

GENETIC ADVANCE AND REGRESSION ANALYSIS IN SUNFLOWER

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The knowledge about the magnitude and nature of variability that is present in a breeding population is an important prerequisite for designing efficient breeding programme in order to improve the yield potential of genotypes. The objective of this research was to evaluate heritability and genetic advance of important quantitative traits in new crosses of sunflower as well as to evaluate ratio of dominant and recessive genes in parental genotypes. The plant material selected for this research consisted of 6 sunflower genotypes, which according to literary data possess important characteristics for the production of sunflower. According to presented results there is significant variability of evaluated quantitative traits. Phenotypic variance was higher than genotypic demonstrating strong environment effect in expression of traits. The broad sense heritability was found very high for plant height (83.25%), high for 1000 seed weight (69.33%), moderate for seed yield/plant (46.53%) and head diameter (56.89%), while low for oil content (29.35%). Genetic advance expressed as a percentage of the mean ranged between 2.23% and 19.96%. Placement of array points displayed that the highest frequency of dominant genes for seed yield/plant, 1000 seed weight and head diameter was found in parental genotype Rodnik. Position of expected line of regression pointed over dominance in inheritance for seed yield/plant, oil content and head diameter, while for 1000 seed weight and plant height additive gene action played role in inheritance

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suggesting that selection in early generations for these traits will be effective. By testing the coefficients of regression interallelic interaction was not determined.

Key words: components of variance, heritability, inheritance, interallelic interaction

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is mainly cultivated because of edible oil, which is considered healthy for human consumption due to the presence of polyunsaturated fatty acids which reduces the risk of cardiac related problems. Being a crop with medium water requirements sunflower has ability to tolerate periods of drought because of well-developed root system and capacity to extract water from the subsoil. Besides, sunflower holds great promise because of its short duration, wider adaptability and higher amount of superior quality oil (RANI *et al.* 2017).

Firstly grown as an ornamental plant for more than two centuries, the phenotypic changes that sunflower has undergone during domestication largely follow the domestication syndrome (RADANOVIĆ *et al.* 2018). During domestication and improvement sunflower has undergone through selection and genetic drift, which has reduced its genetic diversity (TANG and KNAPP, 2003; LIU and BURKE, 2006), with modern cultivars retaining 50–67% of the diversity that is present in wild *H. annuus* populations. Bearing this in mind further improvement of sunflower requires increase of genetic variability focused on the most important economic characteristics. To achieve this, systemic and successful hybridization program is needed for thorough understanding of genetic inheritance of economically important traits, as genetic and non-genetic causes are involved in expression of traits. Regarding this, for designing appropriate breeding procedure adequate information on the genetic structure of the parents and mode of gene action affecting the yield and its related traits are prerequisite for future progress. Bearing in mind that greater variability between the genotypes leads to higher chance for further progress, important precondition for successful breeding program is knowledge about the nature and magnitude of variability which is available. Previous studies reported that rapid improvement in crop breeding depends on the availability of genetic diversity which can be combined in various forms for further improvement (DENTON and NWANGBURUKA, 2011; WANI *et al.* 2011). Most often there is a close correspondence between the magnitude of genetic variability and heterosis (LALITHA REDDY *et al.* 2000; FUZZATTO *et al.* 2002; RAO *et al.* 2004; OLIVEIRA *et al.* 2004). Further, there are also studies reporting that heterosis will not always occur when divergent parents are crossed (DIXIT and SWAIN, 2000; VIVAS *et al.* 2018). For an effective breeding strategy heritability estimates are important indicator about transferability of economically important quantitative traits. Heritability estimates also help in predicting the level of success in upcoming generations by designing the appropriate selection criteria and assessing the level of genetic improvement. High heritability supplemented with high genetic advance indicates reliable crop improvement which is valuable in the process of developing new genotypes with desirable traits.

