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GENOTYPE BY ENVIRONMENT INTERACTION FOR LENGTH OF FLOWERING TIME IN WINTER OILSEED RAPE (*BRASSICA NAPUS* L.) USING ADDITIVE MAIN EFFECTS AND MULTIPLICATIVE INTERACTION MODEL

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Summary

The objective of this study was to assess genotype by environment interaction for length of flowering time in winter oilseed rape cultivars grown in West Poland by the additive main effects and multiplicative interaction model. The study comprised of 25 winter oilseed rape genotypes (15 F_1 CMS *ogura* hybrids, eight parental lines and two European cultivars: open pollinated Californium and F_1 hybrid Hercules), evaluated at five locations in a randomized complete block design, with

four replicates. Across location average length of flowering time of the tested genotypes ranged from 22.3 to 27.4 days. In the AMMI analyses, 78.42% of the length of flowering time total variation was explained by environments, 4.34% by genotypes, and 9.04% by genotype × environment interaction. The line PN68 is recommended for further use in the breeding program due to its low average length of flowering time (24.5 days) and very good stability across environments (ASV=0.37).

Keywords and phrases: AMMI; *Brassica napus*; length of flowering time; stability

Classification AMS 2010: 62P10, 92D10

1. Introduction

Oilseed rape (*Brassica napus* L.) is grown in many parts of the world on 33.7 million hectares with increasing tendency. In Europe, the largest field areas cultivated by oilseed rape belong to Germany, France and Poland (FAOSTAT Database, 2018). It is the most important source of vegetable oil for human nutrition and for industrial products. After the extraction of oil from the seeds, the expeller and oilseed rape meal are commonly used in livestock feeding. High and stable seed and oil yield, oilseed rape yield components (silique number, seed number per silique and seed 1000 weight) as well as yield related traits, such as flowering time and plant height are the most important breeding goal in oilseed rape breeding. Now, when the plant breeders introgressed new genetic material this was done with respect to seed quality and agronomic traits such as early flowering plant with shorter length of flowering and also tolerance to biotic and abiotic stress (Würschum et al. 2012).

Flowering is a primary requirement for plant reproduction and one of the most important agronomic traits for crop production (Tasma et al. 2001). Plants of the same species that grow in different ecological conditions have developed various mechanisms to respond to environmental conditions, such as temperature, vernalization, day length, biotic and abiotic stress. Long et al. (2007) showed that genotype, environments, and genotype-by-environment interaction had significant effects on flowering time in double haploid (DH) segregating populations of oilseed rape. Iniguez-Luy and Federico (2011) reported that length of flowering time is a highly heritable trait and day to flower are still quite dependable upon environmental cues (cold exposure, day length, rainfall). Also Schiessi et. al (2015) used 158 European winter-type *B. napus* inbreed lines, detected that flowering time, plant height and seed yield are strongly influenced by climatic and day-length adaptation in crop plants. The GE interaction may be analyzed by the additive main effects and multiplicative interaction (AMMI) model (Gollob 1968; Gauch 1988, 1992; Zobel et al. 1988; Gauch and Zobel 1990;

Cornelius 1993). The AMMI model combines the ANOVA for the genotype and environment main effects and the principal component analysis (PCA) with multiplicative parameters for GE interaction effects.

The objective of this paper was to assess GE interaction for length of flowering time in winter oilseed rape by the AMMI model.

