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Original scientific article

INFLUENCE OF DIFFERENT ENVIRONMENTAL CONDITIONS AND GIBERELLIC ACID TREATMENT ON FLOWERING TIME OF DIVERGENT GENOTYPES OF CABBAGE (*Brassica oleracea* var. *capitata* L.) AND THEIR F1 HYBRIDS

Slađan ADŽIĆ*, Zdenka GIREK, Suzana PAVLOVIĆ, Bogoljub ZEČEVIĆ, Jelena DAMNJANOVIĆ, Dejan CVIKIĆ, Milan UGRINOVIĆ

Institute for Vegetable Crops, Smederevska Palanka, Serbia

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In the process of cabbage breeding and seed production, one of the most important characteristics is the time of flowering. In order to investigate the influence of genotype, season and term of sowing on the flowering process, as well as the genetic control of this trait, an experiment was conducted with three genotypes of cabbage N, B and Scc of different geographical origin and different vegetation length, as well as three new F1 hybrids N x Scc , B x Scc and B x N obtained by hybridization between genotypes. The experiment was conducted during three temperature different seasons S1, S2 and S3 (average cold, cold and warm seasons), in three different sowing terms: August 15 (I), September 1 (II) and September 15 (III). During the winter period favorable for vernalization, two treatments with 300 ppm GA_3 were performed. A statistically significant influence of all examined factors: genotype, year, sowing term and gibberellin GA_3 treatment, on flowering time was determined. AMMI analysis determined the degree of adaptability of genotypes depending on the growing season,

Corresponding author: Slađan Adžić, Institute for Vegetable Crops, Karadjordjeva 71, 11420 Smederevska Palanka, Serbia,e-mail: sladjan.adzic@gmail.com, Phone: +381 26 317170, +381 64 8834 413

sowing terms and GA_3 treatment for the trait time of flowering. The highest stability based on ASV values for flowering time was shown by hybrid BxN and its parental component genotypes B and N, while the hybrid Scc x B proved to be the most unstable in terms of flowering time. The pattern of relative expression of the most important flower repressor *BoFLC2* gene showed a certain correlation with the flowering time of genotypes. The lowest quantitative expression of this gene was found in genotype B and it had the earliest flowering in all seasons, while genotype N had the highest relative expression of the *BoFLC2* locus and the latest flowering.
Keywords: cabbage, time of flowering, gibberellic acid, expression *BoFLC2* gene

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata* L.) is one of the most economically important vegetable species in the world. Cabbage contain high amounts of vitamins C, K, A, B1, B2, PP, E and folic acid, dietary fiber, flavonoids, proteins, and minerals. Because of good nutritional value and antioxidant and anti-inflammatory properties, it is consumed worldwide in the human diet. Breeding and seed production are complicated because of their two-year head-seed-head cycle.

The main feature of morphological diversity within the genus *Brassica* is the variation of flowering time (SCHRANZ *et al.,* 2002). Flowering time is a particularly important agronomic trait in *Brassica* crops and regulation of the time is one of major factor in the breeding program and seed production. The process of transformation of vegetative into flower meristem in cabbage depends on integration of endogenous factors such as leaf number and gibberellin (GA) biosynthesis (BERNIER and PERILLEUX, 2005; MUTASA-GÖTTGENS and HEDDEN, 2009) and environmental cues such as vernalization, temperature, and photoperiod (ALEXANDRE and HENNIG, 2008; ANDRÉS and COUPLAND, 2012; KUMAR *et al*., 2012).

In the continental climate, the vernalization path of flowering is used for the economical seed production. Activation of the vernalization flowering path requires exposure of plants to low positive temperatures of 5-8˚C for several weeks and is a typical flowering path for overwintering plant species (genera *Brassica, Triticum, Hordeum*...). The vernalization path of flowering is controlled by expression of the floral inducers *FT* (*Flowering locus T*) i *FLC* (*Flowering Locus C*) repressor gene (JOHANSON *et al.,* 2000; ZHANG and NOCKER, 2002; MICHAELS *et al.,* 2004; PODUSKA *et al.,* 2003; RIDGE *et al.,* 2015). Vernalization, reduces the expression of *FLC* genes whose main role is to delay flowering (HELLIWELL *et al.,* 2015). Plants with high *FLC* activity are late flowering because *FLC* directly represses the expression of the floral inducers *FT* and *suppressor of overexpression of CO 1* (*SOC1*) (HELLIWELL *et al.* 2006). A genetic-genomics approach revealed that *BrFLC2* is a major regulator of flowering time in *B. rapa* (ZHAO *et al.,* 2010; XIAO *et al.,* 2013, 2014). Recently, LI *et al.* (2016) reported that mutation in *FLC1* and *FLC2* leading to production of truncated proteins was associated with shortness of flowering time in *B. rapa.*

In conditions when the day is short and the temperature is low positive, the plant in the in rosette stage of development enters a special physiological state whose goal is to survive during the winter. Plants in such conditions record an increase in the levels of endogenous gibberellins that trigger flower induction at the level of the meristem (HILLMAN, 1969). In that way, the plants

pass from the vegetative to the reproductive stage without forming a head, and during March they create flowering trees.

