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Heritability of Qualitative Traits in Forage Pea (*Pisum Sativum* L.)

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Abstract: In breeding process of forage pea (*Pisum sativum* L.) inter variety crosses between two Bulgarian: *Pleven 4* (spring form) and *Pleven 10* (winter form) and two Russian: *Rosacrono* (spring form) and *Shtambovyi* (winter form) varieties are effectuated in field crop experiment during 2011-2013 on the Second experimental field of the IFC, Pleven, Bulgaria. Populations of P_1, P_2, F_2 and F_1 of the crosses *Shtambovyi x Pleven 10* and *Rosacrono x Pleven 4* are investigated. The aim of this study is to establish the inheritance type of some forage quality parameters as protein, fiber components and digestibility between inter variety hybrids of forage pea. For the hybrids of F1 are estimated changes of heterosis effect. The cross: *Shtambovyi x Pleven 10* is characterized with the highest positive real heterosis effect for crude protein content and the cross: *Rosacrono x Pleven 4* have the highest rate for crude fiber, Acid-detergent fiber and Acid-detergent lignin. The parameters crude protein and crude fiber for the both crosses are inherited positively over dominantly. Digestibility *in vitro* dry matter is inherited negatively over dominantly as the qualities of varieties *Pleven 10* and *Rosacrono* are predominated. In the second generation F2 for almost all parameters for *Shtambovyi x Pleven 10* a negative depression is estimated. According to the estimated values for the parameter crude protein contents the plants of the two hybrids are depressed most strongly. Because of parameters crude fiber, Neutral-detergent fiber, Acid-detergent fiber and *in vitro* digestibility of dry matter for the cross *Shtambovyi x Pleven 10* a great her percentage of transgressive plants could be anticipated as it counts for crude protein for the cross *Rosacrono x Pleven 4.* As a result of predominating negative epistatic interaction of cross *Shtambovyi x Pleven 10* for crude protein, Neutral-detergent fiber and Acid-detergent lignin a regression of the degree phenotype exhibition of these signs in comparison with the full additive inheritance is anticipated. It is established that there is a high inheritance coefficient in both crosses for the parameters crude protein, crude fiber, Neutral-detergent fiber and *in vitro* digestibility of dry matter.

Keywords: *Pisum sativum* L., Inheritance, Transgression, Protein, Cell Walls Fibers, *In vitro* Digestibility

Introduction

Grain legume crops are important source of protein, energy, vitamins and minerals and are a trully significant factor in nutrition of ruminants and monogastric livestock. Worldwide the forage pea (*Pisum sativum* L.) is among the four most important annual legume crops together with soybean, peanut and haricot bean (Hulse, 1994; Bansal *et al*., 2011). In Bulgaria forage pea is the most exhibited legume crop in animal nutrition compared to other annual legumes. The above ground plant parts could be used as valuable forage or silage, haylage, hay or dehydrate preparation. Its grain contains 24-26% protein with predominating soluble fractions and could be considered as a positive substitute of soybean meal (Cousin *et al*., 1985; Kirilov, 2005; Gatti *et al*., 2011). The quantity and the quality of the protein in the seeds are important parameters for evaluation of the feeding value

of forage pea. Legume crops investigations show that protein content is characterized with high degree of inheritance, which is a good possibility for selection of high protein forms. The biological characteristics of pea make it possible to be grown successfully in Bulgaria, not only as a spring, but also as a winter crop. Winter pea varieties accumulate more biomass than the spring ones. They assure more stable yield by using the autumn moisture and avoid spring drought (Angelova, 1995).

The protein in the seeds represents the hereditary traits of the genotype and is not influenced by the environment in the vegetation. They could be used as markers for solving certain protein problems. Although yield is the most important criteria in the breeding process, the features concerning the forage quality are becoming increasingly significant. Between different forms there are definitive variations in protein content and other chemical components which define the forage feeding value. The forage plant cell walls fiber components, natural polymer lignin and complexes between them are principal parameters of forage quality because in their degradability, they are the nutritive and energy source for ruminants. Fiber components content determination as new parameters of forage quality is standardized in EC and will be more significant (ENISO 13906, 2008).