As success in breeding process largely depends on initial material that is used for further improvement, evaluation of genetic background is useful for selection of adequate genotypes. The aim of this research was to evaluate heritability and genetic advance of important quantitative traits in new crosses of sunflower as well as to evaluate gene ratio of dominant and recessive genes in parental genotypes.

MATERIALS AND METHODS

The plant material selected for this research consisted of 6 sunflower genotypes, which according to literature data possess important characteristics for the production of sunflower (ŠKORIĆ and JOČIĆ, 2004; GONTCHAROV *et al.* 2006). Sunflower genotypes Azovsky (P1), Kazachy (P2), Harkovski (P3), Lider (P4), Rodnik (P5) and Amaian (P6) were crossed by hand emasculation according to incomplete diallel design. Total of 21 genotypes (6 parental genotypes and their 15 crosses) were sown in randomized complete block design (RCBD) in three replicates at the experimental field Rimski Šančevi of the Institute of Field and Vegetable Crops from Novi Sad. Basic plot size was 10 m², with four 3.6 m long rows and 70x30 cm plant spacing. Harvest was done by hand and the data were recorded on 10 plants from middle rows in each replicate. Seed yield/plant (SYP) and 1000 seed weight (TSW) were recorded in laboratory on a technical scale with an accuracy of 0.01 g. Oil content (OC) was determined by nuclear magnetic resonance (NMR) in pure seed and expressed in percentages (%). Plant height (PH) and head diameter (HD) were measured in the stage of physiological maturity.

Data analysis:

The phenotypic and genotypic coefficients of variation were estimated according to the method suggested by SYUKUR *et al.* (2012) as follows:

$$\text{Environmental variance } (\sigma^2_e) = M_{se}$$

$$\text{Phenotypic variance } (\sigma^2_p) = (\sigma^2_g + \sigma^2_e)$$

$$\text{Genotypic variance } (\sigma^2_g) = (M_{se} - M_{st})/r$$

Where:

M_{se} = mean square error

M_{st} = mean square treatment

r = number of replicates

$$\text{Phenotypic coefficients of variation (PCV)} = (\sqrt{\sigma^2_p X / X}) \times 100$$

$$\text{Genotypic coefficients of variation (GCV)} = (\sqrt{\sigma^2_g X / X}) \times 100$$

Where:

σ^2_p = phenotypic variance

σ^2_g = genotypic variance

X = grand mean of the trait

Broad sense heritability (h^2) was estimated as described by ALLARD (1999) as:

$$h^2_B = (\sigma^2_g / \sigma^2_p) \times 100$$

Where:

h^2_B = broad sense heritability

σ^2_p = phenotypic variance

σ^2_g = genotypic variance

Genetic advance (GA) and percentage of the mean (GAM) assuming selection of superior 5% of the genotypes was estimated in accordance with the methods illustrated by JOHNSON *et al.* (1955) as:

$$GA = (K \times \sqrt{\sigma^2_p \times \sigma^2_g}) / \sigma^2_p$$

Where:

GA = expected genetic advance

k = standardized selection differential at 5% selection intensity (K = 2.063)

σ^2_p = phenotypic variance

σ^2_g = genotypic variance

The genetic advance as percentage of mean (GAM) was computed as:

$$GAM(\%) = (GA/X) \times 100$$

Where:

GAM = genetic advance as percentage of mean

GA = expected genetic advance

X = grand mean of a character

The regression analysis is represented by the $V_r W_r$ graph where the scattering diagram points should be arranged on the part of the expected regression line within the limiting parabola.

$$W_r^2 = V_r \times V_p$$

Where:

V_r = variance of all offspring of each parent

V_p = variance of parents

W_r = covariance between parents and their offspring

Based on the $V_r W_r$ chart it is possible to determine the ratio of dominant and recessive genes which control the observed trait of the parent. The line with the highest number of dominant genes will have the smallest variation of V_r and covariance W_r and corresponds to the point closer to the coordinate start. Contrary, the line with the highest number of recessive genes has the highest variation of V_r and covariance W_r and represents the most distant point from the coordinate start.

