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Cabbage (*Brassica oleracea* var. *capitata* L.) grown under the conditions of the life cycle of winter oilseed rape (*Brassica napus* L.) in order to achieve a stable seed yield

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A B S T R A C T

The expression of genes that induce the transformation of meristems into the reproductive stage in oilseed rape is realized in conditions of low positive temperatures for a certain period of time. Such a flowering process is called the vernalization pathway. A four-factor field trial with 6 genotypes of head cabbage was set up at the Institute of Vegetable Crops in Smederevska Palanka, of which three parental genotypes were divergent by geographical origin: Scc, B and N, and three more F1 hybrids were selected by diallel crossing: Scc x B, Scc x N and B x N. In order to achieve a different vegetative stage, seedlings were sown at three sowing dates: August 15th, September 1st and September 15th. Transplanting was done on October 20th. The results of sowing head cabbage within the sowing period for oilseed rape were the induction of the flower mechanism, the absence of the head formation phenophase, and the realization of a stable seed yield. The experiment was performed *in vivo* in the control version and in the treatment with gibberellic acid – GA_3. The influence of all four factors: season, genotype, sowing date and GA_3 treatment showed statistical significance for the yield components as well as for the yield itself and seed quality. The three seasons in which the experiment was evaluated differed in temperature during overwintering: 2010/2011 was moderately cold, 2011/2012 was extremely cold, while 2012/2013 was warm. In the cold season, the seed yield was low, and reduced to the biological maintenance of the species, while the highest seed yield was achieved in the third – warm (2012/2013) season in the first sowing period. The experiment also confirmed the existence of an identical flower mechanism in the species *Brassica napus* L. and *Brassica oleracea* var. *capitata* L.

Keywords: head cabbage, oilseed rape, vernalization, sowing time, seed yield, overwintering

И З В О Д

Експресија гена који индукују трансформацију меристема у репродуктивни стадијум код уљане репице остварује се у условима ниских позитивних температура у извесном трајању. Такав процес цветања назива се вернализациони пут. У Институту за повртарство из Смедеревске Паланке постављен је четворофакторски пољски оглед са 6 генотипова купуса главичара, од којих су три родитељска генотипа дивергентна по географском пореклу: *Scc*, *B* и *N*, а од њих је диалелним укрштањем селекционисано још три F1 хибрида: *Scc x B*, *Scc x N*, и *B x N*. У циљу постизања различитог вегетативног стадијума расада, сетва је вршена у три рока сетве: 15. август, 1. септембар и 15. септембар. Расађивање је вршено 20. октобра. Резултат сетве купуса главичара у року сетве за уљану репицу била је индукција цветног механизма, изостанак фенофазе формирања главице и реализација стабилног приноса семена. Оглед је постављен *in vivo* у контролној верзији и у третману гиберелинском киселином – GA_3. Утицај сва четири фактора: сезоне, генотипа, рока сетве и третмана GA_3 показао је статистичку значајност на особине компоненте приноса, као и сам принос и квалитет семена. Три сезоне у којима је оглед евалуиран биле су температурно различите у периоду презимљавања: 2010/2011 је била умерено хладна, 2011/2012 је била изразито хладна, док је 2012/2013 била топла. У температурно хладној сезони принос семена био је низак, сведен на биолошко одржавање врсте, док је највећи принос семена остварен у трећој – топлој (2012/2013) сезони истраживања у првом року сетве. Оглед је потврдио и постојање идентичног цветног механизма код врста *Brassica napus* L. и *Brassica oleracea* var. *capitata* L.

Кључне речи: купус главичар, уљана репица, вернализација, рок сетве, принос семена, презимљавање.

1. Introduction

Oilseed rape (*Brassica napus* L.) is a winter crop used to obtain oil from seeds, and the process of flowering is called the vernalization pathway (Antoniou-Kourounioti RL et al., 2021). Cabbage is a biennial crop whose leaves are used for food, while in the second year flowering stems grow from the head, and the typical flowering pathway is an autonomous pathway. The oilseed rape genome is a tetraploid of the

AACC type, created from the genome: *Brassica rapa* L. genome – AA and *Brassica oleracea* L. genome – CC (Nagarahu, 1935; Parkin et al., 1995: Schiessl, 2020), which presupposes the possibility of expressing an identical flower mechanism – the vernalization pathway – in the species *Brassica oleracea* var. *capitata* L. when produced under the same environmental conditions as oilseed rape. In continental climates, oilseed rape is sown in the last ten days of August and the first ten days of September. The transformation of the vegetative into the reproductive meristem in winter oilseed rape is regulated by the vernalization pathway, which is controlled by the expression of the floral integrator gene FT and the repressor gene FLC. This process has been studied in detail in *Arabidopsis thaliana* L. (Burn et al., 1993; Clarke and Dean 1994; Johanson et al., 2000; Kooerneef et al., 1998, Kooerneef et al., 2004; Lee et al., 1993; Michaels et al., 2004; Poduska et al., 2003; Zhang and Nocker, 2002). The most important regulators of this flowering pathway are two genes: FRIGIDA (FRI) and FLC. FRI encodes a nuclear protein that is present only in plants (Johanson et al., 2000, Akter et al., 2021) and increases FLC expression (Michaels and Amasino, 1999; Michaels and Amasino, 2001). Species whose flowering is conditioned by the vernalization process maintain a high level of FLC expression, which strongly delays the expression of the key flowering genes SOC1 (Suppressor of Overexpression of Constans) and FT (Flowering locus T). FLC loses its repressive effect with prolonged exposure to low positive temperatures. Decreased expression is associated with chromatin modifications at the FLC locus under different epigenetic control mechanisms (Kim et al, 2009; Bastow et al., 2004; Sung and Amasino, 2004).