2. Material and Methods

Plant material for field trials consisted of 25 winter oilseed rape genotype entries: 15 F_1 CMS *ogura* hybrids (PN64×PN17, PN64×PN18, PN64×PN21, PN64×PN05, PN64×PN07, PN66×PN17, PN66×PN18, PN66×PN21, PN66×PN05, PN66×PN07, PN68×PN17, PN68×PN18, PN68×PN21, PN68×PN05, PN68×PN07), five restorer lines for Ogura system (PN05, PN07, PN17, PN18, PN21), three CMS *ogura* lines (PN64, PN66, PN68), two European cultivars (Californium and Hercules F_1). The field trials were carried out at five locations: Bąków (E1: 18°18'45'' E, 50°57'58'' N), Borowo (E2: 16°47'19'' E, 52°07'12'' N), Łagiewniki (E3: 17°14'13'' E, 51°45'40'' N), Małyszyn (E4: 18°37'31'' E, 51°14'42'' N) and Zielęcin (E5: 16°22'56'' E, 52°10'19'' N) in the season 2008/2009. All field trials were arranged according to randomized complete block designs with four replicates (Nowosad et al. 2016; Bocianowski et al. 2018). Each genotype was grown in a four row plot of 10.0 m^2 (Borowo and Bąków), 12.0 m^2 (Zielęcin), 9.6 m^2 (Łagiewniki) and 11.2 m^2 (Małyszyn) with a 0.30 row distance and a sowing density of 80 seeds/m^2. Length of flowering time was recorded for each plot. Length of flowering time was defined as the number of days from the onset of flowering (the first flower had opened on 10% of the plants in a plot) to the end of flowering (90% of the plants in a plot had finished flowering period). Plant protection and fertilization was performed according to normal practice at the respective locations, as necessary for the prevailing conditions.

Firstly, the normality of distribution of the length of flowering time was tested using Shapiro–Wilk's normality test. A two-way ANOVA was performed to verify the hypothesis of zero main effects of genotypes and environments and their interaction on length of flowering time. Least-squares means for genotypes and environments were simultaneously produced for the AMMI model. The model first fits additive effects for the main effects of genotypes (G) and environments (E) followed by calculation of multiplicative effects for GE interaction by PCA (Gauch 1992, 2013). The AMMI model (Gauch 1988, 1992; Gauch and Zobel 1990; Nowosad et al. 2016, 2017) is given by:

$$y_{ij} = \mu + g_i + e_j + \sum_{n=1}^{N} \lambda_n \gamma_{in} \delta_{jn} + Q_{ij},$$

where y_{ij} is the mean of the length of flowering time for genotype i in environment j, μ is the grand mean, g_i is the genotypic mean deviations, e_j is the environmental mean deviations, N is the number of PCA axis retained in the adjusted model, λ_n is the square root of the eigenvalue of the PCA axis n, γ_{in} is the genotype score for PCA axis n, δ_{jn} is the score eigenvector for PCA axis n, Q_{ij} is the residual. It was assumed the distribution of the residual Q_{ij} to be normal. The AMMI stability value (ASV) was used to compare the stability of genotypes as described by Purchase et al. (2000):

$$ASV = \sqrt{\left[\frac{SS_{IPCA1}}{SS_{IPCA2}}\left(IPCA_1\right)\right]^2 + \left(IPCA_2\right)^2},$$

where *SS* is the sum of squares, IPCA1 and IPCA2 are the first and the second interaction principal component axes, respectively; and the $IPCA_1$ and $IPCA_2$ scores were the genotypic scores in the AMMI model. ASV is the distance from zero in a two-dimensional scatterplot of $IPCA_1$ scores against $IPCA_2$ scores. The distance from zero is then determined using the theorem of Pythagoras. The higher the IPCA score, either negative or positive, the more specifically adopted a genotype is to certain environments. Lower ASV score indicate a more stable genotype across environments. The significance of the interaction principal component axes in PCA was tested with the *F* test (Dias and Krzanowski 2003). For the AMMI analysis, statistical package GenStat v. 18.2 was used.

3. Results and Discussion

The length of flowering time has a normal distribution. Genotype (G) and environment (E) main effects and GE interaction effects were differentiated highly significantly. The sum of squares for environment main effects represented 78.42% of the total sum of squares for the length of flowering time variation in the multi-environment trials (Table 1). The genotype-main effects explained 4.34% of the total length of flowering time variation, while the effects of GE interaction were responsible for 9.04% of the total variation. The two principal components of GE interaction accounted jointly for 78.79% of the whole G×E interaction effect variation of length of flowering time and were highly significant. The first principal interaction component (IPCA 1) accounted for 55.38% of the variation caused by the interaction, while IPCA 2 accounted for 23.41% of this variation.