Exogenous application of gibberellic acid (GA_3) can cause flowering of plants under vernalization conditions (LUO *et al.,* 2013). YAMAGUCHI *et al.* (2014) found that the use of exogenous GA_3 leads to cessation of vegetative development and inhibition of flower formation in *Arabidospis thaliana* L. Some studies indicate that plants that have not been exposed to low temperatures, and have been exposed to GA_3 treatment *in vitro,* can enter the flowering phase by an independent genetic mechanism of flowering - the gibberellin pathway (KONIG and COMBRINK, 2002).

In this study, GA_3 treatment was applied to plants that overwintered at different temperature conditions to examine its effect on flowering time and *BoFLC2* repressor expression. The application of GA_3 was aimed at confirming the hypothesis that it directly affects the interruption of vegetative development and the occurrence of flowering, as well as confirmation that the flowering time is a highly variable property in different temperature conditions.

MATERIAL AND METHODS

Plant material

Cabbage (*Brassica oleracea* var. *capitata* L.), six different genotypes from the Institute for Vegetable Crops in Smederevska Palanka, Serbia were used in this study. Three genotypes: N, B and Scc of different geographical origin (Pomoravlja, Semberije and europic part of Russia respectively) and different lengths of the vegetation period used in the experiment were provided from the collection of the Institute for Vegetable Crops, Smederevska Palanka. B and Scc are late genotypes from the condition of medium long day and the length of the vegetation period of 125 and 135 days from sowing respectively, while the N is early genotype from the conditions of shorter and colder days, with a length of the vegetation period of 90 days from sowing. Three new F_1 hybrids: N x Scc, B x Scc and N x B were obtained by hybridization (hand pollination) between genotypes. Hybridized mothers were protected with linen isolators to the appearance of the siliques. Harvesting of parental genotypes and F_1 hybrids was carried out successively, as the seeds matured in the siliques. After harvest, the seeds were matured for a period of 45 days.

Estimation of flowering time

The experiment was performed during three temperature-different seasons: 2010/11 (S1), 2011/12 (S2) and 2012/13 (S3). The first season (S1) was characterized by the average daily temperatures that were within the yearly average for the region, the second season (S2) was cold, unfavorable in the winter months (a minimum temperature of -28.4 °C was recorded in mid-February) and in the third season of research (S3) average daily temperature was above the yearly average for January and February which reflected positively on all of the observed parameters (Hydrometeorological Service of Republic Serbia - RHMZS, 2014) (Figure 1).

Sowing was done in three terms: August 15 (I), September 1 (II), September 15 (III), to achieve different ages of seedlings (different biomass). Due to the observed dormancy of seeds in the cabbage, 48 hours before sowing seeds was kept at + 4°C in the fridge. Sowing was carried out in individual pots of 11 cm diameter, filled with sterile substrate, and kept in a protected area. The seedling was maintained in a common manner, i.e. every 15 days fertilized

with NPK 20:20:20 (25g/10l of water) and treated with pesticides as needed. Planting was carried out on October 20 in all three seasons. The plot size was 10 m^2, row-to-row distance was 50 cm and plant-to-plant distance was 70 cm. In each season, 240 plants of each genotype were planted, 80 plants from each term of sowing. In the first decade of December and in the first decade of February, two foliar treatments with 300 ppm gibberellin (GA_3) were performed.

During the experiment, the flowering time was monitored and measured in two ways: 1.) the number of days from the day of sowing and 2.) the number of days from January 1 to the appearance of the flower.

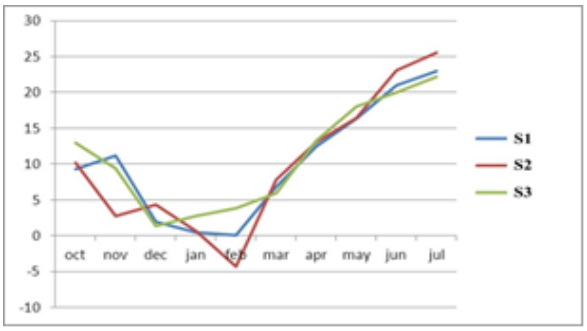


Figure 1. Average daily temperature during the months of three different seasons of experiment (Hydrometeorological Service of Republic Serbia - RHMZS, 2014)

Analysis of BoFLC2 gene expression

The level of *BoFLC2* expression was quantitative determined in all six genotypes using qRT-PCR. The material for the isolation of RNA was taken during the non-inducing vernalization period (mid-November), and during the vernalization period (in December and February 4 weeks after each GA_3 treatment).Total RNA from 100 mg plants leaf tissue was extracted using the GeneJET RNA Purification Kit (Thermo Scientific). After isolated total RNA was quantified spectrophotometric (NanoVue spektrophotometer GE Healthcare Life Sciences), and DNase treatment was performed using the DNA-free DNAse Treatment and Removal kit (Ambion). 1µg of total RNA was subsequently reverse-transcribed into cDNA for each sample using the GeneAmp® RNA PCR Gold Core Kit (Applied Biosystems).