The main characteristic of all biennial species, including biennial species of the genus Brassica, is resistance to winter temperature conditions, overwintering with the aim of biological survival (Kacperska-Palaz, 1987; Palta 1992). The overwintering trait of plants is a common trait of all biennial species of the genus Brassica (Osborn et al., 1997), and is also present in other wintering species (wheat, barley) (Hayes et al., 1993; Pan et al., 1994; Galiba et al., 1995; Storlie et al., 1998). Overwintering and flowering traits are quantitative traits of a group of genes with dominant activity (Kole et al., 2002). Plants in the phenophase of the head are sensitive to low negative temperatures, and this phenophase is a limiting factor in the seed production of cabbage heads. Conditions necessary for the formation of the head are a long day and a higher temperature; when the day is short and the temperature is low positive, the plant in the phenophase of the rosette enters a special physiological state whose goal is survival during winter. During this period, the development of the vegetative phase stops, because FLC stops expressing and at the same time induces flower genes, which is manifested by the appearance of reproductive organs in the meristematic tissue. Thus, the plant without the formation of the head passes from the vegetative to the reproductive stage, and during March the growth of flowering stems is noticed in the plants.

The cost-effective production of head cabbage seeds using an autonomous flowering pathway (which induces flowering in the second year of plant life) is possible only in the Mediterranean area of Europe, where there is no long period of low negative temperatures. An additional difficulty, which causes costs in this way of seed production, lies in removing the leaves of the head from the flowering stem, which often suffers from wet rot (*Erwinia* sp.) after the winter period and which allows the development of the cabbage fly (*Phorbia brassicaceae*) in the flowering stem.

Plants under gibberellin acid (GA_3) treatment can bloom in a special flowering pathway – the gibberellin pathway (*in vitro*) (Konig and Combrink, 2002). In this study, gibberellin treatment was applied to overwintering plants (*in vivo*) in order to examine its effect on other traits.

The advantages of cabbage seed production by using vernalization are economically justified: the first reason is that such a method of production reduces the length of the vegetation period by about 60 days; the second reason is that there are no costs for removing the rotten leaves of the head in the spring when flowering stems sprout; the third reason is that weather conditions favor the growth and development of the plant with reduced pesticide use.

2. Materials and methods

2.1. Materials

Genotypes divergent based on geographical origin were selected for the experiment, including two late genotypes from medium long day conditions, viz. the genotype Scc with a vegetation period of 125 days from sowing, and the genotype B, with a vegetation period of 135 days from sowing, and one early genotype – N, originating from the conditions of a shorter and colder day, with a vegetation period of 90 days from sowing. During 2010, 3 hybrids (Scc x B, Scc x N, B x N) were selected by the diallel crossing of these three genotypes, which were used to examine heterosis. The experiment was set up in 4 replicates in the open field of the Institute of Vegetable Crops in Smederevska Palanka. Four factors were observed: genotype, sowing date, treatment and year. All 6 genotypes were included in the experiment. The experiment was performed in three different temperature seasons: 2010/2011, 2011/2012, 2012/2013. The first season (2010/2011) was characterized by average daily temperatures that were within the long-term average for the region, the second season (2011/2012) was cold, unfavorable in the winter months (a minimum temperature of -28.4 ° C was on Feb 9th, 2012), while in the third season (2012/2013) the average daily temperature was above the long-term average for January and February, which had a positive effect on all observed parameters (RHMSS), Fig. 2. In order to examine the date of sowing for seed yield, three sowing dates were selected: August 15th, September 1st, and September 15th (Mirecki, 2005). A total of 1440 plants were planted, half of which were treated with gibberellic acid and half of the plants were planted as controls. The treated plants were 3m away from the control plants. The treatment of plants with GA_3 (concentration = 300 ppm) was carried out during winter, in the first ten days of December and in the first ten days of February (Mobin et al., 2007). The vegetation space of the plant was 70x50 cm or 28500 plants per hectare. Plants were transplanted on October 20th, in all three seasons.