Length of flowering time of the tested genotypes across the five test locations varied from 14.66 (for PN64×PN17 in E5) to 33.54 days (for Hercules F_1 in E4), with an average of 25.23 days (Table 2). The cultivar Hercules F_1 had the highest average length of flowering time across the five test locations (27.4 days), and the line PN64×PN17 had the lowest average (22.3 days). The across genotype averages length of flowering time at locations varied substantively from 20.29 days in Bąków, to 30.56 days in Małyszyn.

Table 1. Analysis of variance according to main effects and multiplicative interaction model for length of flowering time in the oilseed rape (*Brassica napus* L.) multi-environment trials conducted in the season 2008/2009

Source of variation	d.f.	Sum of squares	Mean squares	*F*-statistic	Variation explained (%)
Treatments	124	9239	74.5	46.22***	91.79
Genotypes	24	437	18.2	11.29***	4.34
Environments	4	7893	1973.2	120.84***	78.42
GE interactions	96	910	9.5	5.88***	9.04
IPCA 1	27	504	18.7	11.59***	55.38
IPCA 2	25	213	8.5	5.28***	23.41
IPCA 3	23	118	5.1	3.17***	12.97
Residuals	21	75	3.6	2.21**	
Error	360	580	1.6		
Total	499	10065	20.2		

** P<0.01; *** P<0.001. IPCA, principal component of interaction.

Environmental conditions strongly differentiate the date of occurrence of subsequent development phases of the oilseed rape plants. It was observed that the difference in the start and end of flowering and also length of flowering time in assessed environments was about a decade. The beginning of flowering depends on the temperature in the month of April. The higher the average temperature of the month, flowering begins usually earlier. The growing season 2008/2009 in Bąków (E1) was characterized by a cooler spring and higher than many years of rainfall. This contributed to poorer plant development and shortening the flowering period of all tested genotypes. At the same time, the deficiency of rainfall in the growing season in Łagiewniki and in Zielęcin could have contributed to the shortening of winter rape flowering length, because under rainfall deficiency conditions generative phases last shorter, which increases the plant's chances of obtaining mature seeds. Adjusting the plant development rate to weather conditions allows for the effective use of genetically conditioned yield potential. In the case of hybrids, it is necessary to select the components so that

the flowering period of the parental forms is synchronized which will allow the production of hybrid seeds. It is also necessary to evaluate new genotypes in many environments, in several growing seasons to assess the ability to absorb nutrients, plant development, reaction to biotic and abiotic stress, and yield potential.. In the case of oilseed rape, the phenomenon of excessive development of the green organ of the plant should be limited, prolonging the flowering time, which prevents the maturation of seeds and hinders harvesting in an optimal time. For this purpose, breeding materials should be tested in several environments for subsequent growing seasons to select early, high-yield genotypes characterized by a high degree of adaptation to environmental conditions. In breeding programs, may accelerate the breeding progress to obtain plants with the desired properties for different traits. For plant breeders, diverse agronomic traits, including flowering time, plant height, as well as seed quality traits in *B. napus* are of highest importance. High oil content is correlated with early flowering and probably shorter length of flowering, as these genotypes have more time to accumulate oil in the seeds.

Length of flowering time in winter oilseed rape (*Brassica napus* L.) is a trait determined by multiple genes that cause variability in the performance of genotypes and diverse response to cultivation environment. Long et al. (2007) indicated in two populations of oilseed rape a quantitative genetic control mechanism, and the variation in flowering time across environments. The results of ANOVA procedure presented by Long et al. (2007) showed that genotype, environment, and GE interaction caused had significant effects on flowering time in both populations. Schiessi et al. (2015) reported that flowering time is strongly influenced by climatic and day-length factors in oilseed rape. Würschum et al. (2014) observed the genotypic variance and the GE interaction variances in the populations of 391 elite oilseed rape lines for flowering time. The heritability (h^2) of flowering time in different populations of oilseed rape ranged from 77 to 90% (Long et al. 2007), 0.91 in elite of 391 oilseed rape lines (Würschum et al. 2014), and 0.84 in 156 winter oilseed rape genotypes (Körber et al. 2012).