For qPCR amplification in the 7500 Real-Time System (Applied Biosystem), each reaction was set up using 20 ng of cDNA with Maxima™ SYBR Green/ROX qPCR Master Mix (Thermo Scientific) and 0.2 µM of each primer. *BoFLC2* specific primers were designed using Primer3Plus program (http://primer3plus.com/cgi-bin/dev/primer3plus.cgi), based on the partial cDNA sequence *BoFLC2* from the database (Acc. No. DQ222850) (BoFLC2f: 5'-AGAGCTTGTCGAAAGTAAGCTTGT-3' and BoFLC2r: 5'-CCTTTTCTTTGAGGCTATCAAACA-3'). The *26S* ribosomal gene was also amplified as an internal control (26Sf: 5'-ATTCCCAAACAACCCGACTC -3' and 26Sr: 5'-GCCGTCCGAATTGTAGTCTG-3'). The qPCR reaction conditions implied 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec, 60°C for 30 sec, 76°C for 1 min. Relative expression is shown as 2^-dCt, dCt (Treshold cycle) - difference of target gene (*BoFLC2*) and internal control

(gene for *26S* RNA). Three technical replicates were performed for each reaction and with non-template control.

Statistical analysis

The trials were conducted in the Randomized Complete Block Design (RCBD) with 4 replications. The influence of 4 factors on the measured parameters was observed: genotype, sowing term, GA3 treatment and season. All data is statistically processed in StatSoft Inc. STATISTICA, version 8.0 (2007). Statistical data processing implied analysis of the variance of the four-factor experiment (ANOVA).

For description of the GxE interaction (GAUCH, 1988; GAUCH, 1992), additive main effect and multiplicative interaction (AMMI) analysis was used to calculate the values of the main components of genotypes and environments representing GxE interaction (NAVEED *et al*., 2007). AMMI1 biplot is comprised from the main effects shown on the abscissa and the first principal component shown on the ordinate, while AMMI2 biplot illustrates the first (PC1) and second (PC2) principal component ratio (GAUCH, 2006; GAUCH *et al*., 2008). To range the genotypes with respect to stability, AMMI stability values (ASV) were calculated (PURCHASE *et al.*, 2000) according to the formula:

$$\text{ASV} = \sqrt{\left[\frac{SSPC1}{SSPC2}(PC1\ value)\right]^2 [PC''\ value]^2}$$

SS = sum of the squares.

PC1 = the first major component.

PC2 = the second major component.

AMMI analysis was performed using the R software, version 3.1.2 (A Language and Environment, Copyright 2014).

RESULTS AND DISCUSSION

Analysis of variance showed highly significant variations of all four factors (year, genotype, GA3 treatment and sowing period) for both forms (number of days from sowing and number of days from 1 January) of the observed trait - time of flowering (Table 1).

Variability of weather conditions between seasons have emerged a larger share of the factor year in the total sum of squares, compared to the remaining three factors. However, the biggest variability was determined in interaction of three observed factors: genotype, year, and sowing date (15.01%, and 14.15% respectively).

Participation of sowing date in the total sum of squares is bigger (8.15%) when the flowering time is observed from sowing compared to the time of flowering observed from 1 January (2.22%). In the case of flowering time observed as number of days from sowing, the statistical significance of the third sowing period at the time of the appearance of the first flower was recorded. In all three seasons, for all genotypes whose sowing was carried out on 15 September was recorded earlier appearance of the first flowers in comparison to the other two sowing dates (15 August and 1 September). The earliest flowering occurred in late genotype B in the first season on the 202 day after sowing, and the latest 268 day after sowing in the early genotype N in the second year (Figure 2, 3 and 4).

Table 1. Four-way ANOVA for the appearance of the first flower (1 - number of days from sowing; 2 - number of days from 1 January)

Source of variations	*Number of days from sowing*			*Number of days from 1 January*		
	df	*SS (%)*	*MS*	*df*	*SS (%)*	*MS*
Blocks	3	0.01	44.97	3	0.01	9.47
Genotype (*A*)	5	5.06	17615.49**	5	7.77	6755.48**
Year (*B*)	2	10.95	95349.71**	2	9.00	19558.79**
GA_3 treatment (*C*)	1	6.08	105875.39**	1	7.51	32673.72**
Sowing date (*D*)	2	8.15	70932.13**	2	2.22	4818.91**
AB	10	8.97	15624.34**	10	11.22	4880.53**
AC	5	4.77	16628.20**	5	4.33	3765.25**
AD	10	7.38	12860.56**	10	6.82	2964.29**
BC	2	9.93	86434.09**	2	12.26	26646.96**
BD	4	2.98	12952.69**	4	3.07	3339.41**
CD	2	0.56	4874.63**	2	0.68	1470.67**
ABC	10	8.52	14832.87**	10	8.98	3904.27**
ABD	20	15.01	13069.02**	20	14.15	3076.61**
ACD	10	3.28	5703.84**	10	3.48	1513.52**
BCD	4	1.33	5793.58**	4	1.71	1855.88**
ABCD	20	6.55	5699.81**	20	6.49	1410.81**
Error	321	0.49	26.48	321	0.31	4.23