Plant cell walls fiber components also determine digestibility of forage dry matter (Brink *et al*., 2007; Fahey and Hussein, 1999). Increased digestibility by 1% may increase animal growth by 10%. The digestibility, determined *in vitro* by enzymes is rapid and promising method and it application in plant sciences, when small quantity of large number accessions from different species, varieties, genotypes, growths, must be evaluated in early stages in breeding process or technological decisions (Casler *et al*., 2000). Biochemical characteristics of Bulgarian and introduced forage pea varieties are established (Naydenova *et al*., 2008; 2012; Najdenova *et al*., 2010; Naydenova and Todorova, 2009; Kirilov *et al*., 2010). Systematization and evaluation of information obtained by biochemical analyses of the distinct pea types provide additional opportunities for increasing of breeding process efficiency (Adsule *et al*., 1989; Buxton, 1996; Humphreys and Theodorou, 2001; Angelova and Stoilova, 2009).

The aim of this study is to establish the inheritance type of forage quality parameters as protein, fiber components and in enzyme *in vitro* digestibility between intervariety hybrids of forage pea.

Materials and Methods

The experimental study is conducted during the 2011-2013 period on the Second experimental field of the Institute of Forage Crops, Pleven, Bulgaria. The parent-form used for crossing are from our collection: Spring forms (*Pisum sativum ssp. sativum*)-Shtambovyi and Pleven 4, winter types (*Pisum sativum ssp. arvense*)-Rosacrono and Pleven 10. The characteristics of these varieties were reported in Table 1. These forms are crossed by hand in 2011.

The parental forms (P_1 and P_2) and first and second generation (F_1 and F_2) are sown at scheme P_1, P_2, F_2, F_1 on a row spacing 70 cm and distance in a row 5 cm. Hand planting is applied with depth of sowing 5 cm. The forage pea is grown by approved technology of the Institute of Forage Crops-Pleven. The plants are harvested at three phenological stages of plant development-budding stage, beginning of flowering stage and full pod formation stage.

Plant sample preparation from the above ground part of the plants is effectuate by air ventilation at 65°C till crumbly at previous fixing for 20 min at 105°C and grinding till particle size 1.0 mm consecutively at laboratory mills QC 136 and QB 114, Labor Mim, Hungary and obligatory screen. A biochemical assessment of the parental forms (P_1 and P_2) and crosses of first and second hybrid generation (F_1 and F_2) is

Table 1. Origin and description of pea genotypes

Variety	Pleven 10	Pleven 4	Shtambovyi	Rosacrono
Origin	Bulgaria	Bulgaria	Russia	Russia
Vine type	Long-prostrate	Long semi-erect	Short semi-erect	Long semi-erect
Flower position	Axial	Axial	Terminal (with fasciation-Fa, FAC)	Terminal (with fasciation-FA, FAC)
Stipule type	Normal	Normal	Double	Double
Leaf type	Normal	Normal	Normal	Normal
Flower color	Purple	White	White	Pink
Crude protein	24.41	23.13	23.02	23.40
Crude fiber	23.63	22.65	24.82	24.31
Neutral-detergent fiber	35.37	33.73	36.03	36.05
Acid-detergent fiber	26.98	28.04	32.48	30.63
Acid-detergent lignin	4.20	4.65	5.71	5.12
In vitro digestibility of dry matter	72.23	70.87	73.19	69.78

performed by the following characteristics: *Crude Protein* (CP) by Kjeldhal method; *Crude Fiber* (CF)-by Heneberg and Stoman method as parameters of Weende systematic analytic procedure (AOAC, 2007) and plant cell walls fiber fractions *Neutral-Detergent Fiber* (NDF); *Acid*-Detergent Fiber (ADF), *Acid*-Detergent Lignin (ADL) as parameters of detergent analysis of Goering and Van Soest (1970; ENISO 13906, 2008). Enzyme *in vitro* Digestibility of Dry Matter (IVDMD) is determined by two stage pepsin-cellulase enzyme method of Aufrere (Todorov *et al*., 2010).

