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Genetic analysis on some yield traits of pea (*Pisum sativum* L.) crosses

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ABSTRACT

The trial was carried during 2011-2013 on the second experimental field of the Institute of Forage Crops – Pleven, Bulgaria. Eight generations (P_1, P_2, F_1, F_2, RC_1 and each reciprocal generation) from a cross between the Shtambovii and Pleven 10 varieties were sown in this study. Five traits were evaluated: number of pods and seeds per plant, seed weight per plant, plant weight and number of fertile nodes per plant were used as components of yield. Positive true heterosis for the investigated traits was found in all hybrids of F_1 generations. At F_1BC - Shtamboviii x Pleven 10 was observed negative heterosis effects for number of pods and seeds per plant and seed weight per plant, and F_1BC - Pleven 10 x Shtambovii for plant weight and number of fertile nodes per plant. In F_2 the plants from Shtamboviii x Pleven 10 had the strongest depression for number of seeds per plant and from Pleven 10 x Shtambovii for plant weight. High level on the indices of transgression was found for all traits. All traits except plant weight and number of fertile nodes per plant had epistatic gene effects. The results showed that for plant weight selection will be more effective if it starts in later hybrid generations. Traits number of fertile nodes and seeds per plant were the characteristics with the highest values for selection.

Key words: additivity, dominance, epistasis, heritability, heterosis

Introduction

Corner stone of the genetics is the Mendel's model that in the present era of the gene engineering technologies serves in genetic analysis of Mendel's discrete units, which in fact are not genes. With other words, the objects of genetic analyses can be not only genes, but also discrete units, which are transferred from the parents to the offspring in probable distribution, but they are not genes (Weir, 1990). The regularity in the inheritance established by Mendel is the fundament on which are build the selection process and improve the methods for selection – genetic assessment of genotypes and the methods of selection (Fisher, 1958; Dragavtcev, 1995; Dragavtcev et al., 2002).

The rise and development of the quantitative genetics is related to clarify the character of genetic control over quantitative trait and the problem with clarifying genetic homeostasis of the genotypes. The effects arise from "genotype – environment" interaction always have been the object of studying and discussion (Bourion et al., 2002;

Arshad et al., 2003; Acikgoz et al., 2009).

The theory for the polymeric genes (polymers) formulated by Nilson-Ele is insufficient for explanation of the inheritance of the quantitative trait. The English-American genetic school developed series quantitative-genetic methods for the needs of selection, which were successfully used all over the world for selection – genetic assessment of the genotypes and for making of selection (Mather, 1949; Allard, 1966; Mather & Jinks, 1982; Hallauer & Miranda, 1982; Hayman, 1954; Griffing, 1956; Gamble, 1962; Fransis & Kanenberg, 1978; Falconer, 1964; Eberhart & Russel, 1966; Tai, 1971; Shukla, 1972; Wrigth, 1952; Becker & Leon, 1988).

The Mendel's model is used in the classical genetics of qualitative traits. At the base of this model are developed methods for Mendel's analysis of quantitative traits as supposed that the last also subordinate the Mendel's laws. In the classic Mendelism in the genetics analysis of the quantitative traits as parameters are reported gene, trait and environment. Unfortunately, the Mendel's system is almost

inapplicable for studying the genetics of the quantitative traits of the main object of selection (Dragavtsev, 1995; Dragavtsev & Kocherina, 2006).

The theory of the genetic analysis of the quantitative traits is developed in the works of Fisher (1958), Mather (1949) and Wrigth (1952).

In the framework of these notions it is possible to be assessing the genetic-statistical parameters of the population only in a given moment from time and in concrete environment. Simultaneously in other moment of the time, in other environment or other sowing density, these parameters are altered in rule rebelled by an unpredictable way. The prognostication value of Mendel's model for quantitative trait is too low. Unfortunately, namely this model lies in the footing of all traditional leaderships and monographs dedicated to the genetics of the quantitative traits and all contemporary books to selection (Hallawer & Miranda, 1982; Mather & Jinks, 1985; Dragavtsev et al., 1984).