2.1.1. Methods, equipment and statistical analysis

Overwintering of plants in the experiment was calculated as the ratio of transplanted and surviving plants, and was presented as a percentage. Vernalization was expressed as the percentage of flowering plants out of the total number of plants planted in October (Adžić et al., 2012). Seed yield was calculated by multiplying the average seed weight per plant by the corrected number of plants from the initial density of 28,500 plants per ha, and correction based on the percentage of vernalized plants.

In order to determine statistically significant differences between genotypes and their hybrids as well as other factors (season, genotype, sowing dates, and gibberellin treatment), analysis of variance (ANOVA) was used, and LSD test was used to test the significance of differences between environments. Since ANOVA does not provide a detailed analysis of the genotype-environment interaction – GxE (Zobel et al., 1998; Mahalingam et al., 2006), we used AMMI analysis (Additive Main Effects and Multiplicative Interaction Models) to describe the GxE interaction (Gauch, 1988). AMMI analysis recalculated the values of the major components of genotypes and environments that represent the GxE interaction (Naveed et al., 2007).

In order to prove the qualitative presence of FLC loci in *Brassica oleracea* var. *capitata* L. (BoFLC 2), m-RNA isolation was performed in the material selected for the research and its qualitative demonstration. To isolate RNA from cabbage leaves, the "GeneJET RNA Purification Kit" (ThermoScientific) was used, according to the manufacturer's instructions. The total RNA was eluted with 40 µlRNAse-free water and the quality and concentration were determined spectrophotometrically (a NanoVue spectrophotometer, GE Healthcare Life Sciences). The removal of gDNA from RNA isolates was performed using the "DNA-free DNAse Treatment and Removal kit" (Ambion, according to the manufacturer's instructions). For first cDNA chain synthesis, 1µg of total RNA was incubated with 0.2 µg Random hexamer primer (ThermoScientific) for 5 min. at 65ºC in a final volume of 12 µl. The primers used in the PCR reactions were designed in the Primer3Plus program (http://example3plus.com/cgi-bin/dev/example3plus.cgi), based on the partial cDNA sequence BoFLC2 from the database (Acc. NoDQ222850) and the 26S gene ribosomal RNA. All PCR reactions were performed in triplicate and with a control that did not retain the matrix. The accumulation of PCR products was detected in real time and analyzed within the 7500 System Software (Applied Biosystems), which determined the efficiencies of the primers used, as well as standard errors.

3. Results and discussions

3.1. Overwintering

The highest percentage of overwintering of 100% was found in the hybrids Scc x N in the warm season (2012/2013) in the third sowing period. The treatment with GA_3 had a negative effect on the percentage of overwintering in all cabbage genotypes, except for Scc x B hybrids, when the use of GA_3 contributed to an increase in the percentage of overwintering plants in the second sowing period in the second (2011/2012) and third (2012/2013) season. Konig and Combrink (2002) reached similar results in their research in 2002, and found that there was no interaction between GA_3 treatment and chicory variety.

The analysis of sowing dates within the season showed statistical significance ($P < 0.05$) for the second sowing period (September 1st) in the second season (2011/2012), in the control of Scc genotype, in relation to the third sowing period (September 15th). Based on the LSD test, the percentage of overwintering in genotype N in the first season (2010/2011) in the third sowing period (September 15th) was statistically significant ($P < 0.01$) compared to the first sowing period. This indicated a direct influence of climatic factors on this trait. The effect of air temperature on plant phenology was reported in another study (Kudo et al., 2004).

For hybrids Scc x N, the percentage of overwintering in the first season (2010/2011) of the second sowing period (September 1st was statistically significant ($P < 0.01$) compared to the other dates; in the second season (2011/2012) the second sowing date had a statistically significantly higher percentage of overwintering plants ($P < 0.01$) than the first sowing date (August 15th). Overwintering of plants in relation to sowing dates in different seasons cannot be related to the statement that the oldest plants always have the best overwintering, but also that plants with a low amount of vegetative biomass are not suitable for overwintering. The results for the overwintering trait showed that season was the decisive factor, followed by genotype, as was confirmed by the results of the AMMI analysis.

Based on the results of the AMMI analysis shown in Fig. 1, the highest stability was observed in the hybrids B x N, and the lowest in the hybrids Scc x N, where the genotype x environment interaction was most pronounced. The influence of year on the stability of the overwintering percentage was pronounced, and genotypes were clearly distributed according to the seasons in which the variability of their interaction with the external environment was the smallest. The application of gibberellin treatment in many situations increases the stability of genotypes in relation to control (normal conditions without the use of GA_3).

