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Evaluation of nodule related components and forage productivity in Pea (*Pisum sativum* L.) genotypes

Viliana Vasileva* and Valentin Kosev
Institute of Forage Crops, 89 "Gen. Vl. Vazov" Str., Pleven 5800, Bulgaria

Abstract

Relationships and variation among nodule related components and forage productivity in pea (*Pisum sativum* L.) genotypes were evaluated in a small-plot trial carried out at the Institute of Forage Crops, Pleven, Bulgaria (2011-2013). Genetic and phenotypic correlations as well as path coefficient analysis were performed. Four parental pea forms were used for crossing: spring type (*Pisum sativum ssp. sativum*) – Shtambovyi and Pleven 4, and winter ones (*Pisum sativum ssp. arvense*) – Rosacrono and Pleven 10. The strongest positive phenotypic correlations were found between aboveground/root mass (weight) and: plant height (r=0.9237), aboveground/root mass (length) (r=0.5517); between root length and: plant height (r=0.8802), aboveground/root mass (weight) (r=0.7955), stems fresh weight (r=0.7593), root dry weight (r=0.6442). The highest genetic correlations were determined between plant height and: aboveground/root mass (weight) (r=0.9418), root length (r = 0.9439), stems fresh weight (r=0.8808), stems dry weight (r=0.8793), aboveground/root mass (weight) (r=0.8354). The strongest and direct positive effects over aboveground fresh weight (leaves+stems) productivity were found for root dry weight (4804.946), stems dry weight (1218.927) and nodule weight per plant (1153.314). The highest positive indirect contribution was in stems dry weight via root fresh weight (8118.055), stem fresh weight (6350.61) and root length (4558.788). Field pea breeders should pay attention to the traits such as stems dry weight, root dry weight and nodule weight per plant, when selecting highyielding genotypes for green mass. The results of the presented study hopefully represent a contribution to a better knowledge on green mass productivity components in field pea.

Key-Words: *Pisum sativum* L., nodules, roots, path analysis, forage productivity

Introduction

Peas (*Pisum sativum* L.) are grown for forage and grain. Field pea genotypes are characterized by a great variety of morphological, phenological and agronomic traits used as parameters for characterization and differentiations of samples, hybrids and lines. Correlations between some traits of genotypes were found, which allow the expression in one of the parameters to be predicted the occurrence of the element, as determined by the same genetic mechanism.

Green mass yield in field pea is a quantitative trait affected by many environmental factors such as temperature, precipitations, soil type, moisture, ect. (Cousin, 1997; Espósito *et al.*, 2009). Therefore, a priority of modern conventional field pea breeding programmes is searching for the donors of desirable traits able to answer all the challenges of specific environment (Mikić *et al.*, 2011).

The appropriateness of the selection directed to increasing of symbiotic potential and selection of promising genotypes for including in breeding process has been demonstrated in studies of Shtark *et al.* (2006), Naumkin (2007), Vishnyakova (2008) ect.
Contemporary breeding programs are using field pea genotypes with a wide genetic base and a diversity of phenological, morphological and economic traits. One of the most important steps in developing novel field pea lines with stable yield is an adequate use of the available germplasm and a proper selection of the parents for hybridisation (Kosev & Mikić, 2012).
The path coefficient analysis initially suggested by Wright (1921) and described by Dewey & Lu (1959), allow partitioning of each correlation coefficient into direct and indirect effects of various traits on the dependent variable and thus helps assessing the cause effect relationship and an effective selection. The presence of correlations is considered an important because they can be used as selection criterion resulting in more effective selection (Togay *et al.*, 2008). Path coefficient analyses correlate coefficients into direct and indirect effects of various seed yield components based on the assumption that relationships

* Corresponding Author
E.Mail: viliana.vasileva@gmail.com

exist among yield components. Statistically, path coefficients are standardised partial-regression coefficients obtained from equations where yield-related variables are expressed as deviations from the means in units of standard deviation (Steel & Torrie, 1982).