Additionally, a $W_r W'$ graph is used to determine the degree of domination among parents. Covariance W' is the mean covariance between the parents and all offspring. The parent with the highest number of recessive genes is located in the first quadrant farthest from the origin, while the parent with the most dominant genes is located closer to the origin. Dispersion diagram

points should be distributed over the expected regression line $b = 1/2$. In case of over dominance, dispersion diagram points will be located in the third quadrant.

A common interpretation of the two graphs makes it possible to discover the existence of interallelic interaction. In the case of an interallelic interaction, the points are below the theoretical regression line $b = 1$ on $VrWr$ graph, while the WrW' graph have the opposite effect, points are located above the theoretical regression line $b = 1/2$. Testing of the coefficient of regression $b = 1$ was done according to AL-NAGGAR *et al.* (2016) through the coefficient of regression (b) and the standard error of regression (sb) according to the formula:

$$t = b-1/sb \text{ for } n-2 \text{ degrees of freedom.}$$

RESULTS AND DISCUSSION

The presented results of parental genotypes and their F1 progeny revealed that there were significant differences in expression of evaluated traits (Table 1). The highest average value regarding seed yield/plant (SYP) of 98.29 g was recorded in parental genotype Azovsky (P1) while the lowest value of 54.81 g was detected in genotype Amaian (P6). Considering cross combinations the highest SYP was observed in combination C13 (95.83 g) in contrast to C14 (56.16 g) with the lowest average SYP. With regard to oil content (OC) the highest average value of 49.78% was recorded in parental genotype Lider (P4), while the lowest (44.35%) in Azovsky (P1). Observing cross combinations the highest mean value of OC was measured in C13 (53.13%) compared to the lowest OC measured in C8 (48.25%). By comparing the average results of 1000 seed weight (TSW) in parental genotypes, the highest average value was expressed in P2 (67.22 g) and the lowest in P3 (50.47 g). With respect to cross combinations the highest mean value of TSW was observed in C1 (65.17 g), while the lowest TSW was determined in cross combination C9 (44.90 g). Average values of plant height (PH) in parental genotypes ranged between 145.43 cm in Harkovski (P3) and 207.27 cm in Azovsky (P1), while in cross combinations PH was between 168.23 cm in C9 and 208.33 cm in C4. Regarding head diameter (HD) the highest mean value of 23.40 cm was measured in parental genotype Azovsky (P1), while Kazachy (P2) had the lowest average value (20.63 cm). Recorded mean values of HD in cross combinations varied from 16.67 cm in C14 to 20.97 cm measured in C11.

To select the appropriate breeding procedure it is necessary to determine the heritable and non-heritable component in expression of traits that are subject of breeding. As presented in previous studies heritable component can be evaluated via phenotypic variance, genotypic variance, heritability and genetic advance (KHAN *et al.* 2007; HASSAN *et al.*, 2012; TRIPATHI *et al.* 2013). In all studied traits phenotypic variance was higher than the genotypic indicating higher environmental effect in expression of traits (Table 2). Earlier findings of TYAGI and TYAGI (2010) are in accordance with our results. Phenotypic and genotypic coefficients of variation (PCV and GCV) were moderate for SYP and TSW, while lower for HD, PH and OC. Previous studies of KALUKHE *et al.* (2010) and MAKANE *et al.* (2011) also reported high PCV and GCV for SYP and TSW. Contrary to our results, RANI *et al.* (2017) observed moderate PCV and GCV parameters for PH and HD, while previous results of SULTANA *et al.* (2005) were in accordance with our study regarding OC. Heritability in the broad sense is the degree of realization of phenotype due to the genetic basis of the individual, and at the level of the gene represents the proportion of total variance attributable to the average effect of the gene, which ultimately

determines the similarity between the relatives. SINGH (2009) categorized heritability estimates as low (<40%), moderate (40-59%), high (60-79%) and very high (>80%).

