EFFECT OF SEMIDWARFING AND CONTROLLING THE VERNALIZATION REQUIREMENTS GENES ON WHEAT RESPONSE TO LATE SOWING

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ABSTRACT

Late emergence, caused by late sowing or delayed germination, following dry or cool weather in autumn, significantly reduces the yield in winter wheat. This research was carried out to determine the effect of reduced height (*Rht1* and *Rht8*) and of vernalization requirement (*Vrn*) genes on genotypic tolerance to late sowing in winter wheat. 76 recombinant inbred lines of the Sincron x F 1054 W cross, polymorphic for *Rht1* and *Rht8*, were tested at normal and late sowing dates. The yield reduction varied from year to year, depending on the weather conditions. *Rht1* semidwarfs yielded more at normal sowing date, but they were equal to the tall lines at late sowing. *Rht8* semidwarfs yielded less and were more susceptible to low winter temperatures. Although tall wheat seems to be more tolerant to late sowing, *Rht1* semidwarfs may be selected, as they produce the same yield at late sowing and under more unfavourable conditions. Two pairs of near isogenic lines different as concerns the vernalization requirements, selected from the Lovrin 34 and Fundulea 4 cultivars, were also tested at normal and late sowing dates. The spring isolines, with no vernalization requirements, yielded significantly more than the lines with high vernalization requirements when sowing was very late, but significantly less when winter was severe. The increased tolerance to late sowing of low vernalization wheat could be used if its cold tolerance would be improved.

Key words: cold tolerance, semidwarf, vernalization, wheat.

INTRODUCTION

Large areas of wheat are annually sown late, over the optimum date, achieving yields lower than in the case of the wheat crops sown in the optimum periods. The late sowing of winter wheat has been mostly determined by reducing the areas cultivated with preceding early crops and by increasing the maize areas which are harvested late in autumn. The application of some supplementary agrotechnical measures such as the increase of number of germinable seeds/m^2 and nitrogen fertilizer rate, crop irrigation, etc., increases the production costs without compensating significantly the tendency of yield decrease. Therefore, one should also operate on soil in view of improving the tolerance to late sowing, so that, even in the case of the late sowing, yields closer to those obtained under right sowing conditions be achieved.

Late sowing leads to an incomplete, non-uniform and delayed emergence (Sipoº et al., 1980), to a limited vegetative development (Murphy et al., 1994) and to shortening and delaying the grain filling period (Randhawa et al., 1993). Mustãþea (1994) states that tolerance to late sowing is a complex character determined by the interaction of genotype with environment, and that this trait may be improved.

To improve the complex characters it is useful to establish their associations with more simple characters, easier to characterize, controlled by major genes.

The purpose of this research was to evaluate the effect of some major genes for reducing the height and controlling the vernalization requirements on wheat response to the late sowing.

MATERIALS AND METHODS

This research was carried out for three years: 1996, 1997 and 1998. The experiments included a set of 76 recombinant homozygous lines (RHL) and two pairs of near isogenic lines for the alleles which determine the reaction to vernalization.

The recombinant homozygous lines were obtained by crossing Sincron line with F-1054 W 7-202 line (Sãulescu et al., 1994). The RHL are different regarding two major genes which control the plant height, *Rht1* and *Rht8*, located on different loci. Having in view the variability degree of the high or short height dominance, it has been established to note with the symbol *Rht* the alleles determining the height decrease and with *rht* the alleles specific to the normal height regardless the dominance (Sãulescu et al., 1988).

Rht1 - a gene of height reduction, is located on the 4AS chromosome and confers to the wheat a semidwarf height and an insensitivity to exogenous gibberellic acid. It is partly dominant and presents the pleiotropic effect on the coleoptile length.

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Rht8 – a gene of height reduction but with a more moderate effect, is located on the 2D chromosome. It confers to the wheat a semidwarf height and sensitivity to exogenous gibberellic acid. It is recessive and does not have pleiotropic effect on the coleoptile length.

The presence of the alleles which reduce the height in a homozygous state to one of the two major genes in the combination *Rht1Rht1 rht8rht8* or *rht1rht1 Rht8Rht8* confers to the wheat a semidwarf height. The presence in a homozygous state of the alleles reducing the height in both major genes *Rht1Rht1 Rht8Rht8* determines the dwarf height of the wheat. The lack of the alleles reducing the height in the both genes *rht1rht1 rht8rht8* gives to the wheat a high height.