The Mendel's model is fundament of the genetics and for analysis discrete inherited units (Weir, 1990) and is commonly genetic base of the selection. The Mendel's analysis of phenotype traits is classic example for genetic analysis. The analysis of genetic data is possible only when it is founded of precise theory or model. The Mendel's model describe and prognosticate the behavior of "grand genes" (oligogenes or block's genes), which perceives as functional genes. According to this model the way "gene-trait" always is synonymous and independent from the environment. The determination of the main genetic parameters of the populations of the base Mendel's model continue to be effective and necessary for creation of fundaments for optimization of the selection programs. It is known now that Mendel's model can be used for analysis of genes, but also for the analysis of all discreet of units that are carried from parents to offspring and dissolve in the inbred offsprings determined by Mendel laws of the inheritance. This is valid from phenotype to molecular level (Weir, 1990).

Until 1958 the theories and methods in the quantitative genetics were based on the Mendel's model (Nilson-Ele, Mather, 1949; Hayman, 1954; Griffing, 1956). The careful analysis of the essence of the Hayman's (1954) and Griffing's (1956) models, which are broadly used in the selection showed that algorithms of the diallel crosses lie at the root of the bases of phenomenological concepts perceived in Mendel's model.

The high influence of the environment over the quantitative trait is known long ago as fact. The pile up data shows the high influence of the environments over the degree of development and variability of quantitative trait at plants. In the development of the genetics after Mendel, the question about the character of the inheritance of the quantitative traits and interaction "genotype – environment" always has been the object of discussion, that continues even now. The origination of the quantitative genetics is related to this discussion. In the selection process are developed and used independent methods of genetic analysis and selection - genetic estimate of the genotypes (Amelin, 2012). The genetic variation of the quantitative and qualitative traits can be resulted from the influence of single genes or of the interaction of multitude genes. The determination of the genetic effect is of high importance for understanding and prediction of the activities of the traits of phenotype level especially when are crossed parents with contrast traits (Changjian et al., 1994; Lou & Zhu, 2002).

Genetic models allow the determination and differentiation of major gene effects. Also those that analyzed the interaction of these effects with an environmental component, permit the selection, with more security, of the types of necessary crosses to increase the presence of important quantitative traits in a crop for the expression of desirable yield traits. This permits the determination of the environmental effect on genotype expression. The genetic information, which is obtained, is fundamental for project of optimal selection program (Gomez & Ligarreto, 2012).

In the present study, a model of generation means was formulated, including maternal effects, to analyze genetic effects on yield traits in pea plants starting from the crossing of two contrasting pea varieties.

Materials and Methods

In this study, two pea varieties were used as parents, which presented morphologically contrasting characteristics in growth habits; the first parental was Shtambovii (P_1) variety (*Pisum sativum* ssp. *sativum*) from Russia and the second one was Pleven 10 (P_2) from Bulgaria variety (*Pisum sativum* ssp. *arvense*) in order to produce the seeds of each one of the eight necessary generations to carry out the genetic model (P_1, P_2, $F_1 = (P_1 \times P_2)$, $F_1R = (P_2 \times P_1)$, $F_2 = (F_1 \times F_1)$, $F_2R = (F_1R \times F_1R)$, $RC_1 = (P_1 \times F_1)$, $RC_2 = (P_2 \times F_1)$). The materials were crossed using the technique of artificial hybridization for emasculation in the pea (Gritton, 1980). Two sowing cycles were performed under field conditions. In