For each studied trait were determined: Heterosis effect in F_1-hypothetical and true and depression (Omarov, 1975), degree of dominance in F_1 (h_{p1}) and in F_2 (h_{p2}) (Romero and Frey, 1973); heritability coefficient in broad sense (H^2), in F_2; Transgression (T_n) Epistasis (E), number of the genes which parental forms are distinguish between for the certain trait (N) (Sobolev, 1976). The statistical processing of the experimental data was performed by the programmes Excel of Microsoft Office 2003 for Windows.

Results

The significance in forage quality evaluation is decreasing of cell walls fiber components content, determined as Neutral-detergent fiber, Acid-detergent fiber, Acid-detergent lignin and increasing *in vitro* digestibility of dry matter are. The structural polyosides in forage plants present from 300 to 800 g kg^{-1} (30-80%) of forage dry matter and they are the general source of nutritional energy for ruminants, but less than 50% of them are digestible and utilized (Fahey and Hussein, 1999). The Neutral detergent fiber present the total content of plant cell walls fiber components of lignin, cellulose, hemicellulose and are laboratory parameter for prediction of forage intake by ruminants. That's why they are criteria in breeding process and evaluation of forage crops (Casler *et al*., 2000). The Acid detergent fiber is the fraction, contained plant cell walls lignin and cellulose which determine forage digestibility.

The basic criteria, effectuated evaluation in forage quality is *in vitro* digestibility by enzymes, characterized by rapidity, reproducibility and heredity, small sample quantity and direct correlation by *in vivo* ruminant digestibility (Fahey and Hussein, 1999). The variation range of *in vitro* digestibility usually is 100 g kg^{-1} dry matter. The breeding process for high protein content led to increased *in vitro* digestibility. Long-period breeding programs for increased protein content are combined with decreasing Acid-detergent fiber (lignocellulose) content. The combined genetic changes may increase digestibility by 16 g kg^{-1} (2.1%). It is proved that the genetic changes in *in vitro* digestibility were due to genetic changes in plant development.

The variability of parameters with broad genetic basis is a good prediction for increasing of the genetic variability and attaining forms with new, not only quantity but also quality parameters (Burstin *et al*., 2011). From the biometric data presented in Table 2 it is seen that for all parameters studied hybrids of first generation develop positive hypothetic heterosis.

Positive real heterosis is established by parameters for crude protein and crude fiber, as for the cross *Shtambovyi x Pleven 10* it reaches up to 11.27% for crude protein and for the cross *Rosacrono x Pleven 4* up to 5.09 for crude fiber. For the crass *Shtambovyi x Pleven 10* values for the other biological and biochemical characteristics Neutral-detergent fiber, Acid-detergent fiber, Acid-detergent lignin and *in vitro* dry matter digestibility are lower compared to the parent with better features. The developed real negative heterosis in this cross is accompanied by lower depression of second generation plants. Crude protein and crude fiber in both crosses are inherited positively dominantly and over dominantly (Fig. 1). For the parameter *in vitro* dry matter digestibility the inheritance type is negatively overdominant as the qualities of variety *Pleven 10* and variety *Rosacrono* predominate. Differences in inheritance parameters Neutral-detergent fiber, Acid-detergent fiber, Acid-detergent lignin are established. Positive overdomination in hybrid *Rosacrono x Pleven 4* is (4.04, 3.54, 3.13% respectively) and negative (171-1.21%) respectively for Neutral-detergent fiber and Acid-detergent fiber and intermediate (- 0.42% for Acid-detergent lignin) in hybrid *Shtambovyi x Pleven 10.* Higher values in domination rate in first generation to second ones (h_{p1}>hp_2) for the cross *Rosacrono x Pleven 4* show that when studied parameters are inherited, dominance has great significance, i.e., the genes determining higher content of parameters dominate. Inheritance of parameters crude protein, Neutral-detergent fiber and Acid-detergent lignin in the cross *Shtambovyi x Pleven 10* is determined by epistatic gene effects (h_{p2}>h_{p1}).