The nearly isogenic lines differing by the vernalization requirements were obtained from the Fundulea 4 and Lovrin 34 wheat cultivars whose genetic constitution is formed in approximately equal proportions of a winter wheat biotype and a spring one.

The lines were separated on the basis of their response to the temperature in the seedling stage, obtaining lines with high vernalization requirements and lines without vernalization requirements.

The lines without vernalization requirements were extracted from the spring biotypes of the two cultivars. The seeds resulted from the two cultivars were cultivated under controlled environmental conditions, in phytotron, at temperatures of 22°C by day and 18°C by night, under long day conditions. The plants belonging to the spring biotypes, having no vernalization requirements, passed in the development stage. The matured ears were harvested, and then the seeds were multiplied. The lines without vernalization requirements were denominated F4 *Vrn* and Lv 34 *Vrn*, after the *Vrn* symbol of the genes which confers the intensitivity to vernalization.

The lines with high vernalization requirements were extracted from the winter biotypes of the two cultivars. The plants belonging to the winter biotypes having requirements to positive low temperatures did not pass to the development stage. They were separated and subjected for 20 days to the positive low temperatures, needed to satisfy the vernalization requirements. At the end of the vernalization stage, the plants were kept under natural conditions, in glasshouse, till the maturation, and then the seeds were multiplied. The lines with high vernalization requirements were named F4 *vrn* and Lv34 *vrn*, according to the *vrn* symbol of alleles which confers them sensitivity to vernalization.

The two separated biotypes (*Vrn* and *Vrn*) were tested along with the original cultivars which represent a mixture of the two forms.

The recombinant homozygous lines were sown at different dates depending on the agroclimatic characteristics of the respective autumn, in 5 m^2 plots, with a density of 550 wheat grains/m^2, using an experimental scheme (blocks) with the repetition of the two parental forms and of the Flamura 85 cultivar control every 20 plots.

The nearly isogenic lines differing by the vernalization requirements were sown at two different dates, excepting the year 1998 when the sowing was made in late period, in three repetitions.

In order to establish the effect of each gene on the yield and other characteristics, the lines were grouped in terms of the alleles corresponding to the respective gene. The averages of the two groups of lines were compared and the significance of the differences was determined on the basis of the "t" test.

RESULTS AND DISCUSSIONS

I. Effect of some height reducing major genes on wheat response to late sowing

The analysis of the figure 1 shows that sowing delay leads to the height reduction of the lines.

The classification of the four groups of lines, from the height viewpoint, remains the same regardless the sowing date, but the variation amplitude due to genes, which decrease the height diminishes at the same time with the decrease of the thermic resources.

Under reduced thermic resources conditions in autumn of 1997 and under the action of the minimum winter temperatures, the semidwarf lines carrying the *Rht8* gene