Agronomic stability (named usually shortly ‘stability’) of a genotype (genotype entry) for a quantitative trait (such as crop yield, different attributes of crop productivity, yield quality or environmental adaptation) is considered as consistent response to changing environmental conditions, biotic and abiotic stresses, agronomic factors as well as weather conditions being usually measured by environmental averages of this trait (Wang et al. 2015). The AMMI 2 biplot (Fig. 1) shows relative variation of GE interaction effects regarding each of the studied genotype and environment. Among the tested genotypes, IPCA 1 values ranged from -1.814 (G08 – hybrid PN64×PN17) to 1.012 (G03 – line PN17), while among tested environments ranged from IPCAe1=-1.923 (Małyszyn) to IPCAe1=2.559 (Zielęcin) (Fig. 1). Obtained results show large variability

of studed genotypes and environments as well as large reaction particular genotypes in individual environments. In hybrid breeding it is necessary selection of early parental components: male sterile CMS *ogura* lines and paternal lines with the restorer gene, in order to obtain early high-yielding F1 hybrids. CMS *ogura* line – PN 68 as maternal component is useful for breeding, because hybrids also have the shortest flowering period regardless of the paternal form used.

Fig. 2 presents the AMMI 1 biplot for length of flowering time. The lines PN05 (G06) and PN64 (G07) interacted positively with the E1 location (Bąków), but negatively with the E5 – Zielęcin (Figures 1 and 2). The hybrids PN64×PN21 (G10), PN66×PN18 (G15) and PN66×PN05 (G17) interacted positively with the E3 location(Łagiewniki), but negatively with the E1 (Bąków) and E4 (Małyszyn) locations. Length of flowering time of restored and composite hybrids CMS *ogura* in field trials in two localities varied significantly between years, locations and climatic conditions (Liersch 2006). The similar results were reported by Wójtowicz (2013) and Wielebski (2013) for different cultivars of winter oilseed rape. Some genotypes have high adaptation; however, most of them have specific adaptability.

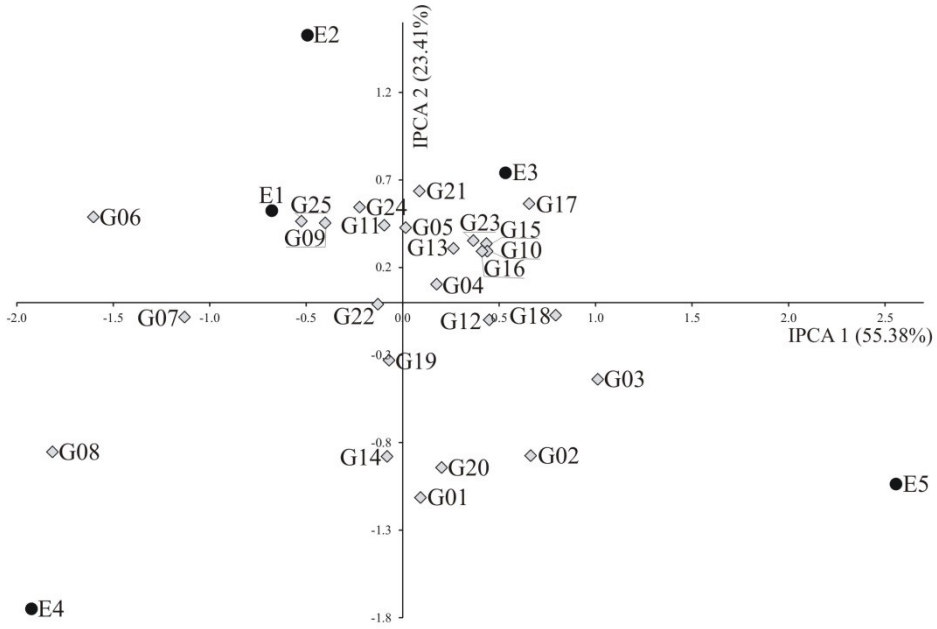

Fig. 1. AMMI 2 biplot for genotype by environment interaction of length of flowering time in 25 winter oilseed rape (*Brassica napus* L.) lines and hybrids and five environments, showing the effects of the first and the second principal interaction components (IPCA 1 and IPCA 2, respectively)