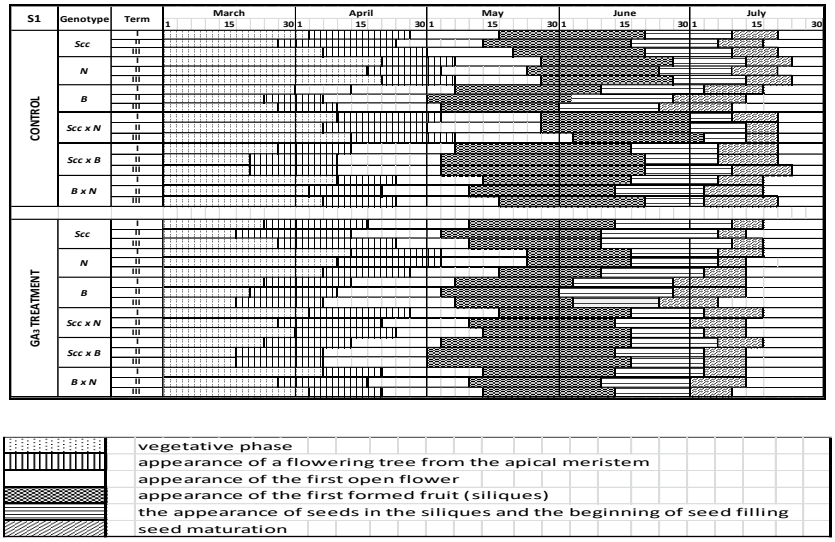


Figure 2. Duration of morphogenetic stages of the reproductive phase, in number of days, of the control and GA3 treatment in Season 1

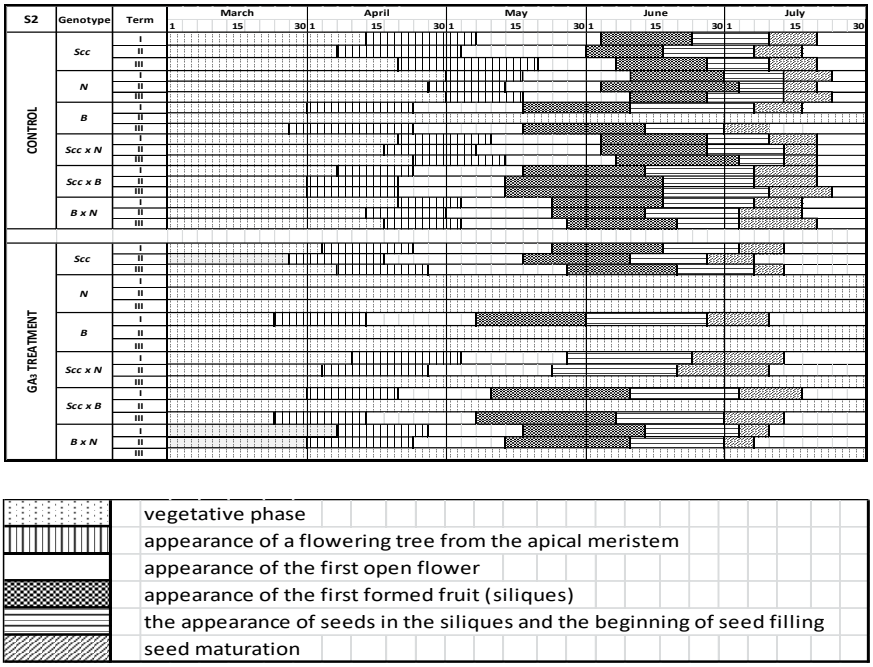


Figure 3. Duration of morphogenetic stages of the reproductive phase, in number of days, of the control and GA3 treatment in Season 2

Observing the late genotype Scc, for the same sowing dates but when different seasons are observed, was recorded the largest time differences in the period from sowing to the appearance of the first flower. The largest observed difference (27 days) in the time of appearance of the first flower in this genotype was recorded between the second and third season of research in the third sowing period. The least variation in flowering time was recorded in genotype B for the first and third examined seasons (Figure 2, 3 and 4).

For the cold season (S2) was observed later flowering in all sowing dates, 12 days later than the in the first season and 10 days later in relation to the third season. The biggest difference in the days until the beginning of flowering was noticed at the third sowing date, where this difference between the cold season (S2) and the other two was 13 days. The smallest difference was recorded in the second sowing period, where the difference in the number of days until the beginning of flowering between the cold season (S2) and the average cold season (S1) is 11 days and 8 days in relation to the warm season (S3) (Figure 2, 3 and 4). ADAMSEN and COFFELT (2005) determined the influence of different sowing times on the time of the appearance of the first flower in oilseed rape, while EDWARDS and MARTIN (2008) in their research on yield and time and length of flowering in oilseed rape found an average difference of 20 days between year in terms of the appearance of the first flower as well as 18 days of reduction of the flowering period in the second studied season when flowering started later. They also found that the time of

harvest did not varied significantly and did not depend of the factor year, which coincides with these studies. Statistically significant variation in the appearance of the first flower in the weather conditions of the three examined seasons is explained by the fact that the organogenesis of flowering is conditioned by environmental conditions and is a characteristic of genotype in the way of time required to perform it in physiological terms (KUMAR *et al*., 2009).