Table 1. Average values of quantitative traits of sunflower

GENOTYPE	SYP (g)	OC (%)	TSW (g)	PH (cm)	HD (cm)
P1	98.29 ^{±34.43}	44.35 ^{±3.69}	65.86 ^{±14.68}	207.27 ^{±11.00}	23.40 ^{±3.65}
P2	68.41 ^{±24.22}	45.43 ^{±3.90}	67.22 ^{±13.58}	158.07 ^{±11.96}	20.63 ^{±3.35}
P3	46.52 ^{±21.27}	46.11 ^{±3.71}	50.47 ^{±10.28}	145.43 ^{±7.86}	21.57 ^{±4.07}
P4	75.49 ^{±32.93}	49.78 ^{±3.21}	52.62 ^{±15.68}	174.30 ^{±11.54}	21.07 ^{±3.63}
P5	66.66 ^{±28.09}	49.17 ^{±4.14}	56.15 ^{±13.09}	159.73 ^{±10.60}	21.27 ^{±4.10}
P6	54.81 ^{±34.92}	45.41 ^{±3.72}	52.77 ^{±15.82}	155.87 ^{±11.55}	21.23 ^{±3.95}
C1 (P1xP2)	62.51 ^{±23.99}	50.37 ^{±2.99}	65.17 ^{±9.69}	189.77 ^{±9.04}	17.37 ^{±2.95}
C2 (P1xP3)	85.91 ^{±36.04}	51.38 ^{±2.74}	61.38 ^{±12.51}	191.80 ^{±10.20}	19.37 ^{±2.63}
C3 (P1xP4)	91.71 ^{±29.81}	49.47 ^{±3.67}	62.73 ^{±14.00}	207.10 ^{±11.06}	19.77 ^{±1.87}
C4 (P1xP5)	81.69 ^{±32.40}	51.87 ^{±3.68}	56.73 ^{±10.52}	208.33 ^{±10.56}	19.53 ^{±2.98}
C5 (P1xP6)	85.52 ^{±30.11}	50.41 ^{±4.27}	56.00 ^{±12.57}	199.67 ^{±10.60}	20.60 ^{±2.67}
C6 (P2xP3)	83.69 ^{±33.39}	49.83 ^{±3.76}	64.72 ^{±12.56}	189.13 ^{±9.50}	20.67 ^{±2.92}
C7 (P2xP4)	63.61 ^{±25.14}	49.35 ^{±2.98}	56.95 ^{±12.13}	191.47 ^{±11.22}	17.47 ^{±3.12}
C8 (P2xP5)	75.11 ^{±34.69}	48.25 ^{±5.85}	59.26 ^{±9.75}	186.03 ^{±11.36}	20.53 ^{±2.98}
C9 (P2xP6)	62.76 ^{±43.37}	48.84 ^{±3.19}	44.90 ^{±13.20}	168.23 ^{±11.21}	18.93 ^{±2.95}
C10 (P3xP4)	89.60 ^{±35.73}	50.79 ^{±3.46}	59.25 ^{±10.73}	184.70 ^{±10.32}	20.73 ^{±2.16}
C11 (P3xP5)	85.91 ^{±39.41}	50.77 ^{±3.15}	58.80 ^{±14.29}	172.00 ^{±12.91}	20.97 ^{±2.09}
C12 (P3xP6)	64.16 ^{±30.75}	52.00 ^{±2.43}	49.49 ^{±9.70}	171.83 ^{±7.35}	19.00 ^{±2.21}
C13 (P4xP5)	95.83 ^{±31.20}	53.13 ^{±3.22}	57.57 ^{±11.42}	191.83 ^{±11.93}	19.57 ^{±2.81}
C14 (P4xP6)	56.16 ^{±13.93}	51.74 ^{±2.81}	47.13 ^{±7.74}	193.90 ^{±12.87}	16.67 ^{±2.02}
C15 (P5xP6)	73.81 ^{±28.19}	51.94 ^{±1.61}	50.91 ^{±8.35}	178.60 ^{±6.20}	19.50 ^{±3.17}
LSD _{0.05}	19.81	2.54	7.26	9.31	1.86
LSD _{0.01}	26.47	3.41	9.71	12.49	2.49