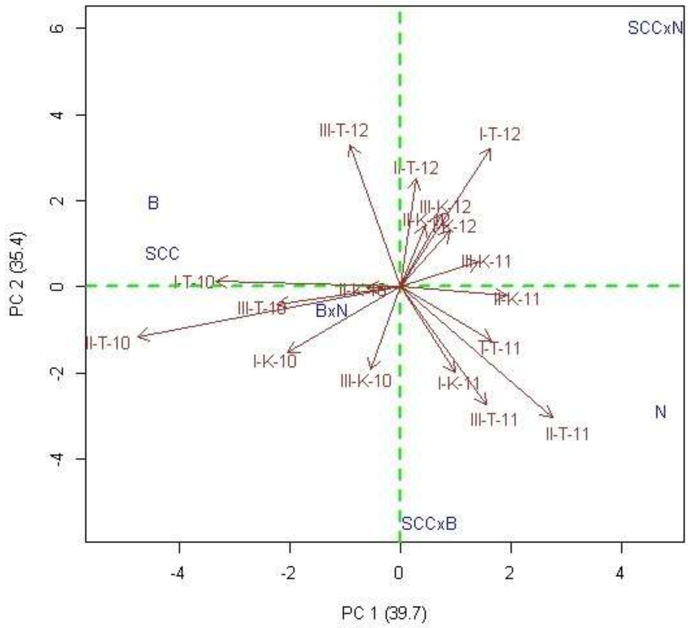


Fig. 1. AMMI 2 biplot for 6 cabbage genotypes

Legend: Sowing dates: I – August 15th; II – September 1st; III – September 15th; T – treatment with gibberellin – GA3; K – control variant; Research years: 10 – 2010/2011, 11 – 2011/2012, 12 – 2012/2013; Parental genotypes: Scc, N, B; F1 hybrids: Scc x N, Scc x B, B x N.

Genotypes were grouped into 3 groups according to the values of the first main component and the average values of the overwintering percentage. The parent Scc, as well as the hybrid B x N, were characterized by a negative value of PC1 and average values of the overwintering percentage (with Scc being slightly below and B x N slightly above the total average), Fig. 3. The genotype N and the hybrid Scc x N had positive values of PC1 and average values lower than the total average, while the hybrid Scc x B stood out in a special group with low positive values of PC1 and average values of the overwintering percentage above the value of the total average.

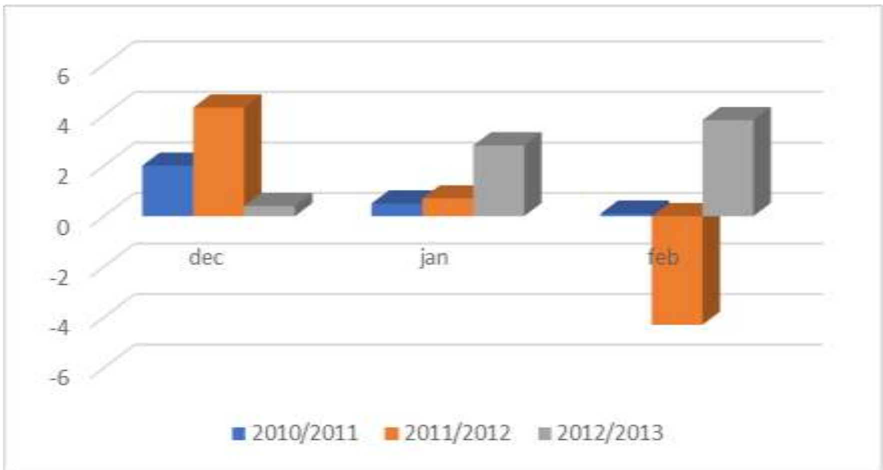


Fig. 2. Average value of air temperatures in December, January and February in the seasons: 2010/2011, 2011/2012, 2012/2013

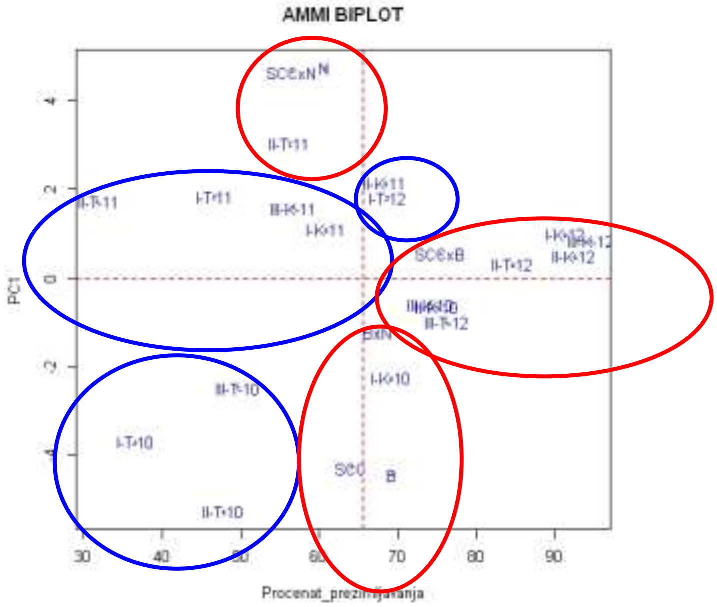


Fig. 3. AMMI 1 biplot for 6 cabbage genotypes

Legend: Sowing dates: I – August 15th; II – September 1st; III – September 15th; T – treatment with gibberellin – GA3; K – control variant; Research years: 10 – 2010/2011, 11 – 2011/2012, 12 – 2012/2013; Parental genotypes: Scc, N, B; F1 hybrids: Scc x N, Scc x B, B x N.

