS in 2005 was 8.1, in 2006 higher reaching 8.3 for the lines tested for two years. However, SCI was lower in 2006 (0.53) compared with that in 2005 (0.64). This relationship together with a considerable difference in the range of SFS (1.7–2.1) and SCI values (7.1) highlighted the advantage of SCI for selection of genotypes tolerant to pre-harvest sprouting.

When the most susceptible lines on the 5th day of testing in both years had severely sprouted (SFS 8–9) the most resistant only just started to germinate (SFS 2–3). Lines with the lowest SFS have great advantage in the case of seed production under rainy weather conditions during harvesting, because under field conditions constant rains rarely continue more than several days. A PHS test of one week duration should match the worst scenario of rainy weather.

Breeding for PHS resistance is complicated because of PHS due to the role of QTL. Many studies show that in hexaploid wheat about half of PHS-related QTL comprise clearly defined loci such as vivipary (*PV-1*), red colour of grain coat (*R*), and late-maturity alpha-amylase located on the third and sixth chromosome groups (Flintham *et al.*, 2002). For a long time, the red grain coat colour has been used in wheat breeding as a marker linked with PHS resistance (Flintham, 2000), but recent studies by the Mares group (Himi *et al.*, 2002; Mares *et al.*, 2002) have shown a strong but breakable relation between grain colour and dormancy. Many European winter wheat cultivars as well as our lines are red grained. However, the above-mentioned relation

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could suggest rejecting newly created lines with white grains in the breeding process when white grained cultivars are not highly preferred.

However, the complete mechanism of PHS resistance-related traits is still not clear. The least studied genetic relationships are mechanisms in seed and glumes. Lan *et al.* (2005) showed that seed germination depended on germination method. Germination percentages decreased gradually: embryo, seed with pierced coat, normal seed, seed with destroyed glume and intact spike. It is likely that seed germination was affected by the pericarp, the content of seed and glumes. This indicated the importance of the vegetative structures of the ear in enhancing sprouting resistance in the investigated genotypes. This suggests that PHS resistance tests should be mainly conducted with intact ears.

The resistance to PHS is an advantage of a high quality, bread wheat cultivar. Because the nature of sprout damage is such that a small amount of sprouted grain, if mixed with sound grain, causes a disproportionately greater extent of damage to the entire consignment. Wheat usually does not ripen in a field completely evenly. Different ripening is influenced by soil differences in field, plant damage by diseases and pests, which appear in patches and some technological inaccuracy.

Therefore, PHS resistance of new cultivars should be one of the traits of resistance to abiotic stresses under conditions of changing climate.

Based on our results, it can be concluded that most of the tested lines in this study have low to moderate level of pre-harvest sprouting resistance. The tested genotypes indicate that incorporating resistance to pre-harvest sprouting into desirable wheat phenotypes is an attainable objective. Lower SFS and SCI values of some lines showed that genes or QTL's for resistance exist in germplasm and could be effectively exploited. Therefore, selecting lines with good resistance to pre-harvest sprouting will reduce the chances of diminished grain quality and discounts due to PHS.

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TOLERANCE SĒKLU DĪGŠANAI VĀRPĀS LABĀKAJĀS LIETUVAS ZIEMAS KVIEŠU SELEKCIJAS LĪNIJĀS

Analizēta tolerance sēklu dīgšanai vārpās 131 Lietuvas izcelsmes ziemas kviešu selekcijas līnijās. Apspriesta dažādu metožu pielietojamība šīs īpašības noteikšanai.

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