the first cycle, direct and reciprocal crosses were carried out between the Shtambovii and Pleven 10 varieties to obtain seed F_1. 60 plants of each variety were sowed in a row spacing 70 cm and distance in row 5 cm. Hand planting was applied with depth of sowing 5 cm. The seeds from F_1, were sowed in the second cycle, 50 seeds of the direct crosses (F_1) and 50 seeds of the reciprocal ones (F_1R); and 50 seeds of each one of the parents were also sowed, self-pollination was allowed in some flowers of F_1 plants to generate F_2; direct and reciprocal backcrosses were carried out with the remaining flowers toward both parents (RC_1, RC_2). Each material was sown in independent rows space. Five characteristics from each generation were evaluated. The number of pods per plant, number of seeds per plant, seed weight per plant (g), plant weight (g) in maturity and number of fertile nodes per plant were measured as yield components. To evaluate genetic effects and genetic interaction effects, a lineal model was used employing the six basic generations P_1, P_2, F_1, F_2, backcrosses and the reciprocal of these generations were determinated: six types genetic effects, (components of the heterosis): genetic background (m) in F_2 and two types genetic effects - a (aditive), d (dominance), and three types epistasis (c): aa (aditive x aditive), ad (aditive x

dominance), dd (dominance x dominance). Genetic analysis according to Gamble (1962) was used. Reported was the meaning of cytoplasmic effect. Determinates were main genetic parameters in Mendel's genetics: coefficients of heritability narrow sense (h^2) following the method proposed by Warner (Warner, 1952), minimum number of genes on that, distinguish the parents (n) Burton (1952), heterosis effect in F_1, hypothetical and true and depression (Omarov, 1975), degree of dominance in F_1 (h_{p1}) and F_2 (h_{p2}) (Romero & Fray, 1973), degree (Tn) and frequency of transgression (T_f) in F_2 (Voskresenskaya & Shpota, 1971).

Results

No significant differences were found between the parentals in all studied variables (Table 1). In the generations F_1 did not present significant differences between direct and their reciprocal for all traits. Cytoplasmic effect was found for number of pods per plant. In F_2 the differences were found only for seed weight per plant and in the backcrosses for plant weight in maturity and the number of fertile nodes per plant.

Table 1. *Description of pea genotypes (P_1; P_2; F_1; F_2; F_1BC).*

Hybrids	P_1	P_2	F_1	F_2	F_1BC
number of pods per plant					
Shtamboviii x Pleven 10	5.3 [a]	14.25 [abc]	18 [bc]	24.85 [cd]	9.45 [ab]
Pleven 10 x Shtambovii			10.7 [ab]	30 [d]	15.85 [abc]
number of seeds per plant					
Shtamboviii x Pleven 10	13.4 [a]	46.15 [ab]	61.75 [ab]	89.1 [bc]	29.85 [a]
Pleven 10 x Shtambovii			31.4 [a]	139.85 [c]	45.68 [ab]
seed weight per plant (g)					
Shtamboviii x Pleven 10	4.7 [a]	6.58 [ab]	11.55 [b]	11.3 [b]	2.99 [a]
Pleven 10 x Shtambovii			6.32 [ab]	22.68 [c]	7.09 [ab]
plant weight (g) in maturity					
Shtamboviii x Pleven 10	9.6 [a]	16.8 [abc]	21.7 [bcd]	26.63 [cd]	28.65 [d]
Pleven 10 x Shtambovii			29.79 [d]	27.56 [d]	13.75 [ab]
number of fertile nodes per plant					
Shtamboviii x Pleven 10	1.52 [a]	4.41 [a]	10.41 [b]	12.41 [b]	10.27 [b]
Pleven 10 x Shtambovii			10.25 [b]	11.32 [b]	4 [a]

a,b,c,d - statistically proven differences in P=0.05

Inheritance of traits determining the yield of pea (Pisum sativum L.)

The data for pod number per plant (Table 2) showed that the hybrids (F_1; reciprocal and F_1BC-Pleven 10 x Shtambovii displayed positive real and hypothetical heterosis. Only at F_1BC - Shtamboviii x Pleven 10 was observed negative heterosis effects (-3.32; -33.68). The plants of both crosses were with comparatively high negative depression. At this trait prevalent the epistatic gene effects ($hp_1 < hp_2$). The seed number per plant was inherited in an overdominant at Shtamboviii x Pleven 10 and epistatic way in its reciprocal. The hybrid with mother's form Shtamboviii manifested strong true heterotic effects (107.39%). In the reciprocal backcross was observed opposite heterosis behaviour - Pleven 10 x Shtambovii (240.90%) and very low depression (-183.76%).