AMMI stability values (ASV) revealed variations in length of flowering time stability among the 25 genotypes (Table 2). According to Purchase et al. (2000), a stable variety is defined as one with ASV value close to zero. Consequently, the hybrid PN68×PN21 (G22) with ASV of 0.30 and line PN68 (G19) with ASV of 0.37 were the most stable, while the hybrid PN64×PN17 – G08 (4.38) and line PN05 – G06 (3.83) were the least stable (Table 2). Hybrid PN68×PN18 (G21) and line PN68 (G19) had the lowest averages of length of flowering time, and low AMMI 1 principal component scores (Figure 2, Table 2). Significant for flowering time, GE interaction in this study is concordant with previous research (Fletcher et al. 2015; Lou et al. 2007; Shi et al. 2009). Fletcher et al. (2015) analysed 225 doubled haploid lines population in wet and dry treatments; Lou et al. (2007): seven populations (F_2, F_3, DH lines, BC) in six environments; Shi et al. (2009): 202 doubled haploid lines in 10 microenvironments.

The average performance of oilseed rape genotypes in Bąków (E1) was characterized by lowest length of flowering time in comparison to this performance in Małyszyn (E4), Borowo (E2) and Łagiewniki (E2). In conditions of light texture soils of Borowo, Małyszyn and Łagiewniki, the length of flowering time depended on genotype (inheritance of length of flowering time) and weather conditions, especially rainfall in the spring season (Nowosad et al. 2016).

The AMMI 1 biplot allows the visualization of the main effects of the genotypes and environments, in addition to the most important GE interactions (de Oliveira et al. 2014). The AMMI model provides a useful tool in analysing GE interaction patterns and improving the accuracy of response estimates (Farshadfar et al. 2011).

4. Conclusions

1. The presented study illustrated how AMMI analyses permits proximity of interaction effect of a genotype in each environment for length of flowering time in winter oilseed rape and it helps to identify genotypes best suited for specific environmental conditions with respect to the studied trait. The AMMI stability value exposed high genotypes stability.
2. CMS *ogura* line – PN 68 as maternal component is useful for breeding, because hybrid also have the shortest flowering period regardless of the paternal form used.

Table 2. Average length of flowering time (days), for genotypes and environments, interaction principal component analysis scores of tested oilseed rape (*Brassica napus* L.) lines and hybrids and AMMI stability value (ASV)