Heritability estimates in broad sense (H^2) were very high for PH, high for TSW, moderate for SYP and HD, and low for OC. The characteristics having high heritability estimates are of immense importance as selection at phenotypic level can be more successful in prediction of breeding value (or genetic merit) from phenotypic data. Confirming the results from our study, previous research from KHAN *et al.* (2007), ARSHAD *et al.* (2010) and ANDARKHOR *et al.* (2012) reported high values of heritability for TSW and PH indicating a high proportion of the genetic basis in the expression of these traits. High values of heritability for SYP, OC and moderate for HD were observed by MAKANE *et al.* (2011). Heritability estimates coupled together with genetic advance (GA) is much better approach for selection of desirable individuals (IBRAHIM and HUSSEIN, 2006; OGUNNIYAN and OLAKOJO, 2014; MOHAMMED *et al.* 2019). High values of heritability and genetic advance indicate success of selection towards

improvement of a trait based on phenotypic performance (SYUKUR *et al.* 2012). JOHNSON *et al.* (1955) classified GA as low (0-10%), moderate (10-20%) and high (>20%). Moderate values of GAM were recorded for SYP, TSW and PH, while low for OC and HD (Table 2). As earlier stated by KHAN *et al.* (2007) and TANEVA *et al.* (2019) that high GA indicates that H^2 is mainly due to additive gene effect, meaning that less genetic gain is expected through selection of characters depicting high heritability estimates with low genetic advance while considerable genetic gain is expected through selection for traits possessing high GCV, moderate heritability and high genetic advance.

Table 2. Components of variance, broad sense heritability (H^2) and genetic advance as a % of mean (GAM)

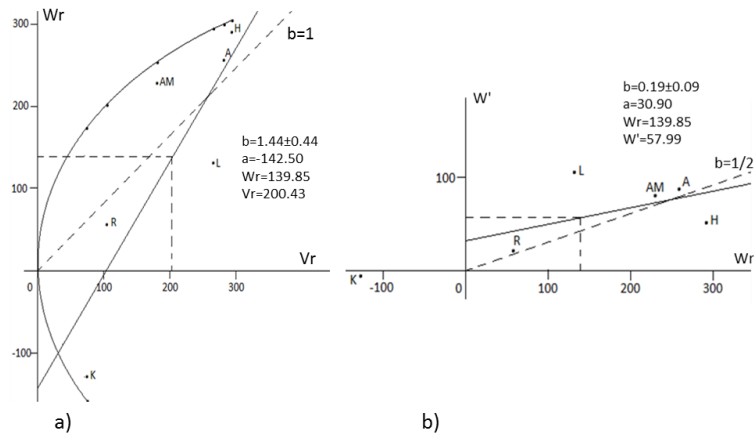
Traits	Average	σ^2_p	PCV(%)	σ^2_g	GCV(%)	H^2 (%)	GAM(%)
SYP (g)	77.56	260.86	20.82	121.37	14.20	46.53	19.96
OC (%)	50.67	3.49	3.67	1.02	1.99	29.35	2.23
TSW (g)	56.75	47.78	12.18	33.12	10.14	69.33	17.40
PH (cm)	188.29	161.19	6.74	134.19	6.15	83.25	11.56
HD (cm)	19.38	2.45	8.08	1.40	6.11	56.89	9.47

SYP – seed yield per plant; OC – oil content; TSW – thousand seed weight; PH – plant height; HD – head diameter; σ^2_p – phenotypic variance; σ^2_g – genotypic variance; PCV(%) – phenotypic coefficient of variation; GCV(%) – genotypic coefficient of variation