For the other studied triats F_1 hybrids also showed positive heterosis as hypothetical (from 11.30% in Shtamboviii x Pleven 10 for seed weight per plant to 27.56% in Pleven 10 x Shtambovii for plant weight in maturity) so real (from 12.06% in Pleven 10 x Shtambovii for seed weight per plant to 251.10% in Shtamboviii x Pleven 10 for number of fertile nodes per plant). In the backcrosses high positive real heterosis was obtained only for number of fertile nodes per plant (132.88%) and plant weight in maturity (70.54%) in Shtamboviii x Pleven 10. With low depression were characterized the crosses in those traits with exception on Pleven 10 x Shtambovii for plant weight (g) in maturity where the depression was the higtest (7.49%). The inheritance of those traits can be interpreted as epistatic gene effects ($hp_1 < hp_2$).

All crosses (Figure 1) showed high positive behavior of degree of transgression (above 51%). This probably was due to the rich inheritable base of the parent's forms. The values on this index in direct cross (Shtamboviii x Pleven 10) were higher than reciprocal of number of pods and seeds per plant and seed weight per plant respectively 91%; 183% and 197%. The duration of cross have influence over the productive values on almost all traits. The values for frequency of transgression also were high for all traits with exception on Shtamboviii x Pleven 10 for plant weight in maturity. According to the observed data for these parameters in both crosses can be expected homozygous genotypes with increased characteristics on the studies traits.

Table 2. *Biometrical data of the quantitative traits of the investigated crosses in F_1 and F_2.*

Hybrids	Heterosis F_1, (%)		Heterosis F_1BC, (%)		Depression in F_2 (%)	Degrees of dominance		
	hypothetical	real	hypothetical	real		in F_1 h_{p1}	in F_2 h_{p2}	$hp_1 >$ $<hp_2$
number of pods per plant								
Shtamboviii x Pleven 10	24.85	84.14	-3.32	-33.68	-38.06	1.84	6.74	<
Pleven 10 x Shtambovii	30	9.46	62.15	11.23	-180.37	0.21	9.04	<
number of seeds per plant								
Shtamboviii x Pleven 10	38.35	107.39	0.25	-35.32	37.89	1.95	1.05	>
Pleven 10 x Shtambovii	89.10	5.46	53.42	240.90	-183.76	0.10	7.25	<
seed weight per plant (g)								
Shtamboviii x Pleven 10	11.30	104.79	-46.99	-54.56	2.16	6.29	12.04	<
Pleven 10 x Shtambovii	22.68	12.06	25.71	7.75	-258.86	0.72	36.26	<
plant weight (g) in maturity								
Shtamboviii x Pleven 10	26.63	64.39	117.05	70.54	-22.72	2.36	7.46	<
Pleven 10 x Shtambovii	27.56	125.68	4.17	-18.15	7.49	4.61	7.98	<
number of fertile nodes per plant								
Shtamboviii x Pleven 10	12.41	251.10	246.37	132.88	-19.21	5.15	13.07	<
Pleven 10 x Shtambovii	11.32	245.70	34.91	-9.30	-10.44	5.04	11.56	<

Heterosis: real - (F_1-HP/) HP*100; hypothetical - (F_1-MP)/MP*100; MP - mid parent value (P_1+ P_2)/2; HP - best parent value; Depression (%) - (F_1-F_2)/F_1*100; Degrees of dominance h_{p1-} (F_1-MP)/D; hp_2 -(F_2-MP)/D; D-(HP-MP)

RESEARCH ARTICLE

Analysis of genetic effects of the genes on yield traits of pea (Pisum sativum L.)

Genetic analysis by Gamble (1962) discloses in detail most gene effects that are parts of heterosis and have contradictory action. The set of locuses, the effects of which determinated aditive genetic variability of species represent the genetic formula of trait. Each mutability of environmental conditions endangers ecological mutability as there are no changes only in the activity of locuses of constant genes from polygene systems (Dragavtcev, 1995).