Positive values for transgression parameter in all characteristics in both crosses are established. This suggests that in the distributing hybrid generations the higher percentage of homozygous genotypes will have higher values for the parameters in comparison with the primary varieties. For the cross *Shtambovyi x Pleven 10* a greater percentage of transgressive plants could be anticipated relating to crude fiber, Neutral-detergent fiber, Acid-detergent fiber and *in vitro* dry matter digestibility and for the cross between variety *Rosacrono* and variety *Pleven 4*- relating to crude protein (3.78%).

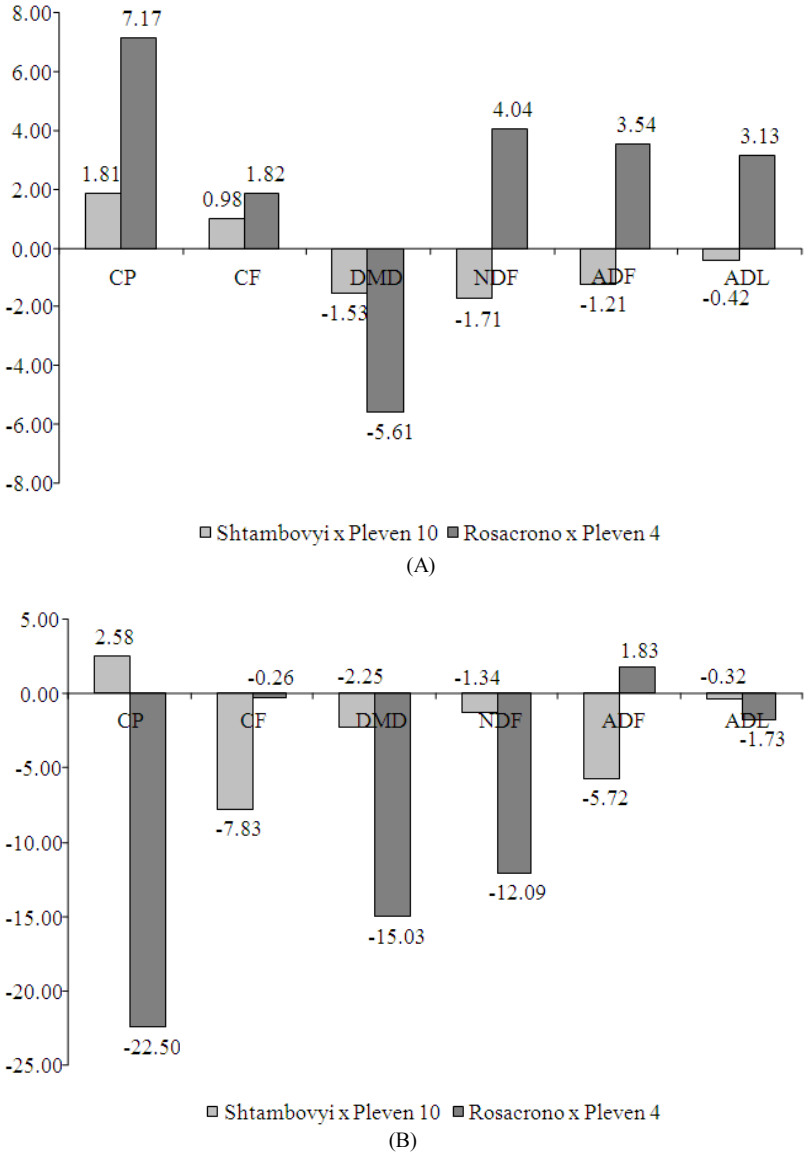

Fig.1. Degrees of dominance (A) degree of dominance in F1 (hp1) (B) degree of dominance in F2 (hp2); CP-Crude Protein; CF-Crude Fiber; NDF-Neutral-Detergent Fiber; ADF-Acid-Detergent Fiber; ADL-Acid-Detergent Lignin; IVDMD-*in vitro* Digestibility of Dry Matter