3.2. Percentage of vernalized plants

The highest percentage of vernalized plants occurred in the Scc x B hybrid in the control, and was 82.5% in the first season (2010/2011) of the experiment at the second (September 1st) and third sowing dates (September 15th) as well as in the third season (2012/2013) at the first sowing date (August 15th). In the conditions of the cold season (2011/2012), it showed resistance to winter and gave a very high percentage of vernalized plants in the first and second sowing period, 72.5% and 77.5%, respectively. Deviations in all sowing dates except the third date (September 15th) of the season 2011/2012 were not statistically significant. In the season 2011/2012, a lower percentage of vernalization was expressed at all sowing dates and in all genotypes, except for the hybrid Scc x B. Also, the influence of the cold weather factor on vernalized plants was confirmed by Martinez-Zapater et Somerville (1990); Johnson (2011); Sandile Manzi Ngwenya (2016).

The higher percentage of vernalized plants in the early genotype N, in the control, was influenced by the later sowing date (September 15th) in all seasons: 2010/2011 – 65.0%; 2011/2012 – 72.5%; 2012/13 – 52.5%. The late genotype B in the control in more stable years (seasons 2010/2011 and 2012/2013) responded favorably to earlier sowing, with the percentage of vernalized plants of 70.0% and 72.5%, respectively, which indicated that sowing dates statistically significantly affected the vernalization percentage. It proved to be resistant to prolonged exposure to low temperatures in the second test season (2011/2012). The influence of genotype on vernalized plants was confirmed by Zhiyuan et al. (2000).

The analysis of the values of the percentage of vernalized plants in the third, warm (2012/2013), season of the experiment in the control revealed stabilization of the percentage of vernalized plants in all hybrid combinations at all sowing dates. The value of the percentage of vernalized plants across sowing dates was not statistically significant.

Based on the results of the AMMI analysis, Fig. 4, the genotype B x N exhibited the greatest stability, i.e. the lowest level of interaction between genotypes and the external environment (year, sowing date, control and treatment), especially in the second (cold) season (2011/2012) in early and medium early sowing periods.

The lowest stability was observed in the early parent B, where the genotype x environment interaction was highest. The influence of years was very pronounced; therefore, genotypes were clearly distributed across seasons in which the variability of their interaction with the external environment was the smallest: genotypes Scc, Scc x B and B, first – moderately cold season; genotypes N and Scc x N, third – warm season; hybrid B x N, second – cold season.

Genotypes were grouped into 4 groups according to the values of the first main component and the average values of the percentage of vernalization, graph 5. The parents B and N, as well as their hybrid B x N were characterized by average values of the percentage of vernalization, N had a high positive value and B x N had an average PC1 value, while B had a low negative PC1 value. The parent N and the hybrid Scc x N were characterized by positive PC1 values and average values lower than the total average, although genotype N was much closer to the total average trait. The Scc x B hybrid was singled out in a separate group with mean negative PC1 values and average vernalization percentage values above the total average value. A special group included the late genotype Scc, which had similar PC1 values as the hybrid Scc x B (medium, negative), but the average values of vernalization were lower than the total average.

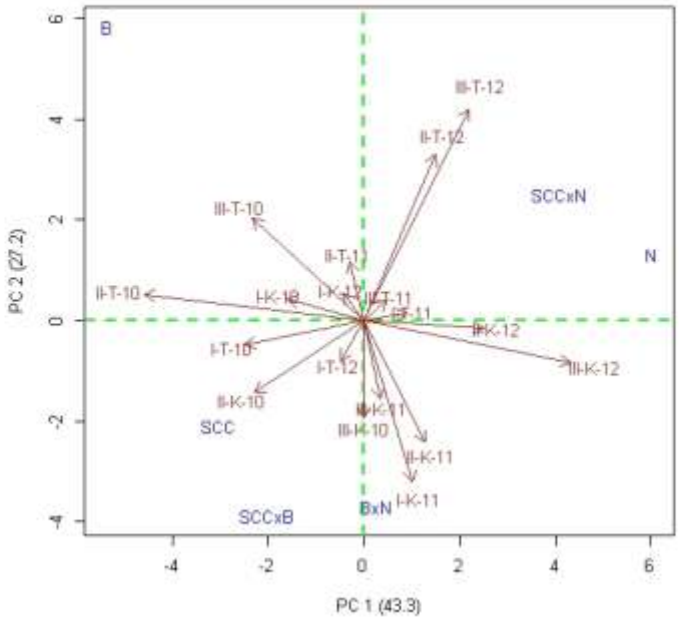


Fig. 4. AMMI 2 biplot for 6 cabbage genotypes

Legend: Sowing dates: I – August 15th; II – September 1st; III – September 15th; T – treatment with gibberellin – GA3; K – control variant; Research years: 10 – 2010/2011, 11 – 2011/2012, 12 -2012/2013; Parental genotypes: Scc, N, B; F1 hybrids: Scc x N, Scc x B, B x N.