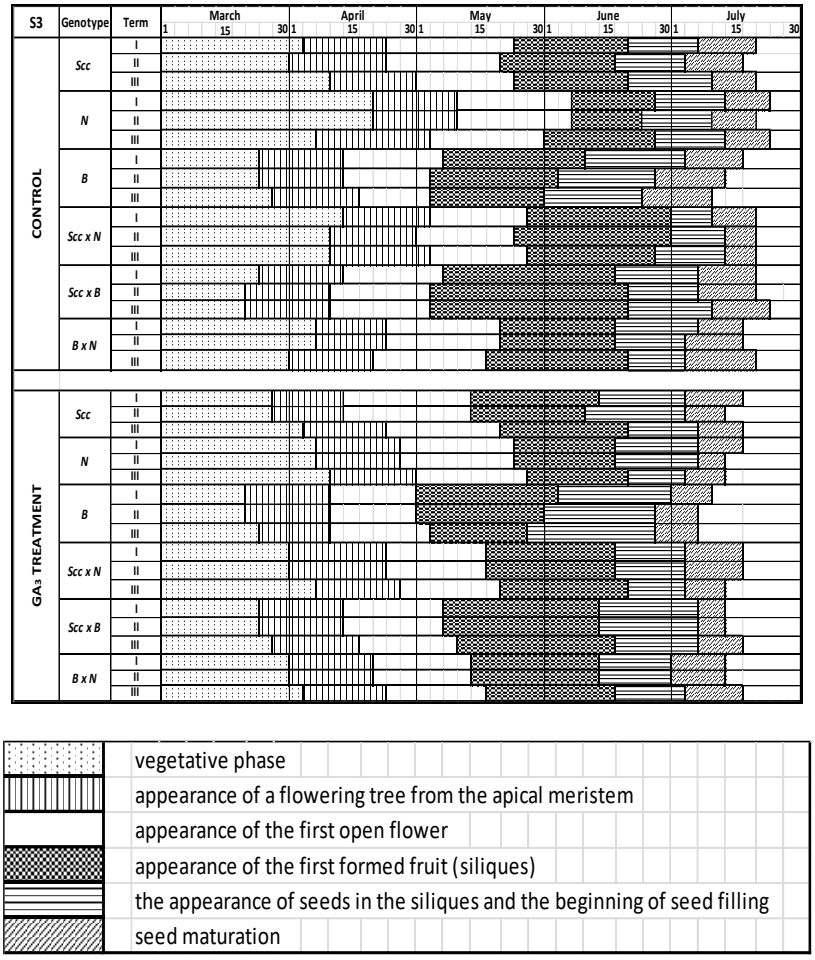


Figure 4. Duration of morphogenetic stages of the reproductive phase, in number of days, of the control and GA3 treatment in Season 3

Effect of the GA3 treatment in almost all the cases, where was find that was statistically significant as a factor, was to reduce the period of appearance of the first flower on the plant. compared to the control (GUAN *et al*., 2019; LINWATTANA *et al*., 1997). When observing its effect on the reduction of flowering time in relation to the length of the vegetation period (number of days after sowing) was recorded that, in eleven cases was statistically significantly reduced the time of appearance of the first flower compared to the control, and more than half of the cases occurred in the third season. Exogenous application of GA3 induced an earlier appearance of flowers compared to the control. Endogenous gibberellins are among the most important bioactive substances that are responsible for the start of flowering in plants that for this process require conditions of vernalization (REGNAULT *et al*., 2016). Analyzing the influence of GA3 treatment on the shortening of flowering time in the examined genotypes and their F_1 hybrids, compared to January 1, 25 statistically very significant changes in the decrease in the value of the tested trait were observed. A statistically significant increase of the value of the observed trait was found in the late genotype B in the first season and its hybrids (B x Scc and B x N) in the third season (Figure 2, 3 and 4). In the cold season (S2) the effect of exogenous gibberellin had the effect of interrupting vegetative development and the absence of flowering in all genotypes except Scc (Figure 2) (YAMAGUCHI *et al*., 2014).