The parental components participating in the crosses differ in the number of genes in most of the studied features which probably is due to heredity abilities of primary varieties that participate in the selection of the parents. Significant variations of this parameter are established between varieties *Rosacrono x Pleven 4* as well as by parameters crude protein (5), Neutral-detergent fiber (23), Acid-detergent lignin (9) and *in vitro* dry matter digestibility (16) and between varieties *Shtambovyi x Pleven 10* by crude fiber (16) and Acid-detergent lignin (8). In the analysis of values for parameter of interallelic interactions (E) it could be seen that for greater part of the studied parameters epistasis is positive. It is negative only for the cross *Shtambovyi x Pleven 10* for crude protein, Neutral-detergent fiber and Acid-detergent lignin and it could be assumed that this would decrease the rate of phenotype exhibition in comparison with their full additive inheritance.

Coefficients of inheritance for both crosses show that there is greater genetic part in phenotype exhibition of parameters of crude protein, crude fiber, Neutral-detergent fiber content and *in vitro* dry matter digestibility. It could be assumed that there is strong influence of environmental factors on phenotype expression of Acid-detergent lignin number for both hybrids and Acid-detergent fiber for hybrid *Rosacrono x Pleven 4.*

Table 2. Biometrical data of the qualitative traits of the crosses

Hybrids	F_1	F_2	Heterosis in F_1 (%)		Depression in F_2 (%)
			Hypothetical	Real	
Crude protein					
Shtambovyi x Pleven 10	18.30	21.71	21.08	11.27	4.76
Rosacrono x Pleven 4	20.96	21.94	19.73	4.08	3.49
Crude Fiber					
Shtambovyi x Pleven 10	22.71	23.73	21.21	2.17	-0.04
Rosacrono x Pleven 4	22.44	24.26	23.00	5.09	2.23
Neutral Detergent Fiber					
Shtambovyi x Pleven 10	36.83	36.42	37.02	-2.63	-4.11
Rosacrono x Pleven 4	37.18	38.34	36.02	2.49	1.86
Acid Detergent Fiber					
Shtambovyi x Pleven 10	30.29	30.09	28.53	-3.67	-6.49
Rosacrono x Pleven 4	29.26	31.44	30.18	5.72	4.04
Acid Detergent Lignin					
Shtambovyi x Pleven 10	5.15	5.24	5.28	-1.23	-4.03
Rosacrono x Pleven 4	5.09	5.40	5.10	4.55	3.05
***In vitro* dry matter digestibility**					
Shtambovyi x Pleven 10	68.78	67.94	68.58	-3.45	-5.59
Rosacrono x Pleven 4	70.23	68.8	68.21	-2.47	-2.89

F1 and F2 - first and second generation;

Table 3. Values of the gene parameters for the quantitative traits of the investigated crosses in F_2 generation

Crosses/Indicators	T_n	N	E	H^2
Crude protein				
Shtambovii x Pleven 10	1.59	1.00	-0.46	0.27
Rosacrono x Pleven 4	3.78	5.00	1.94	0.53
Crude fiber				
Shtambovii x Pleven 10	2.48	16.00	4.35	0.42
Rosacrono x Pleven 4	1.62	3.00	1.18	0.25
Neutral detergent fiber				
Shtambovii x Pleven 10	4.26	1.00	-0.11	0.58
Rosacrono x Pleven 4	1.02	23.00	5.94	0.43
Acid detergent fiber				
Shtambovii x Pleven 10	2.39	8.00	2.56	0.24
Rosacrono x Pleven 4	0.90	3.00	1.19	0.09
Acid detergent lignin				
Shtambovii x Pleven 10	0.14	1.00	-0.06	0.04
Rosacrono x Pleven 4	0.20	9.00	2.61	0.13
***In vitro* dry matter digestibility**				
Shtambovii x Pleven 10	2.07	1.00	0.63	0.37
Rosacrono x Pleven 4	1.57	16.00	4.62	0.32

H^2-heritability coefficient in broad sense; T_n-transgression; E-epistasis; N-number of the genes which parental forms are distinguish between for the certain trait;

Demonstration of heterosis effect is established in the hybrid of first generation. The cross *Shtambovyi x Pleven 10* is characterized with a high positive real heterosis for crude protein content and the cross *Rosacrono x Pleven 4*-for traits crude fiber, Acid-detergent fiber and Acid-detergent lignin content. The parameters crude protein and crude fiber for both crosses are inherited positively overdominantly. Digestibility *in vitro* dry matter (IVDMD) is inherited negatively overdominantly as the characteristics of varieties *Pleven 10* and *Rosacrono* predominate.