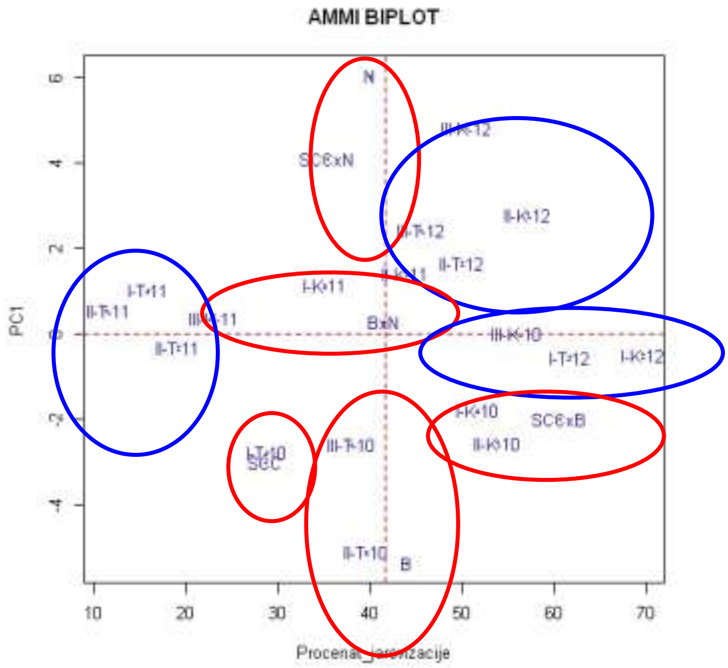


Fig. 5. AMMI 1 biplot for 6 cabbage genotypes

Legend: Sowing dates: I – August 15th; II – September 1st; III – September 15th; T – treatment with gibberellin – GA_3; K – control variant; Research years: 10 – 2010/2011, 11 – 2011/2012, 12 –2012/2013; Parental genotypes: Scc, N, B; F1 hybrids: SccxN, SccxB,

3.3. Seed yield

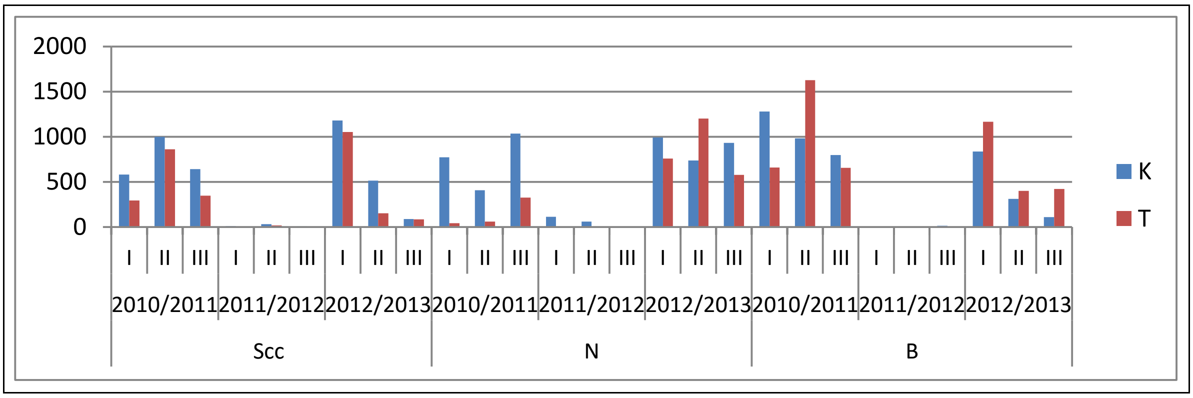
Across research seasons, sowing dates and genotypes, in general, the highest yield was achieved in the third season (2012/2013), which was characterized by average daily temperatures higher than the long-term average. Also, in the cold season (2011/2012), there was a statistically significant advantage of older plants or the earliest sowing date (August 15th). In the moderately cold season (2010/2011), the second sowing period gave a slightly higher seed yield, Figs. 6 and 7. The high influence of climatic conditions on seed yield was also confirmed by Kumar et al. (2009) and Baghdadi et al. (2012).

The highest Hr value was calculated for the late Scc x B hybrid 189%, Table 1. Heterosis significantly affected seed yield in the cold season (2010/11), in which hybrid plants gave seed yields two to thirty times higher than the parental genotypes.