Table 2. Inheritance of time of flowering in cabbage

F_1 hybrid	P_1	P_2	*MP*	F_1	$F_{0,05}$	$F_{0,01}$
Scc x B	121	104	113	104	-d	-d
B x N	104	127	116	116	i	i
Scc x N	121	127	124	125	i	i

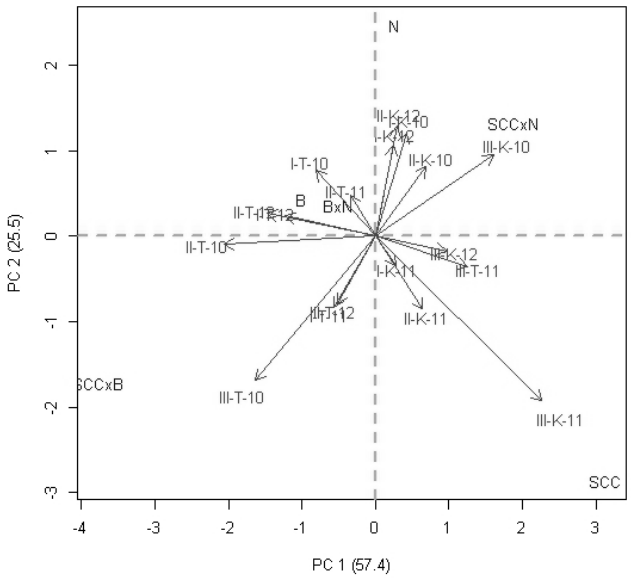
P_1, P_2 - mean values for parents during flowering, MP - mean value of sum (of mean values) of flowering time, F_1 - flowering time of hybrids

Table 3. Mean values, AMMI stability values and ranking order of stability for 6 cabbage genotypes

Number	Genotype	Trait		*PC1*	*PC2*	*ASV*	
		Mean	Rank			Value	Rank
1	Scc	239,51	3	3,1384	-2,8676	7,62	5
2	B	222,33	6	-1,0060	0,4299	2,30	2
3	N	244,64	1	0,2578	2,4642	2,53	3
4	Scc x B	224,71	5	-3,7720	-1,7166	8,65	6
5	B x N	233,64	4	-0,5053	0,3627	1,19	1
6	Scc x N	240,78	2	1,8871	1,3274	4,45	4

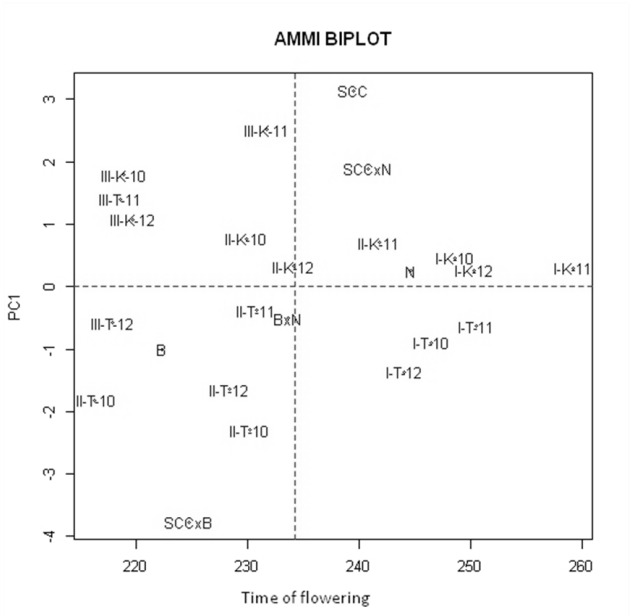
The mode of inheritance of flowering time for hybrids in which one of the parents is the early genotype N - Scc x N and B x N is an intermediate - additive. Earlier authors found that the time of flowering is a quantitative trait (JUNG *et al*., 2018; SHU *et al*., 2018; LIU *et al*., 2019). For hybrid created by crossing late parents Scc x B, it was determined that the flowering time is dominant (under the dominance of parent B; Table 2). With AMMI analysis, it was determined

that of all parental genotypes, genotype B had the most stable values of flowering time for all seasons (Figure 5), so based on all these, it can be justified to conclude that its dominance was also manifested in the hybrid Scc x B. The lowest stability was observed in hybrids Scc x B as well as in one of the parents, genotype Scc. In these two genotypes, the genotype - environment interaction is the highest, ie the stability coefficient of the AMMI model is the highest in rank (Table 3). The effect of year was expressed in the way that the genotypes were distributed throughout the seasons in which the variability of their interaction with the external environment was the smallest (except for the most stable genotypes: B, B x N).



Legend: Sowing dates: I - 15 August, II - 1 September, III - 15 September; T - treatment with gibberellin - GA3; K - control; Research years: 10 - 2010, 11 - 2011, 12 -2012; Parental genotypes: Scc, N, B; F_1 hybrids: SccxN, SccxB, BxN
Figure 5. AMMI 2 biplot for 6 cabbage genotypes

Analysis of *BoFLC2* gene expression in the non-inducing period for vernalization showed that the late genotype B had a lower level of expression compared to N and Scc (Figure 7). In late parent hybrids (B x Scc) the *BoFLC2* expression level is between the gene expression levels in the parents, while in the early parent hybrid N (N x Scc and N x B), the *BoFLC2* gene expression level is higher than in the parents. This pattern of expression is maintained during vernalization too (Figure 8).