In the second generation F2 for almost all parameters in hybrid *Shtambovyi x Pleven 10* a negative depression is established (Table 3). The plants of the two hybrids are most strongly depressed by the parameter of crude protein content. For the cross *Shtambovyi x Pleven 10* by the parameters crude protein, crude fiber, Acid-detergent fiber and *in vitro* dry matter digestibility a greater percentage of transgressive plants could be anticipated, while in crude protein content-in cross *Rosacrono x Pleven 4.* As a result of predominating negative epistatic interactions in the cross *Shtambovyi x Pleven 10* for crude protein, Neutral-detergent fiber and Acid-detergent

lignin content it could be assumed that there might be a decrease of phenotype manifestation rate of these characteristics compare to their full additive inheritance. For both crosses it is established that there is a high coefficient of inheritance for the parameters crude protein, crude fiber, Neutral-detergent fiber and *in vitro* dry matter digestibility.

Discussion

Inheritance of biochemical parameters is important factor for performing effective selection of genotypes in quality characteristics. According to Saxena *et al*. (2002; Frimpong *et al*., 2009) the agroclimatical conditions significantly influence the protein content in grains for most legume crops. Other authors (Matthews and Arthur, 1985) report about relatively constant protein content and similar gene actions in forage pea genotypes grown in different environmental conditions. Singh *et al*. (1986) analyzing the data of diallelic analysis in forage pea varieties report about predominating in additive gene actions in terms of crude protein content. Noubissie *et al*. (2012) observed the similar trends for crude protein in common bean (*Phaseolus vulgaris* L.) cultivars. In soybean, Tajuddin (2005) noted that seed protein content was quantitatively inherited and controlled mainly by genes with additive genetic effects. In contrast, the crude protein content appeared to be controlled by overdominance effects and associated with recessive genes in cowpea (Tchiagam *et al*., 2011). Ceyhan *et al*., (2014) also reported for the prevalence of nonadditive gene action for crosses protein in pea crosses.

Conclusion

The cross: *Shtambovyi x Pleven 10* is characterized with the highest positive real heterosis effect for crude protein content and the cross: *Rosacrono x Pleven 4* have the highest rate for crude fiber, Acid-detergent fiber and Acid-detergent lignin. The parameters crude protein and crude fiber for the both crosses are inherited positively over dominantly.

Digestibility *in vitro* dry matter is inherited negatively over dominantly as the qualities of varieties *Pleven 10* and *Rosacrono* are predominated. In the second generation F2 for almost all parameters for *Shtambovyi x Pleven 10* a negative depression is estimated. According to the estimated values for the parameter crude proteins content the plants of the two hybrids are depressed most strongly. Because of parameters crude fiber, Neutral-detergent fiber, Acid-detergent fiber and *in vitro* digestibility of dry matter for the cross *Shtambovyi x Pleven 10* a great her percentage of transgressive plants could be anticipated as it counts for crude protein for the cross *Rosacrono x Pleven 4.*

As a result of predominating negative epistatic interaction of cross *Shtambovyi x Pleven 10* for crude protein, Neutral-detergent fiber and Acid-detergent lignin a regression of the degree phenotype exhibition of these signs in comparison with the full additive inheritance is anticipated.

It is established that there is a high inheritance coefficient in both crosses for the parameters crude protein, crude fiber, Neutral-detergent fiber and *in vitro* digestibility of dry matter.

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Author's Contributions

Valentin Kosev: Wrote the manuscript, participated in all field experiments, conceived carried out of the statisticall analysis, read and approved the final manuscript.

Yordanka Naydenova: Wrote the manuscript. Analysis in forage quality traits. The author read and approved the final manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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