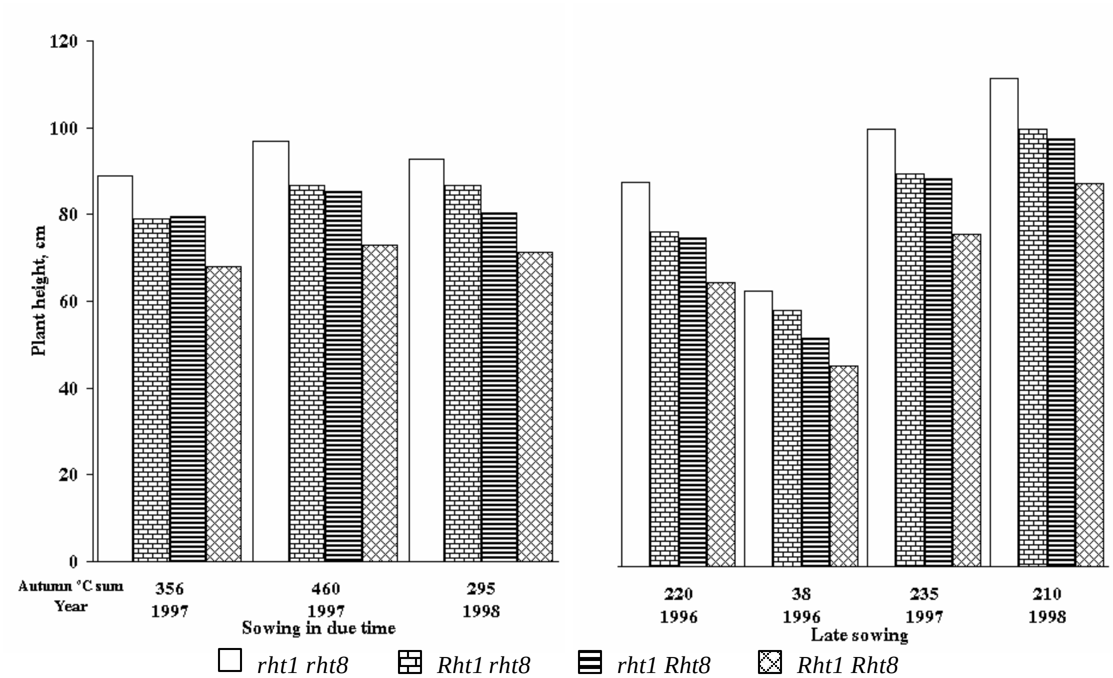

Figure 1. Effect of *Rht1* and *Rht8* genes on plant height depending on autumn °C sum

reduced their height more than the semidwarf lines carrying the *Rht1* gene, as compared with what was observed in the other years, at both seeding dates, which means that they respond in a stronger manner to the decrease of the thermic resources in autumn and to the minimum temperature action in winter.

The semidwarf lines carrying the *Rht1* gene are superior to the semidwarf lines carrying *Rht8* gene or the other combinations of genes, regarding the yield obtained under the nearly normal growing conditions of 1996 and 1998 and under the very suitable conditions for growing and development in 1997. Simultaneously with the sowing delay, the level of yields achieved with all the four combinations of genes as well as the advantage of the lines carrying the *Rht1* gene decrease. The *Rht1* gene does not seem to be inferior to the other genes or combinations of genes neither as concerning the sowing delay (Figure 2). The highest decrease of yields occured in 1995-1996 due to autumn limited thermic resources, lack of some “winter windows” and spring drought. In the agricultural year 1996-1997, the yield reduction was lower. Under these conditions, the semidwarf lines carrying the *Rht1* gene maintain a light advantage as compared to the semidwarf lines carrying the *Rht8* gene and the dwarf lines (as well as in the next year), and they do not seem to be inferior to the high height lines. On the contrary, in 1997-1998, under sowing delay conditions, the semidwarf lines carrying the *Rht8* gene and the dwarf lines responded stronger, reducing much more their yield as compared with the yield recorded in the other years.

Under normal growing conditions in autumn, the semidwarf lines carrying the alleles of the *Rht1* gene present a significantly distinct advantage regarding the yield, as compared to the lines carrying the alleles of the *rht1* genes corresponding to the normal height (P=0.8%** and P=0.52**) in 1996 and 1997 and a significant advantage (P=2.1%*) in 1998.

The sowing delay leads to the decrease of yields and to cancellation of this advantage, the average differences between the lines carrying the two alleles being insignificant (Figure 3).

Significant differences between the lines carrying the alleles of the *Rht8* gene were not