Legend: Sowing dates: I - 15 August, II - 1 September, III - 15 September; T - treatment with gibberellin - GA3; K - control; Research years: 10 - 2010, 11 - 2011, 12 -2012; Parental genotypes: Scc, N, B; F_1 hybrids: SccxN, SccxB, BxN

Figure 6. AMMI 1 biplot for 6 cabbage genotypes

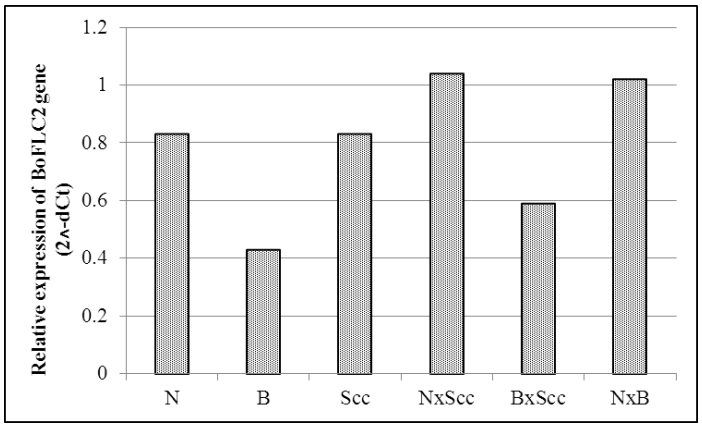


Figure 7. Relative expression of *BoFLC2* gene in parental (N, B and Scc) and hybrid genotypes (NxScc, BxScc, NxB) in the non-inducing period for vernalization.

AMMI biplot analysis showed that the average time of flowering for genotype B is the lowest compared to other genotypes, and that in genotypes Scc and N the mean values of time of flowering are higher than the average value, which coincides with the results of analysis of expression of this gene (Figure 6). Based on these data, it can be concluded that lower levels of FLC expression correlate with shorter time of flowering (SHELDON *et al*., 1999; MICHAELS and AMASINO, 1999; SHELDON *et al*., 2000; GAZZANI *et al*., 2003; MICHAELS *et al*., 2003).

Analysis of *BoFLC2* gene expression 4 and 8 weeks after the occurrence of stable *in vivo* conditions of vernalization and 4 weeks after each GA_3 treatment showed that in parental late genotypes, originating from these areas, and their hybrids (B, Scc and B x Scc) *BoFLC2* expression decreases over time (Figure 8), which is in accordance with the theory of its repressive effect on the flowering process (FINNEGAN *et al*., 2005). The level of *BoFLC2* expression in genotypes B and B x Scc, in all three sowing terms, is lower compared to genotype Scc. Such expression profile is correlated with the time of flowering that occurs earlier in the genotypes B and B x Scc compared to the Scc genotype. Based on AMMI analysis, it was concluded that the relative expression of *BoFLC2* in these genotypes correlated with time of flowering (Figure 6).

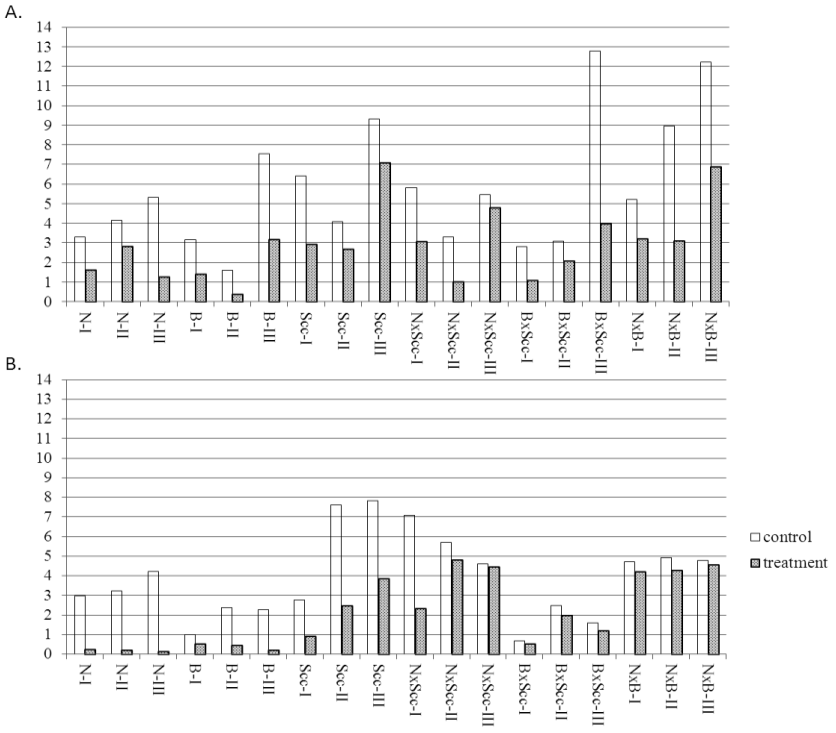


Figure 8. Expression of *BoFLC2* gene in parental lines (N, B and Scc) and F1 hybrids (N x Scc, B x Scc, N x B) from 3 sowing terms (I, II, III), without GA_3 treatment (control) and with GA_3 treatments. A. Level of expression 4 weeks after first GA_3 treatment B. Level of expression 4 weeks after second GA_3 treatment

Based on the analysis in early genotype N, in plants of the second and third sowing terms, as well as in hybrids in which it participates as a parent (SccxN and BxN), higher expression of *BoFLC2* gene is observed compared to plants of the first sowing term. Transmission of genotypes from different day length conditions causes different reactions of plants. LEMPE *et al*. (2005) studying the reaction of a large number of genotypes of *Arabidopsis thaliana* L. (177 wild and 32 floweing mutants) originating from the area of the different conditions of day length and temperature, concluded that plants originating from long day conditions bloom later in shorter day conditions and vice versa. The occurrence of mutations at certain loci involved in different pathways of flowering regulation is not excluded.