The graphical (VrWr) representation of the evaluated traits shows that regarding SYP, OC and HD expected line of regression intercept the Wr axis below the coordinate origin which represents over dominance in the inheritance of SYP, OC and HD (Figure 1a, 2a, 5a). Vice versa, regarding TSW and PH the expected regression line cuts the Wr axis above the coordinate start (the positive value of the parameter "a") and points to the partial domination in the inheritance of TSW and PH (Figure 3a, 4a). The regression analysis did not determine the presence of interallelic interactions in the inheritance of SYP, OC, TSW, PH and HD since it was found that the regression coefficient did not deviate significantly from the unit slope ($b = 1.41 \pm 0.44$; $b = 0.54 \pm 0.20$; $b = 0.51 \pm 0.21$; $b = 0.88 \pm 0.23$ and $b = 0.54 \pm 0.26$). None of the parental genotypes were at the cross-section point of the limiting parabola and the expected line of regression indicate that they contained an uneven number of dominant and recessive genes regarding evaluated traits. The distribution of array points on regression line in terms of SYP, TSW and HD indicated that genotype Rodnik, due to proximity to the origin, possessed the highest frequency of dominant genes. By contrast, the highest number of recessive genes for SYP, TSW and HD was determined in genotypes Harkovski, Kazački and Azovsky, respectively (Figure 1a, 3a, 5a). In terms of OC and PH, due to the distance from coordinate origin the highest number of dominant genes were determined in genotypes Lider and Azovsky, while the highest frequency of recessive genes for OC and PH were determined in genotypes Azovsky and Rodnik (Figure 2a, 4a).

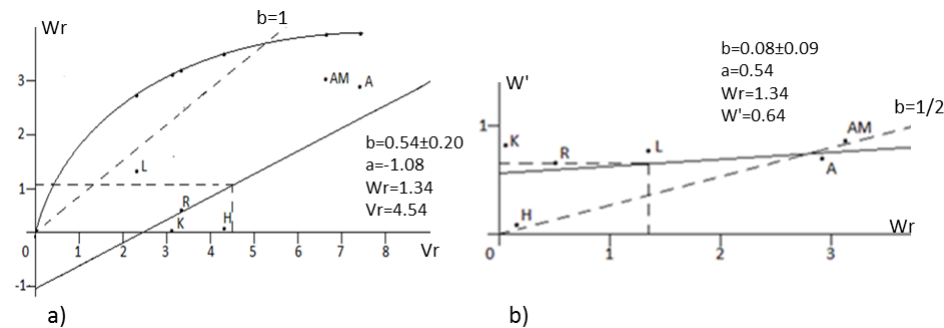
Degree of dominance between parents for evaluated traits are graphically presented on WrW' graph. The joint interpretation of both diagrams allows a more precise determination of interallelic interaction. The sequence of the scattering diagrams on the WrW' graph is the same as on the VrWr chart, but the position of the individual points relative to the regression line is different (Figure 1b, 2b, 3b, 4b, 5b). Namely, such a change in the distribution and position of

the points relative to the regression line indicates the presence of interallelic interaction but the presence of epistasis has not been determined by testing the regression coefficients (b) because the calculated (t) values were less than the critical in the t -distribution tables. On figure 1b it is noticeable that genotype Kazački is located in the third quadrant of WrW' graph which means that it was over dominant in terms of SYP. Related to other evaluated traits, there were no points located in third quadrant of the WrW' meaning that none of the parental genotypes were over dominant for analysed traits.