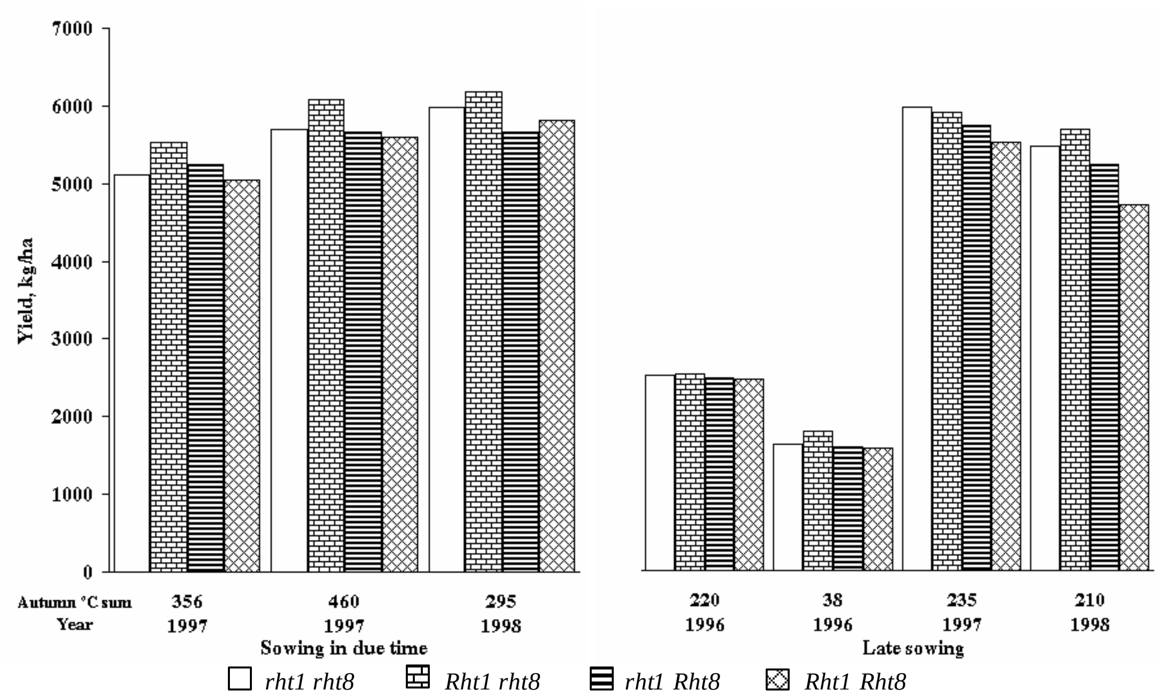

Figure 2. Effect of *Rht1* and *Rht8* genes on yield depending on autumn °C sum

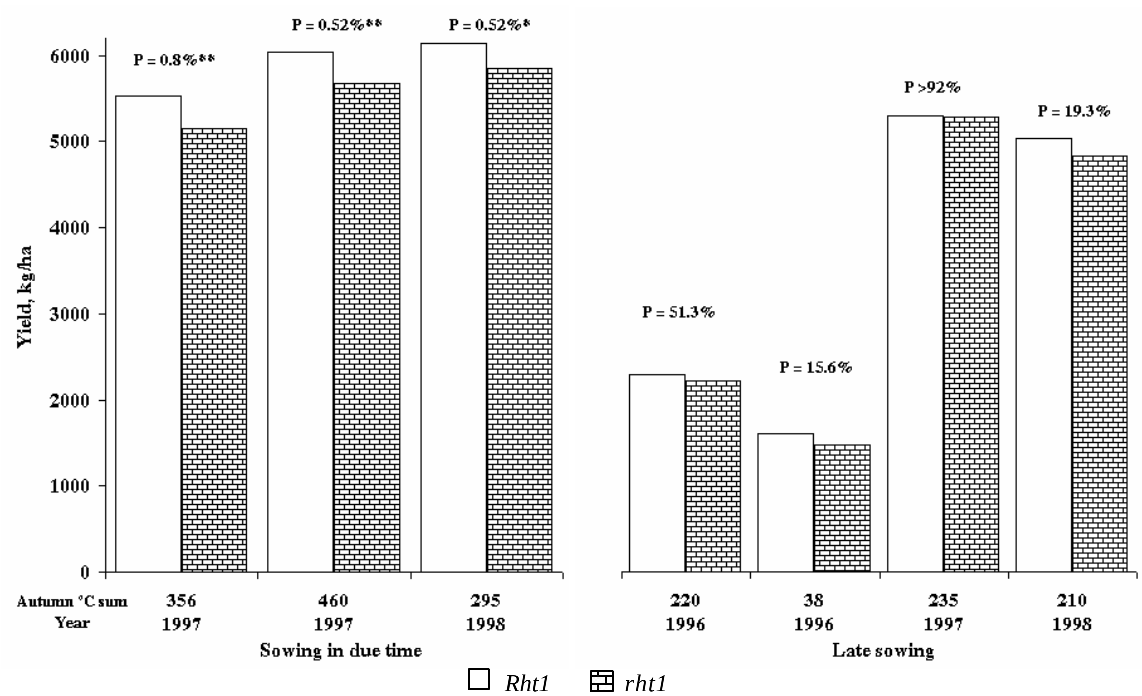

Figure 3. Effect of *Rht1* gene on yield depending on autumn °C sum

observed regarding the yield in 1996 and 1997 both under autumn normal growing conditions and the sowing delay (Figure 4).

Under the year 1997-1998 conditions characterized by reduced thermic resources in autumn and low minimum temperatures in winter, the lines carrying the *rht8* alleles were significantly more productive than the lines carrying the *Rht8* alleles in the both cases of sowing dates (P=1.4%*, P=2.1%*, respectively).