It has been found that in *Ath* mutants that do not have an *FLC* locus, vernalization continues to affect flowering and that there are *FLC*-dependent and independent flowering mechanisms (MICHAELS and AMASINO, 2001). Usually, as a consequence of all the above, there is a time delay flowering that is in a statistically significant correlation with the length of the day and temperature, which is exactly the case with the examined genotype N. SHELDON *et al*. (2006) examining the level and stability of *FLC* repression during the vernalization period concluded that cold treatment of plants over winter affects the level but not the stability of *FLC* repression, which may explain the difference in *BoFLC2* expression of the first and second expression measurements in early parent N.

GA_3 treatment resulted in decreased expression of *BoFLC2* gene in all genotypes. The most pronounced decrease in *BoFLC2* gene expression was measured in N genotype 4 weeks after second GA_3 treatment, while the smallest decrease in gene expression was observed in hybrids B x Scc and B x N. GOLLDBERG-MÖELLER *et al*. (2013) have also reported a negative influence of exogenous GA_3 treatment on the level of *FLC* expression in the final time of vernalization.

CONCLUSION

Based on the results of this research, it can be concluded that the flowering time of cabbage in vernalization conditions is typical for each genotype and depends on the environmental conditions. As the expression level of *BoFLC 2* is correlated with the time of flowering, it has been shown that the assessment of the level of its expression can predict the time of appearance of the first flower as well as the length of the flowering process. Treatment with gibberellin in *in vivo* GA3 conditions affects on the correction of flowering time. The study of the relative expression of *BoFLC 2* and the application of GA3 hormones in a group of genotypes can be useful for the best selection of genotypes with identical flowering time for most effective pollination, all in order to increase the yield of hybrid seed.

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UTICAJ RAZLIČITIH USLOVA SREDINE I TRETMANA GIBERILINSKOM KISELINOM NA VREME CVETANJA DIVERGENTNIH GENOTIPOVA KUPUSA GLAVIČARA (*Brassica oleracea* var. *capitata* L.) I NJIHOVIH F_1 HIBRIDA

Slađan ADŽIĆ, Zdenka GIREK, Suzana PAVLOVIĆ, Bogoljub ZEČEVIĆ, Jelena DAMNJANOVIĆ, Dejan CVIKIĆ, Milan UGRINOVIĆ

Institut za povrtarstvo, Smederevska Palanka, Srbija

Izvod

U procesu selekcije i proizvodnje semena kupusa, jedna od najznačajnijih karakteristika je vreme cvetanja. U cilju ispitivanja uticaja genotipa, sezone i datuma setve na proces cvetanja, kao i načina nasleđivanja ove osobine sproveden je eksperiment sa tri genotipa kupusa N, B i Scc različitog geografskog porekla i različite dužine vegetacionog perioda, kao i tri nova F1 hibrida N x Scc, B x Scc i B x N dobijena hibridizacijom između genotipova. Eksperiment je sproveden tokom tri temperaturno različite sezone S1, S2 i S3 (prosečno hladna, hladna i topla sezona), sa tri različita termina setve: 15. avgusta (I), 01.septembra (II) i 15.septembra (III). Tokom zimskog perioda povoljnog za vernalizaciju uzvršena su dva tretmana 300 ppm GA3. Utvrđen je statistički značajan uticaj svih ispitivanih faktora: genotipa, godine, roka setve i tretmana giberelinom, na vreme cvetanja. *AMMI* analizom utvrđen je stepen adaptabilnosti genotipova u zavisnosti od sezone gajenja, rokova setve i tretmana giberelinom za osobinu vreme cvetanja. Najvišu stabilnost na osnovu *ASV* vrednosti kod vremena cvetanja pokazao je hibrid *B*x*N* i njegove roditeljske komponentne genotipovi *B* i *N*, dok se kao najnestabilniji u vremenu cvetanja pokazao hibrid *Scc* x *B*. Patern relativne ekspresije najznačajnijeg cvetnog represornog gena *BoFLC*2 lokusa pokazao je korelativnu vezu sa vremenom cvetanja genotipova. Najniža ekspresija ovog lokusa utvrđena je kod genotipa *B* i on je imao u svim sezonama najranije cvetanje, dok je genotip *N* imao najvišu relativnu ekspresiju lokusa *BoFLC*2 i najkasnije cvetanje.

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