In Table 3 are presented the results from measuring of comparative parts of different types gene effects: aditive (a), dominance (d) epistasis (c) – include aditive x aditive (aa), aditive x dominance (ad) and dominance x dominance (dd) in inheritance of investigated traits. For all traits was high significant the influence and the participation of aditive, dominance and epistasis gene action (aa and ad). Significant differences were not found for „dd" gene action.

Number of pods and seeds per plant. From the complex analysis of the relative part of the genetic effects on inheritance of these traits was determined that epistasis gene interactions (ad) making to reinforcement on characters, aditive (a) and dominant (d) gene effects reduce their behaviour. Differently was degree of influence of the epistasis gene actions on the crosses, as more higher it was for number seeds per plant.

Seed weight per plant. The analysis for relatively part of the gene effects for seed weight showed explicit prevail ascendancy of epistatic gene interaction. Very low influence on additive and dominant effects over this trait was established.

Plant weight in maturity. In the inheritance on this trait additive gene effects (a) were the most important followed by epistatics (ad), which were with relatively high significant. Dominant gene actions (d) were depressed trait activity and in both hybrids.

Number of fertile nodes per plant. From the data for specific gene effects could be seen that traits were under conditions of complex genetic control. The additive gene effects were with clear domination toward other gene effects. The epistatic gene effects were significant but with weaker influence.

The participation in crosses of parental components distinguishes number of genes (Figure 2) at the most traits, which probably is due to the hereditary data of initial varieties participated in the selection of the parents as the mother's effect of the cytoplasm. Essential differences in this indicator between direct and reciprocal crosses for number of pods and seeds per plant were obtained. The parental varieties were distinguished for relatively smaller number of genes for fertile nodes per plant and they showed similarity for seed weight per plant and plant weight.
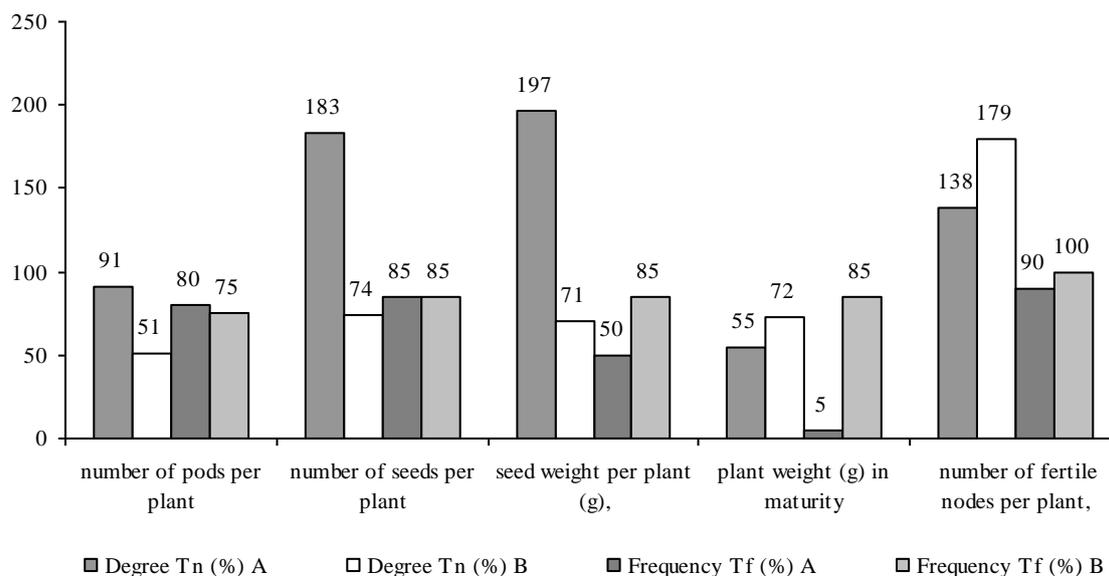