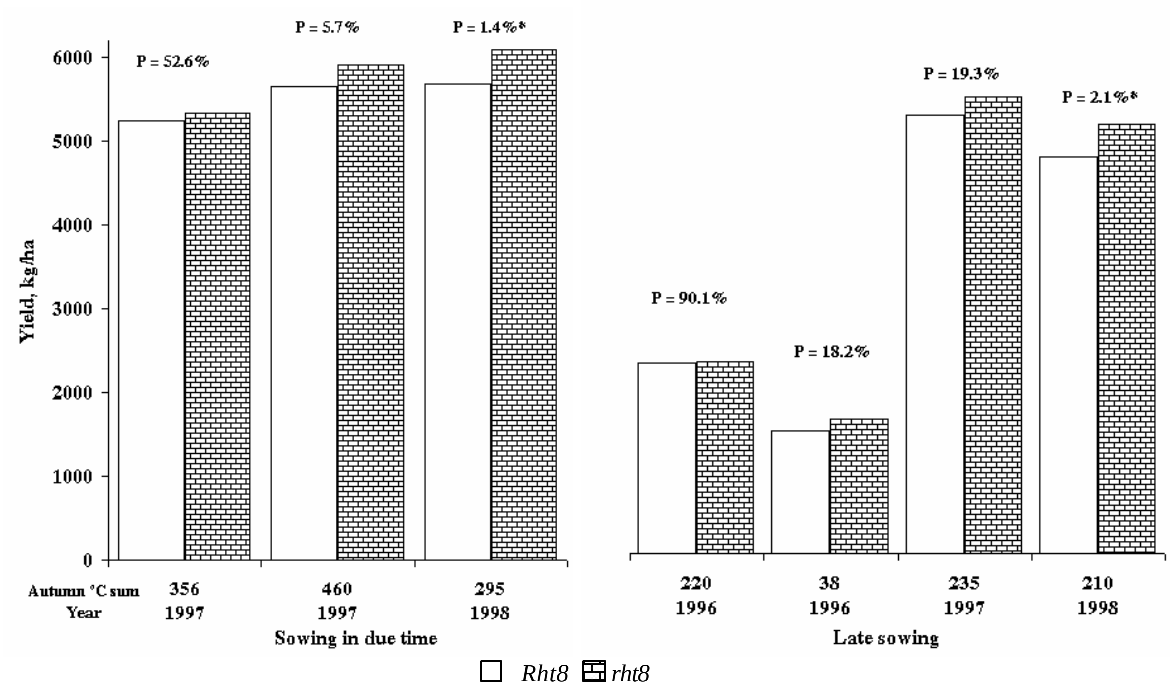

Figure 4. Effect of *Rht8* gene on yield depending on autum n °C sum

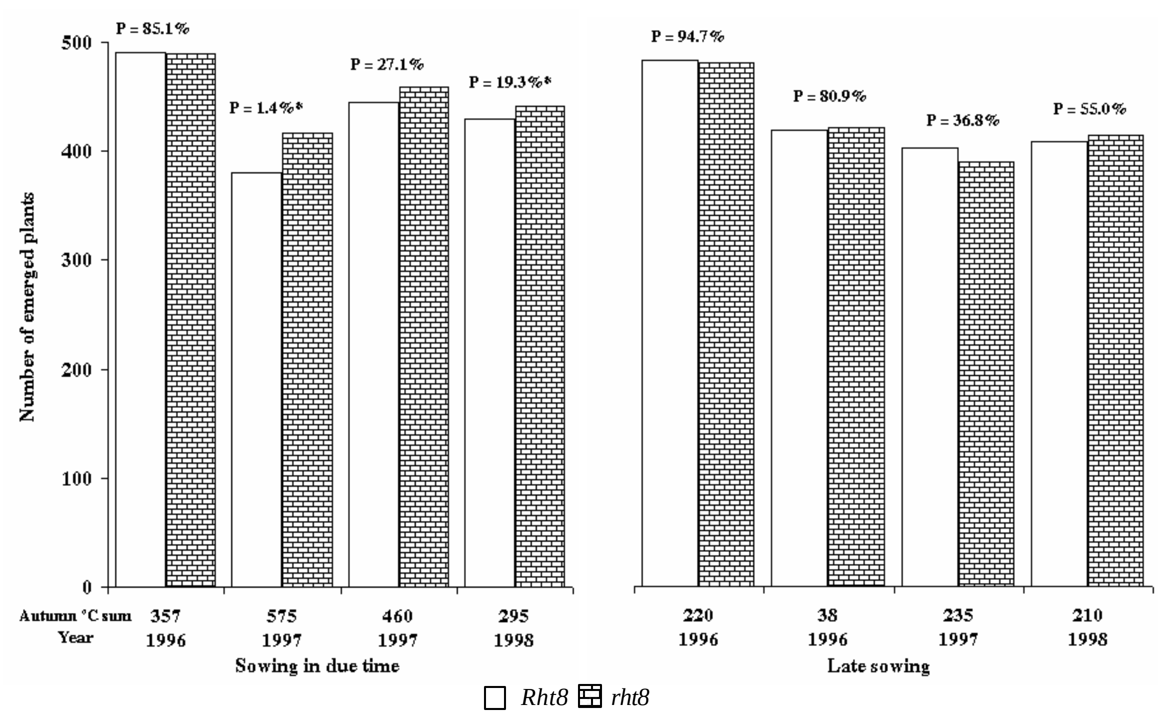

Figure 5. General effect of *Rht1* gene on number of emerged plants

The significant yield differences recorded between the two groups of lines may be attributed to their different sensitivity to the minimum reduced temperatures in winter, taking into account that the semidwarf lines carrying the *Rht8* allele strongly responded also regarding the height reduction at the two sowing dates. Actually, the semidwarf lines carrying the *Rht8* allele proved to be more sensitive to the frost action as compared to the lines carrying the *rht8* allele, the differences being significant at the both sowing dates (P=4.8%*, P=2.8*, respectively). No significant differences were observed among the lines carrying the alleles of the *Rht1* gene regarding the tolerance against the winter conditions at both sowing dates. As in the case of the lines carrying the alleles of the *Rht8* gene, in the case of the sowing delay, the sensitivity to the action of the minimum low temperatures increases, due to the different growing stage of lines at the two sowing dates.

The previous researches showed that, because *Rht1* has a pleiotropic effect on the coleoptile length (the presence of *rht1* determines the manifestation of the short coleoptile character) in years with unfavourable conditions for emergence, the genotypes carrying the *Rht1* gene are disadvantaged, producing a lower number of emerged plants (Allan, 1980).

From the figure 5, it may be observed that, under normal growing conditions in autumn, there are no significant differences between the lines carrying the *Rht1* allele (with short coleoptile) and the lines carrying the *rht1* allele (with long coleoptile).