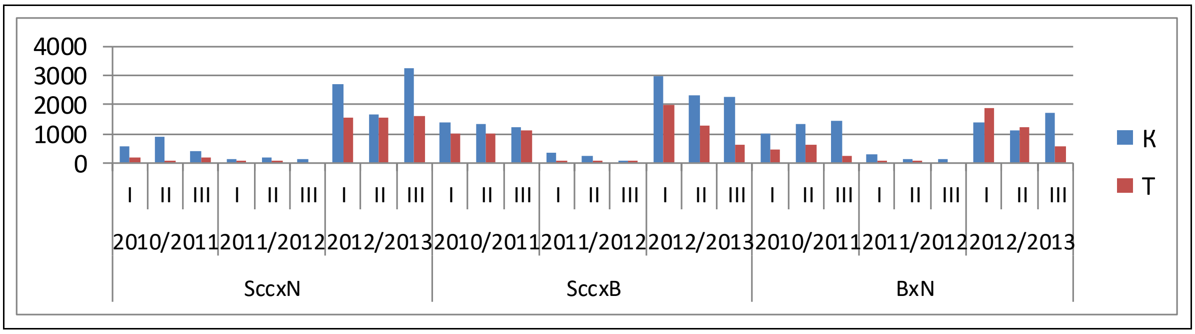
The LSD test determined the significance of the effect of GA_3 treatment in relation to the control of seed yield, Figs. 6 and 7. GA_3 treatment had a generally negative effect on seed yield compared to control results. In some situations, an increase in yield under the influence of GA_3 treatment was shown compared to the control, and therefore no definitive conclusion can be drawn about the negative effect of the treatment on seed yield.

Graph 1 shows the highest stability in the hybrids B x N, the lowest level of interaction between genotypes and the environment (year, sowing date, control and treatment), especially in the second (cold) season (2011/2012) at the I and II sowing dates of the control and III sowing date in the control in the first season (2010/2011). In this hybrid, the grouping of many environments is noticeable, which also testifies to its stability.

The influence of years was very pronounced; therefore, genotypes were clearly distributed across seasons in which the variability of their interaction with the external environment was the smallest; genotypes: Scc x N, Scc x B, and N, third – warm season; Scc and B in the moderately cold season; while the hybrid B x N was stable in the moderately cold and cold season. The high dependence between climatic factors and the seed yield of cabbage was confirmed by Kudo et al. (2004), Kumar et al. (2009) and Johnson (2011).



Graph 1. Seed yield (kg ha^{-1}) of parental genotypes across sowing dates (I – August 15th; II – September 1st; III – September 15th) and sowing seasons – the comparison of average values of plant weight in control (blue) and in GA_3 treatment (red)



Graph 2. Seed yield (kg ha^{-1}) of F1 hybrid genotypes across sowing dates (I – August 15th; II – September 1st; III – September 15th) and sowing seasons – the comparison of average values of plant weight in control (blue) and in GA_3 treatment (red)

Table 1.
Average values and heterosis for seed yield (kg ha^{-1})

*F*1 hybrid	Head weight (*kg*)				Heterosis (%)	
-	*P1*	*P2*	*MP*	*F1*	*Ha*	*Hr*
SccxN	451.00	562.00	506.50	1102.00	595.50	117.57
SccxB	451.00	481.00	466.00	1348.00	882.00	189.27
BxN	481.00	562.00	521.50	963.00	441.50	84.66

P1, P2 – mean parental values for seed yield, MP – mean value of sum (mean values) of seed yield, F1 – seed yield in hybrids, Ha – absolute heterosis, Hr – relative heterosis

Genotypes were grouped into 5 groups, Fig. 7, according to the values of the first main component and the average values of seed yield. The Scc x B hybrid stood out from all the others and had a yield above the total average and a positive PC1 value. Interestingly, heterosis, Table 1, was present in all hybrids for seed yield traits, clearly separated parents from hybrids in terms of the relationship with the average value, which was higher in hybrids than the total average value, and they were also characterized by positive PC1 values (unlike parents, which had lower-than-average values of the trait and negative PC1 values).