Figure 1. *Degree Tn (%) and frequency T_f(%) of transgression in F_2, (A) Shtamboviii x Pleven 10; (B) Pleven 10 x Shtambovii.*

Table 3. *Estimates of the genetic effects and parameters governing the inheritance of investigated traits.*

Crosses	Gene effects					
	genetic background	aditive	dominance	epistazis		
	M (F$_2$)	a	d	aa	ad	dd
number of pods per plant						
Shtamboviii x Pleven 10	24.85***	-6.40***	-40.58***	-48.80***	6.64***	53.75[ns]
Pleven 10 x Shtambovii	30***	-6.4***	-68.47***	-69.4***	7.13***	59.75[ns]
number of seeds per plant						
Shtamboviii x Pleven 10	89.10***	-15.83***	-173.36***	-205.33***	90.69***	237.31[ns]
Pleven 10 x Shtambovii	139.85***	-15.83***	-406.70***	-408.33***	207.96***	379.613[ns]
seed weight per plant						
Shtamboviii x Pleven 10	11.30***	-4.10***	-17.95***	-25.04***	2.17***	36.92[ns]
Pleven 10 x Shtambovii	22.68***	-4.10***	-69.90***	-70.57***	5.27***	74.35[ns]
plant weight (g) in maturity						
Shtamboviii x Pleven 10	26.64***	14.91***	-13.24***	-21.74***	10.76**	6.75[ns]
Pleven 10 x Shtambovii	27.56***	14.90**	-8.86***	-25.45***	9.50*	26.64[ns]
number of fertile nodes per plant						
Shtamboviii x Pleven 10	12.42***	6.28***	-13.67***	-21.12***	1.01***	19.33[ns]
Pleven 10 x Shtambovii	11.32***	6.27***	-9.46***	-16.74***	0.63***	14.63[ns]

** significant differences at P≤0.01; *** significant differences at P≤0.00; ns: non-significant parameter
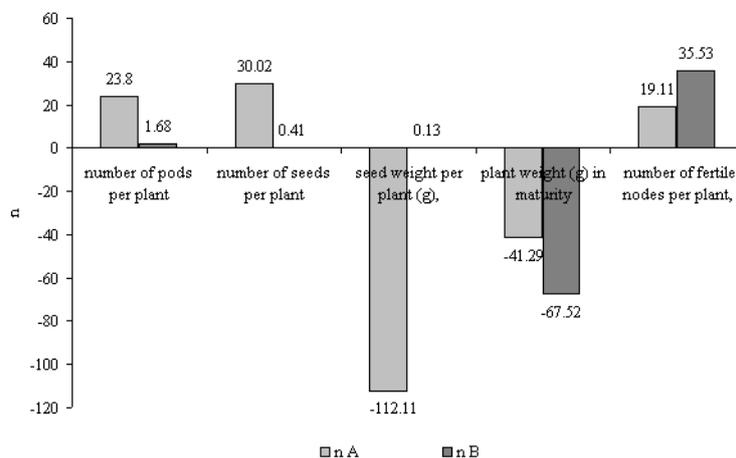
Figure 2. *Minimum number of genes (n) on that are distinguished from the parents (Burton, 1952), (A - Shtamboviii x Pleven 10; B- Pleven 10 x Shtambovii) n = ((0.25*(0.75 h+(h*h))*(D*D))/(S$^2_{F2}$-S$^2_{F1}$); h= (F$_1$-P$_1$)/D; D=P$_1$-P$_2$.*

The part from common variability (Figure 3) conditioned from genetic differences was determined through using coefficient of heritability (h^2). The inheritance is the character of the relative part of the genetic differences and these which are result of the action of the environment in the phenotypic diversity. At change of genotype or the environment follow and variation of the assessment for inheritability.