At the same time with the sowing delay, the number of emerged plants decreased also, but without noting significant differences between the two groups of lines with different coleoptile length.

The highest reduction took place at the spring emergence in 1996, with about 100 less emerged plant per area unit (plot). However, the reduction of the emerged plant number, under the conditions of the three experimental years, was not as high as compared to that observed in other years. Only under the 1996 autumn conditions, when sowing was carried out in dry soil, on 10.05.1996, the semidwarf lines carrying the *Rht1* allele with short coleoptile produced significantly a lower number of emerged plants as compared to the lines carrying the *rht1* allele with high height and long coleoptile. Therefore, the superiority of the long coleoptile character occurs especially under dry autumn conditions.

Significant differences concerning the number of the emerged plants were not observed between the two groups of lines carrying the alleles of the *Rht8* gene (both with long coleoptile), neither in the case of sowing in dry soil.

The quantification of tolerance to the sowing delay of the wheat genotypes may be based on the grain yield achieved as such in the absence of a specific tolerance mechanism. Therefore, from the set of 76 RHL, a number of 7 lines were selected which provided yields similar or higher to those of the Flamura 85 control cultivar, a cultivar with a large extent in the agriculture of Romania and enough tolerant to the sowing delay (Mustăţea, 1994).

Out of the 7 RHL, which besides the high yields under the conditions of sowing in due time provide also good yields when the sowing is delayed, 5 are semidwarf lines carrying the *Rht1* gene, and 2 are with high height.

