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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Received 22 July 2022; Accepted 15 December 2022

ABSTRACT

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, 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 – GA3. The influence of all four factors: season, genotype, sowing date and GA3 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

ИЗВОД


1. Introduction

Oilseed rape (Brassica napus L.) is a biennial crop used to obtain oil from seeds, and the process of flowering is called the vernalization pathway.
AACC type, created from the genome: Brassica rapa L. genome – AA and Brassica oleracea L. genome – CC (Nagarahau, 1935; Parkin et al., 1995; Schiesl, 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; Sturlie 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 its function changes from a repressor to an activator (Michaels and Amasino, 1999; Michaels and Amasino, 2004). The expression of the repressor gene FLC in the reproductive phase is maintained by a complex of three repressor proteins (Koolen et al., 2005). These proteins are: FOS, FUS, and LEC (Koolen et al., 2005). FOS is encoded by the FLC gene, which is expressed in all tissues of the plant, and the function of FUS and LEC is to repress the expression of FLC, which is not expressed in the vegetative phase of the plant. The complex of these three proteins in the reproductive phase of the plant is responsible for the repression of FLC expression and the initiation of flowering. The formation of a head requires the repression of FLC expression, which is maintained by the complex of three repressor proteins (FOS, FUS, and LEC) (Koolen et al., 2005). The repression of FLC expression in the reproductive phase of the plant is maintained by the complex of three repressor proteins (FOS, FUS, and LEC). The repression of FLC expression in the reproductive phase of the plant is maintained by the complex of three repressor proteins (FOS, FUS, and LEC).

A total of 1440 plants was plated, 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 GA3 (concentration = 300 ppm) was carried out during winter, in the first ten days of December and in the first ten days of February (Mohin et al., 2007). The vegetative space of the plant was 70x50 cm or 28500 plants per hectare. Plants were transplanted on October 20th, in all three seasons.

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 (Sc 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 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 (Mirecz, 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 GA3 (concentration = 300 ppm) was carried out during winter, in the first ten days of December and in the first ten days of February (Mohin et al., 2007). The vegetative 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 sowing in September 15th P was statistically significant in their research, with similar results in their research (Konig and Combrink, 2002). The treatment, care Life Sciences). 

The primers used, as well as standard errors. Before the statistical analysis, 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 65°C 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 (Access. NoDQ222850) and the 26S gene ribosomal RNA. All PCR products were 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 Sc x N in the warm season (2012/2013) in the third sowing period. The treatment with GA3 had a negative effect on the percentage of overwintering in all cabbage genotypes, except for Sc x B hybrids, when the use of GA3 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 GA3 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 Sc 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 Sc 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 Sc 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 GA3).
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.
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 15th) and third sowing dates (September 15th) as well as 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.
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 GA₃ treatment in relation to the control of seed yield, Figs. 6 and 7. GA₃ treatment had a generally negative effect on seed yield compared to control results. In some situations, an increase in yield under the influence of GA₃ 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 (2011/12) at the I and II sowing dates of the control and III sowing date in the control in the first season (2010/11). 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 GA₃ 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 GA₃ treatment (red)

**Table 1.**

<table>
<thead>
<tr>
<th>F1 hybrid</th>
<th>Head weight (kg)</th>
<th>Heterosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P1</td>
<td>P2</td>
</tr>
<tr>
<td>SccxN</td>
<td>451.00</td>
<td>562.00</td>
</tr>
<tr>
<td>SccxB</td>
<td>451.00</td>
<td>481.00</td>
</tr>
<tr>
<td>BxN</td>
<td>481.00</td>
<td>562.00</td>
</tr>
</tbody>
</table>

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 – GA3; 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 – GA3; 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 26S F / 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 from 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.

Acknowledgment

This research was conducted with the support of the Ministry of Education, Science and Technological Development of the Republic of Serbia (contract number: 451-03-68 / 2022-14 / 200216).

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