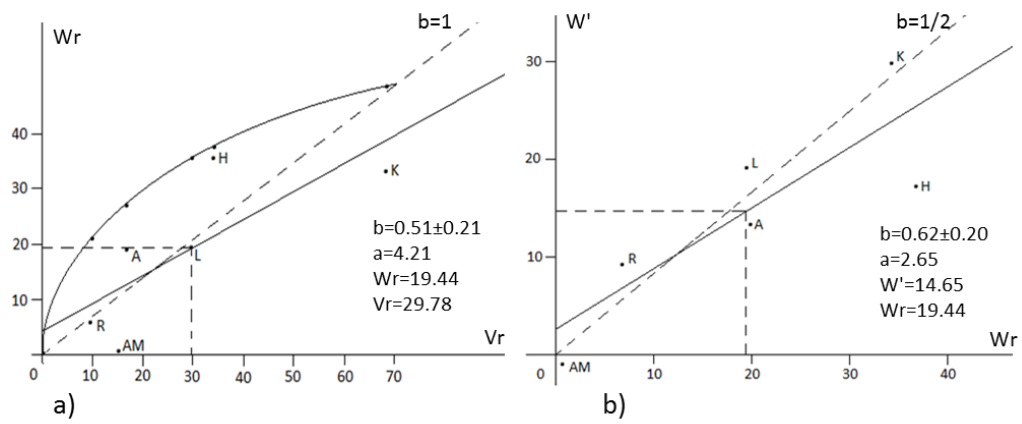
A – Azovsky; K – Kazachy; H – Harkovski; L – Lider; R – Rodnik; AM – Amaian

Figure 1: (a) $VrWr$ regression analysis for seed yield/plant (SYP); (b) WrW' regression analysis for seed yield/plant (SYP)



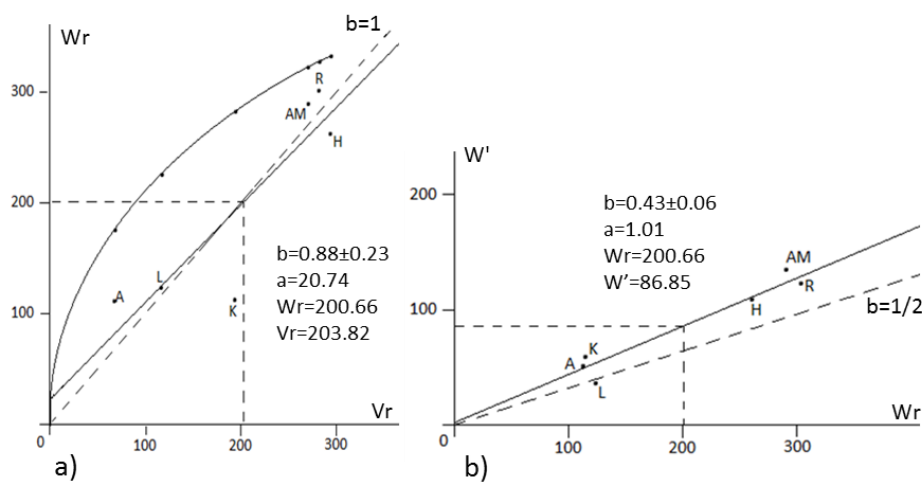
A – Azovsky; K – Kazachy; H – Harkovski; L – Lider; R – Rodnik; AM – Amaian

Figure 2: (a) $VrWr$ regression analysis for oil content (OC); (b) WrW' regression analysis for oil content (OC)



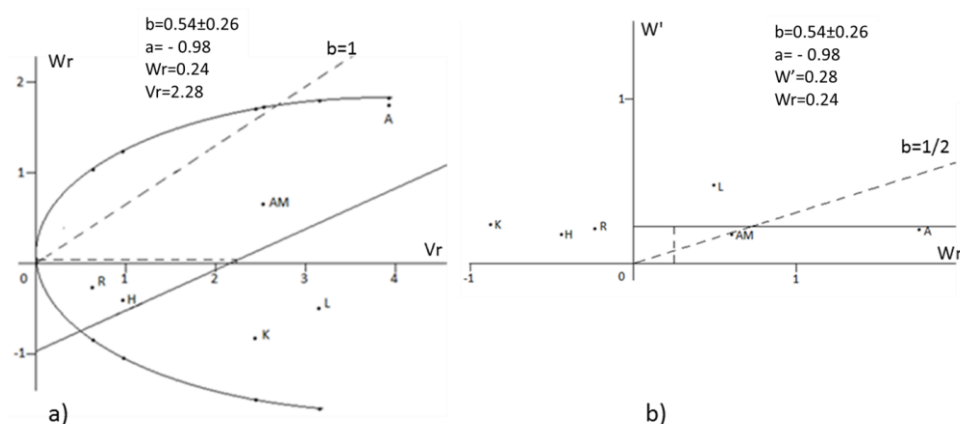
A – Azovsky; K – Kazachy; H – Harkovski; L – Lider; R – Rodnik; AM – Amaian