Table 1. Characterization of some recombinant homozygous lines tolerant to late sowing

Genotype (RHL)	Height genetic control	Yield at optimum sowing date kg/ha	Yield at late sowing date kg/ha
Coleo 10	*Rht1 rht8*	6362	3663
Coleo 24	*rht1 rht8*	6382	3751
Coleo 35	*Rht1 rht8*	6657*	3591
Coleo 49	*rht1 rht8*	6131	3955*
Coleo 59	*Rht1 rht8*	6017	3860
Coleo 69	*Rht1 rht8*	6812**	3919*
Coleo 70	*Rht1 rht8*	6512*	3619
Flamura 85		6106	3747
LSD 5%		332	162

It may be observed the good behaviour of the Coleo 49 and Coleo 69 recombinant homozygous lines which provide significant yield increases as compared to the Flamura 85 cultivar control under the late sowing conditions. The Coleo 49 line is a line with high height, and the Coleo 69 is a semidwarf line carrying the *Rht1* allele. Therefore, a good behaviour, under the late sowing conditions, may have both the lines with high height and the semidwarf lines carrying the *Rht1* gene.

The sowing delay, under the conditions of a modern agriculture, should be considered as a technological mistake or as an accidental factor or determined by a certain conjuncture. That is why the breeding accent should be put on creating cultivars with genetic potential of high production, able to secure high yields by sowing in due time, and yields closer to them in case of late sowing. The Coleo 69 semidwarf line carrying the *Rht1* gene has a good behaviour both under optimum and late sowing conditions, obtaining significant yield gains as compared to Flamura 85 cultivar control.

The Coleo 49 line, with significant yield gains as compared to Flamura 85 cultivar control under the late sowing conditions, has a similar behaviour with that of Flamura 85 cultivar control under the optimum sowing conditions.

Although the tall wheat cultivars have a good behaviour at a late sowing, their use on a large scale in the breeding programmes could lead to the reduction of the lodging resistance and of the genetic productive potential.

The dwarf lines (*Rht1 Rht1 Rht8 Rht8*) do not present interest for the improvement of tolerance to late sowing due to both the low level of their yields and the harvesting difficulties determined by the plant small height.

The semidwarf lines carrying the *Rht8* gene present interest in the breeding programmes for the plant height, but especially for the coleoptile length. However our data suggest that the replacement of the *Rht1* gene with the *Rht8* gene in order to create semidwarf forms with long coleoptile risks to have an unfavourable effect on the productive capacity.

II. The effect of genes controlling the vernalization requirements on wheat response to late sowing

The transition of the winter wheat from the vegetative to generative stage is conditioned by the long action of the low positive temperatures, a process called "vernalization". The primordial flowers occur only after this thermic period. The perceptive locus place of the low positive temperatures proved to be the active embryo or the apical bud. The vernalization may be achieved both with the imbibed seeds and the seedlings.

Influencing the duration of the development stages, the vernalization and light requirements largely determine also the response of cultivars to the environmental conditions and especially to the variation of the sowing date (Stern and Kirby, 1979).

Sown in optimum time, under limited thermic resource conditions in autumn and the long winter specific to 1996 year, the