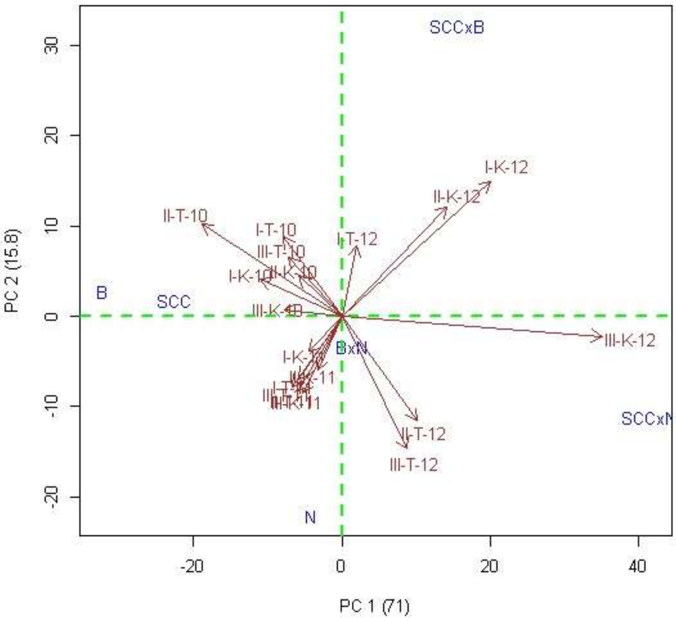


Fig. 6. AMMI 2 biplot for 6 cabbage genotypes

Legend: Sowing dates: I – August 15th; II -–September 1st; III – September 15th; T – treatment with gibberellin – GA_3; K – control variant; Research years: 10 – 2010, 11 – 2011, 12 – 2012; Parental genotypes: Scc, N, B; F1 hybrids: Scc x N, Scc x B, B x N.

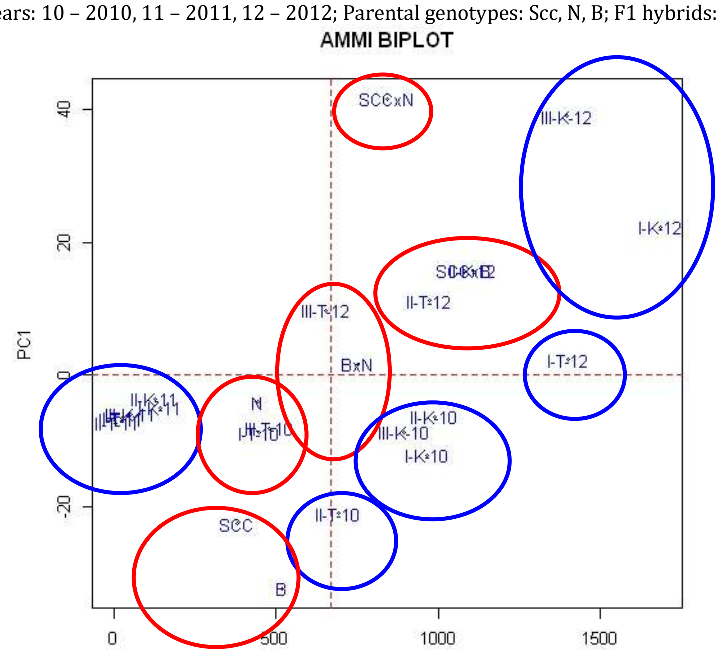


Fig. 7. AMMI 2 biplot for 6 cabbage genotypes

Legend: Sowing dates I – August 15th; II – September 1st; III – September 15th; T – treatment with gibberellin – GA_3; K – control variant; Research years: 10 – 2010, 11 – 2011, 12 – 2012; Parental genotypes: Scc, N, B; F1 hybrids: Scc x N, Scc x B, B x N.

3.4. Qualitative proof of the presence of BoFLC 2 repressors

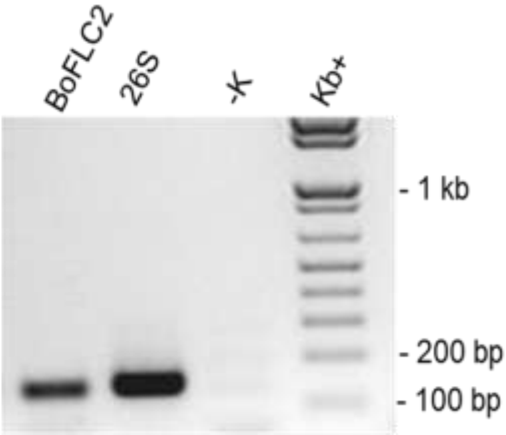


Fig. 8. Electrophoretic analysis of PCR reaction products

Performed with BoFLC2f / BoFLC2r and 26Sf / 26Sr primers on cDNA of genotype N as a template. -K is a template-free reaction (negative control). Arrows indicate the length of DNA marker fragments (Kb + Ladder, Invitrogen)

The primers designed for the expression analysis were first tested in a qualitative PCR reaction on cDNA synthesized on RNA from the genotype N. The electrophoretic analysis showed that both pairs of primers gave a unique product of the corresponding length of 150 bp, Fig. 8, which confirmed their specificity and homologous presence of the same gene as in *B. napus* L., which was also expressed in head cabbage in identical living conditions. As a negative control, a PCR reaction that did not contain a DNA template was used, which indicated that these primers did not make dimers.

4. Conclusions

The use of vernalization for the purpose of stable seed production is possible in continental climates, but it is noteworthy that the seasonal factor can be limiting in terms of the occurrence of extremely low negative temperatures that destroy the crop. The experiment proved the positive effect of heterosis in many traits, especially seed yield and overwintering, which was especially significant in seasons with high deviations from the long-term standard normal in terms of average daily temperatures.

Previous discoveries in the field of FLC gene expression regulation in oilseed rape have opened up the possibility of using an identical flower mechanism in cabbage in order to facilitate seed production in agro-ecological conditions of a continental climate.

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