The aim of the study was to evaluate the relationships and variation among nodule related components and forage productivity in pea genotypes using correlations and path coefficient analysis.

Material and Methods

A small-plot trial was carried out from 2011 to 2013 at the experimental field of the Institute of Forage Crops, Pleven (43.41°N, 24.61°E), situated in the Central part of the Danube hilly plain of Bulgaria. Parental forms used for crossing were from our collection: spring type (*Pisum sativum ssp. sativum*) – Shtambovyi and Pleven 4, winter ones (*Pisum sativum ssp. arvense*) – Rosacrono and Pleven 10, characteristics of which are shown in Table 1. These forms were crossed by hand in 2011. The parental forms (P1 and P2) and first and second generation (F1 and F2) were sown at scheme P1, P2, F2, F1 on a row spacing 70 cm and distance in a row 5 cm. Sowing by hand was applied with depth of 5 cm. The field pea was grown according to the technology approved in the Institute of Forage Crops, Pleven.

For measurements soil monoliths were taken at two phenological stages of plant development – budding and beginning of the flowering (Beck *et al.*, 1993). There were 15 plants from each hybrid or parent fell into the monolith. Biometrical assessment of the parental forms (P1 and P2) and crosses of first and second hybrid generation (F1 and F2) was done by the following characteristics: plant height, (cm); stems fresh weight, (g); stems dry weight, (g); root length (cm); nodule number per plant; nodule weight per plant (g); root fresh weight (g); root dry weight (g); specific root length (root length/root weight, cm g^{-1}); specific nodulating ability (g nodules/g root weight); aboveground fresh weight (leaves + stems); aboveground/root mass (length); aboveground/root mass (weight).

Genetic and phenotypic correlations were calculated by two-factor analysis of variance (ANOVA) and path analysis (Williams *et al.*, 1990). Broad sense heritability (Hbs) was calculated using the formula proposed by Mahmud & Kramer (1951). Genotypic coefficient of variation (CVg) and phenotypic coefficient of variation (GVe) were estimated by the formula suggested by Burton (1952).

The computer software GENES 2009.7.0 for Windows XP (Cruz, 2009) was used for all analysis.

Results and Discussion

Correlation Analysis

The phenotypic correlation is conditioned by the relationship among individual characters and the influence of environmental factors. The genotypic correlation is a function of the pleiotropic action of the genes involved and their related inheritance. Linked genes have additive, dominant and epistatic actions. It is generally regarded that the additive genes are of greatest value in breeding (Zhelyazkov & Tsvetanova, 2002).

From the breeding point of view the presence of correlations is particularly important because of the possibility to use them as an important criterion for the implementation of more efficient and simultaneous selection by phenotype of two or more traits (Zhuchenko *et al.*, 1980).

The performed analysis of the relationships among the tested traits showed that the coefficients of phenotypic dependence for most of the traits were lower as compared to the coefficients of genotypic correlations (Table 2 and Table 3). Strong positive phenotypic correlations were found between aboveground/root mass (weight) and: plant height (r=0.9237), aboveground/root mass (length) (r=0.5517); between root length and: plant height (r=0.8802), aboveground/root mass (weight) (r=0.7955), stems fresh weight (r=0.7593), as well root dry weight (g) (r=0.6442). Relatively low phenotypic correlations were detected between root length and specific root length (r = 0.3646). Positive but weak were the relationships between root length and aboveground/root mass (r = 0.2374) and root fresh weight (r = 0.1944). Negative correlations were found between specific nodulating ability and: aboveground fresh weight (leaves + stems) (r = –0.6786), stems fresh weight (r = –0.5918) and aboveground/root mass (weight) (r = –0.5244).