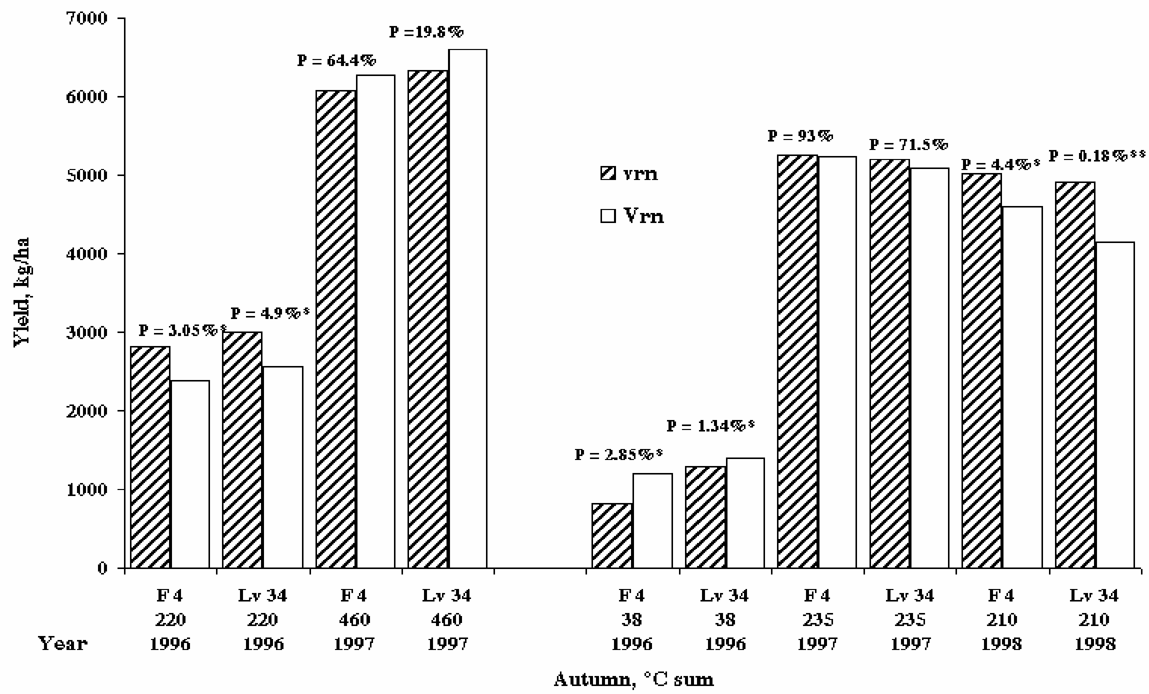

Figure 6. Influence of vernalization requirements at different sowing dates

lines with high vernalization requirements were significantly more productive than the lines without vernalization requirements (P=3.05%* and P=4.9%*, respectively). Even if damages due to wintering were not observed, the thermic resources in autumn and the long winter represented however a stress for the lines without vernalization requirements (Figure 6).

Under the very favourable growing conditions in autumn and winter, specific to 1997 year, significant differences (P=64.4%, P=19.8%) were not observed between the lines with high vernalization requirements and lines without vernalization requirements. With the late sowing, which, under 1996 conditions, led to a very late emergence, in spring, the differences regarding the vernalization requirements caused a differentiation of the yields, the lines without vernalization requirements being significantly more productive than the lines with high requirements of vernalization (P=2.85*, P=1.34%*, respectively).

Under the favourable conditions of the year 1997, significant differences were not observed between the two groups of lines with different vernalization requirements neither regarding the late sowing.

On the contrary, in 1998, under the late sowing conditions and reduced thermic resources in autumn, and under the winter unfavourable conditions (frost), the differences regarding the vernalization requirements determined differences concerning the degree of frost resistance, that led to yield differentiation. The yield level achieved was determined by the degree of frost resistance, a distinctly significant negative correlation (r= -096**) between yield and wintering resistance being observed. The lines without vernalization proved to be sensitive (F 4 *Vrn*) and very sensitive (Lv 34 *Vrn*) to the frost action during winter, as compared to the lines with vernalization requirements. As a result, the lines with high vernalization requirements were significantly and distinct significantly more productive than the lines without vernalization requirements (P=4.4%*, P=0.18%**) (Figure 6).

This aspect emphasizes the idea that the genes inducing frost resistance are linked with the genes which induce the sensitivity to vernalization (*vrn*) (Brule-Babel and Fowler, 1988).

The experimental results illustrate the complexity of this problem, the effect of the vernalization requirements being in a strong interaction with the specific conditions of every year. The use of the spring forms without vernalization is dependent on the possibility of improving their wintering resistance.

The development of spring lines with wintering resistance similar or close to the typic winter lines looks like being difficult, but the studies carried out by some scientists suggested a perfect correlation between the resistance to frost and the vernalization requirements (Fowler and Limin, 1997).

CONCLUSIONS

The sowing delay has resulted in a more or less important reduction of wheat yield, depending on the unfavourable climatic conditions varying from year to year.

The semidwarf lines carrying the *Rht1* gene have been more production as compared to the semidwarf lines carrying the *Rht8* gene, while the dwarf lines and the tall ones only under sufficient vegetative growing conditions in autumn, but they were not inferior to the other genotypes even when the sowing was delayed.

The semidwarf lines carrying the *Rht8* gene proved to be less productive than those carrying the *Rht1* and more sensitive to the action of the minimum low temperatures in winter, reacting stronger regarding the plant height and the yielding capacity.

Under spring emergence conditions, the lines with high vernalization requirements are less productive than the lines without vernalization requirements. Under late sowing conditions and when the vernalization stage occurs in autumn followed by a mild winter, the differences concerning the vernalization do not influence the yields. In autumns followed by winters with minimum low temperatures, the lines with vernalization requirements are significantly more productive than the lines without vernalization requirements.

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