Genotype	Code	E1	E2	E3	E4	E5	Mean	IPCA1	IPCA2	ASV
Californium	G01	20.9	27.11	25.46	32.9	23.38	25.95	0.093	-1.114	1.14
Hercules F_1	G02	19.59	27.91	30.92	33.54	25.04	27.4	0.664	-0.875	1.80
PN17	G03	21.18	28.56	28.08	31.08	25.85	26.95	1.012	-0.439	2.44
PN18	G04	20.53	28.47	27.14	30.56	21.8	25.7	0.177	0.104	0.43
PN21	G05	20.5	28.75	27	30	20.75	25.4	0.016	0.427	0.43
PN05	G06	19.98	28.51	26.35	32.32	15.34	24.5	-1.602	0.488	3.83
PN64	G07	19.35	27.41	26.19	32.41	17.14	24.5	-1.130	-0.084	2.68
PN64×PN17	G08	18.89	24.86	20.69	32.4	14.66	22.3	-1.814	-0.854	4.38
PN64×PN18	G09	20.96	28.79	25.73	30.26	19.51	25.05	-0.401	0.454	1.05
PN64×PN21	G10	21.61	29.39	27.05	29.97	22.98	26.2	0.435	0.336	1.09
PN64×PN05	G11	20.88	28.62	25.57	29.59	20.33	25	-0.095	0.441	0.50
PN64×PN07	G12	20.2	27.8	26.47	30.01	22.51	25.4	0.450	-0.100	1.07
PN66	G13	19.64	28.12	27.46	29.65	21.13	25.2	0.264	0.308	0.70
PN66×PN17	G14	19.35	26.15	25.05	31.72	21.23	24.7	-0.080	-0.880	0.90
PN66×PN18	G15	21.14	28.87	26.57	29.59	22.58	25.75	0.439	0.292	1.08
PN66×PN21	G16	20.57	28.43	26.41	29.29	22.04	25.35	0.412	0.292	1.02
PN66×PN05	G17	20.53	28.96	27.63	28.81	22.58	25.7	0.656	0.563	1.65
PN66×PN07	G18	19.63	27.61	27.3	29.47	23.25	25.45	0.794	-0.073	1.88
PN68	G19	20.24	27.02	24.12	30.32	20.81	24.5	-0.070	-0.330	0.37
PN68×PN17	G20	20.38	26.88	25.44	32.03	23.03	25.55	0.202	-0.944	1.06
PN68×PN18	G21	19.91	28.09	25.59	28.33	19.82	24.35	0.087	0.636	0.67
PN68×PN21	G22	20.35	27.89	25.79	30.6	20.62	25.05	-0.128	-0.011	0.30
PN68×PN05	G23	20	28	26.13	28.81	21.3	24.85	0.367	0.353	0.94
PN68×PN07	G24	19.95	28.32	26.37	29.58	19.29	24.7	-0.223	0.544	0.76
PN07	G25	20.99	28.99	26.23	30.75	19.28	25.25	-0.524	0.464	1.33
Mean		20.29	27.98	26.27	30.56	21.05	25.23			
IPCAe1		-0.677	-0.492	0.534	-1.923	2.559				
IPCAe2		0.523	1.526	0.740	-1.751	-1.038				

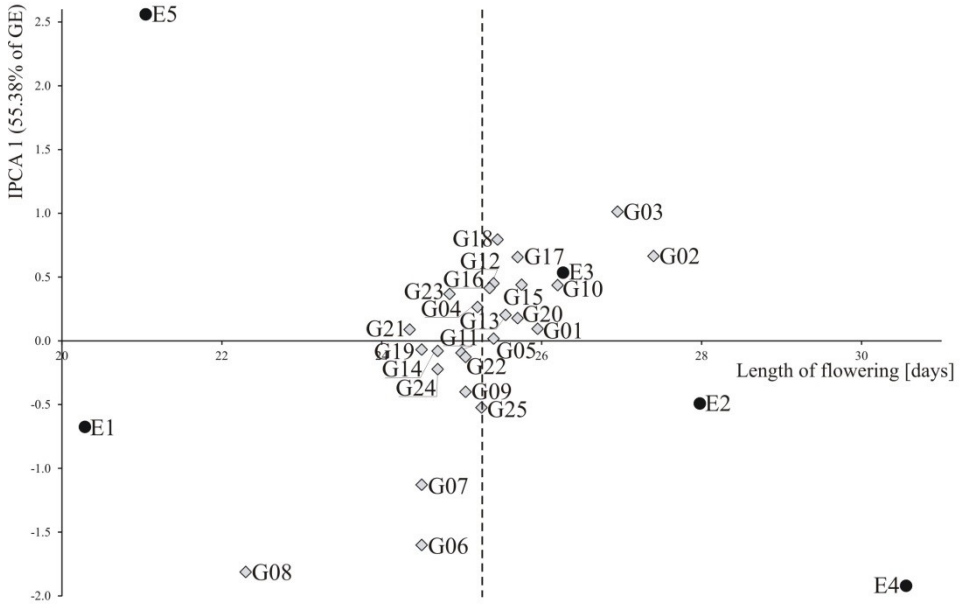

Fig. 2. AMMI 1 biplot for the first principal component of interaction (IPCA 1) and average oilseed rape (*Brassica napus* L.) length of flowering time (days). Vertical line at the centre of biplot is the general grand mean calculated for all genotypes across environments.

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