For plant weight were established low value of narrow sense heritability, which show that the environmental conditions have high influence in trait determination and selection will be more effective if it starts in later hybrid generations (F$_6$-F$_7$). The results for other traits showed relatively high heritability, stronger manifest in reciprocal hybrid. The highest heritability was obtained for fertile nodes per plant, which indicate that individual selection can begin in the early generations (F2 – F3).
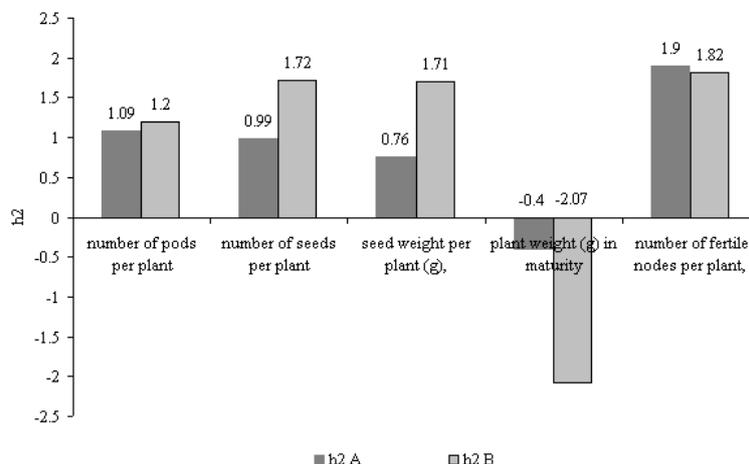
Figure 3. *Coefficients of heritability narrow sense (h^2) (by Warner, 1952), (A - Shtamboviii x Pleven 10; B- Pleven 10 x Shtambovii) $h^2 = 2S^2_{F2} - (S^2_{RC1} + S^2_{RC2})/S^2_{F2} *100$; where S^2_{P1}; S^2_{P2}; S^2_{F1}; S^2_{RC1}; S^2_{RC2} and S^2_{F2} are the variances of P_1, P_2, F_1, RC_1, RC_2 and F_2.*

Discussion

The obtained experimental data were in agreement with published results by other authors (Borah, 2009), which reported for behavior of negative or feebly positive heterosis for traits of pea. Espinosa & Ligarreto (2005) reported on activity of negative heterosis for 1000 seed mass and for different heterosis for seed weight per plant. Other studies have shown that number of pods and seeds per plant presents a higher additive effect than the non-additive action (Gomez & Ligarreto, 2012). Narsinghani et al. (1982) and Sharma et al. (2013) reported that additive genetic variance in pea was significant for seed yield per plant, while epistatic gene action was positive for number of pods and seeds per plant. There was a positive additive, dominance and over dominance gene action for seeds per plant.

For self-pollinated crop (as a pea *Pisun sativum* L.), is preferable to be used narrow sense heritability which reflects additive genetic contribution to phenotypic expression of traits, because only additive gene effects can be fixed to progeny. When triats have high values for narrow sense heritability, it is recommended to begin selection in F2 and in these cases can be successful applied pedigree selection (Lupu et al., 2012). As a general rule, traits controlled by a small number of genes show high heritability in early generations, permitting the fixation of distinct genotypes by using a small number of selfing generations (Anand & Torrie 1963; Shivali et al., 2013). Obtained values for coefficients of heritability confirm results by other authors (Shinde, 2000),

which reported for moderate to high inheritance of investigated from them quantitative traits.

Positive true heterosis heterosis for the investigated traits was found in all hybrids of F_1 generations. At F_1BC - Shtamboviii x Pleven 10 was observed negative heterosis effects for number of pods and seeds per plant and seed weight per plant and F_1BC - Pleven 10 x Shtambovii for plant weight and number of fertile nodes per plant. In F_2 the plants from Shtamboviii x Pleven 10 had the strongest depression for number of seeds per plant and from Pleven 10 x Shtambovii for plant weight. High level on the indices of transgression was found for all traits. All traits except plant weight and number of fertile nodes per plant had epistatic gene effects. The results showed that for plant weight, selection will be more effective if it starts in later hybrid generations (F_6-F_7). On traits number of fertile nodes and seeds per plant were the characteristics with the highest values for selection.

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