Figure 3: (a) VrWr regression analysis for 1000 seed weight (TSW); (b) WrW' regression analysis for 1000 seed weight (TSW)



A – Azovsky; K – Kazachy; H – Harkovski; L – Lider; R – Rodnik; AM – Amaian

Figure 4: (a) VrWr regression analysis for plant height (PH); (b) WrW' regression analysis for plant height (PH)



A – Azovsky; K – Kazachy; H – Harkovski; L – Lider; R – Rodnik; AM – Amaian

Figure 5: (a) $V_r W_r$ regression analysis for head diameter (HD); (b) $W_r W'$ regression analysis for head diameter (HD)

CONCLUSIONS

Presented results indicated significant genetic divergence in quantitative traits between sunflower genotypes. Phenotypic variance was higher than genotypic demonstrating strong environment impact in expression of traits. High and moderate heritability estimates were determined for PH, TSW, SYP and HD. Heritability values coupled together with moderate genetic advance for SYP, TSW and PH indicated that selection for these traits can give improvement. Regression analysis for evaluated traits showed that parental genotypes are characterized with uneven distribution of dominant and recessive genes. Placement of array points indicated that the highest frequency of dominant genes for SYP, TSW and HD was found in parental genotype Rodnik. Position of expected line of regression pointed over dominance in inheritance for SYP, OC and HD, while for TSW and PH additive gene action played role in inheritance suggesting that selection in early generations for these traits will be effective. Interallelic interaction was not determined since it was found that regression coefficients did not deviate significantly from unit slope.

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GENETIČKI NAPREDAK I REGRESIONA ANALIZA KOD SUNCOKRETA

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Izvod

Poznavanje veličine i prirode varijabilnosti koja je prisutna u selekcionoj populaciji važan je preduslov za dizajniranje efikasnog oplemenjivačkog programa u cilju poboljšanja potencijala za prinos genotipova. Cilj ovog istraživanja bio je ocena heritabilnosti i genetičkog napretka važnih kvantitativnih osobina u novim ukrštanjima suncokreta kao i procena odnosa dominantnih i recesivnih gena u roditeljskim genotipovima. Biljni materijal odabran za ovo istraživanje se sastojao od 6 genotipova suncokreta, koji prema literarnim podacima imaju važne karakteristike za proizvodnju suncokreta. Prema prikazanim rezultatima postoji značajna varijabilnost ispitivanih kvantitativnih osobina. Fenotipska varijansa bila je veća od genotipske, što je pokazalo snažan efekat spoljašne sredine u ekspresiji osobina. Mera naslednosti u širem smislu je bila veoma visoka za visinu biljke (83,25%), visoka za masu 1000 semena (69,33%), umerena za prinos semena/biljci (46,53%) i prečnik glave (56,89%), dok je za sadržaj ulja bila niska (29,35%). Genetički napredak izražen kao procenat sredine kretao se između 2,23% i 19,96%. Postavljanjem tačaka nizova prikazano je da je najveća frekvencija dominantnih gena za prinos semena/biljci, masu 1000 semena i prečnik glave pronađena unutar roditeljskog genotipa Rodnik. Položaj očekivane linije regresije ukazao je na dominaciju u nasleđivanju za prinos semena/biljci, sadržaj ulja i prečnik glave, dok je za masu 1000 semena i visinu biljke aditivno delovanje gena igralo ulogu u nasleđivanju sugerišući da će selekcija u ranim generacijama za ove osobine biti efikasna. Testiranjem koeficijenata regresije nije utvrđena interalelna interakcija.

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