High genetic correlations were detected between plant height and: aboveground/root mass (weight) (r=0.9418) and root length (r = 0.9439), between root length and: stems fresh weight (r=0.8808), stems dry weight (r=0.8793) and aboveground/root mass (weight) (r=0.8354). Low correlations were detected between root length and root fresh weight (r = 0.2742), as well aboveground/root mass (length) (r = 0.2024). Negative genetic correlation coefficients were detected between specific nodulating ability and: aboveground fresh weight (leaves + stems) (r = –0.8343), stems fresh weight (r = –0.7181), aboveground/root mass (weight) (r = –0.647), aboveground/root mass (weight) (r = –0.5244); between nodule weight per plant and

aboveground/root mass (weight) (r = –0.2493).
The presented data demonstrated that the green mass productivity in field pea is a complex trait and is the result of the combined effect of all components.

Path Coefficient Analysis
The path coefficient analysis (Table 4) revealed that root dry weight (4804.946), stems dry weight (1218.927) and nodule weight per plant (1153.314) were the productivity components possessing the highest positive direct effects on green mass. They can be important criterion for selecting of desirable traits for the genetic improvement of the field pea crop.
In our study the strongest indirect effects were found for stems dry weight via root fresh weight (8118.055), stem fresh weight (6350.61) and root length (4558.788). Negative indirect effects were found in stems fresh weight via fresh weight (–7771.94) and root length (–3917.41).

Regression Analysis
The results of carried out multiple regression analysis showed that the linear component in the regression of green mass productivity per plant in respect to the investigated quantitative traits was significant and reliable (Table 5).
From the complex study of the traits was obtained a model (1) which demonstrated the complexity character of the change of productivity depending on the variation of investigated quantitative traits.
The common type of the obtained equation of regression was:

(1) $Y=2.31877 + 0.0555098*X1 - 53.4903*X2 + 16.8452*X3 -0.37158*X4 + 10.0761*X5 - 0.84037*X6 + 0.0353759*X7 +1.78682*X8 - 0.142933*X9+ 0.22818*X10 -0.00789512*X11 - 0.0831974*X12$

The applied analysis showed that for the aboveground fresh weight formation the highest influence had the specific nodulating ability (16.84) and root fresh weight (10.07), followed by stems fresh weight (1.786).
In the regression equation (1) the dependence between the productivity of aboveground fresh weight and nodule weight per plant (-53.49) was with negative value, i.e. every increasing of the nodule weight per plant led to decrease in the productivity. The highest relative share on aboveground fresh weight productiveness had the indicator of specific nodulating ability. The individual productiveness is increased by nearly 16.84 g if the specific nodulating ability is increased by an unit.

Coefficients of Variation
The comparison of characters as regards to the extent of genetic variation could be better judged by the estimation of genotypic coefficient of variation (CVg) in relation to their respective phenotypic coefficient of variation (CVe). Amongst the investigated characters very small difference between CVg and CVe was observed for the traits like root length, root fresh weight, specific root length, stem fresh weight, stems dry weight, aboveground/root mass (length), aboveground fresh weight (leaves + stems) (Figure 1). It indicates that the observed variations for the trait were mostly due to genetic factors. However, the environment played a little role on the expression of this trait.
On the other hand, large difference between CVg and CVe was observed for the characters as nodule number per plant, nodule weight per plant; specific nodulating ability, root dry weight, aboveground/root mass (weight), plant height. This indicated the role of environmental influence over this characters. In this experiment, high CVg was observed in character like specific root length, stems dry weight, stem fresh weight, specific nodulating ability and aboveground fresh weight (leaves+stems). The high CVg for these traits indicated further selection could improve the genotypes.
Biomass formation of the plants and their organs are result of assimilation activity of photosynthetic issues, but from the activity of root system as well (Novikova, 2012). To increase symbiotic nitrogen fixation and plant productivity should be working on the selection of high productive genotypes with increased symbiotic nitrogen fixing potential. Sidorova *et al.* (2012) demonstrated possibility to conduct simultaneous selection for increasing both, nodulating ability and productivity in pea. Correlation coefficient between root weight and aboveground organs weight at the sixth true leaf stage in the field conditions reached r= +0.921 (Naumkina, 2007).
Similar results were obtained in *Phaseolus vulgaris* genotypes for fresh nodule weight and fresh root weight. Some authors reported for strong positive correlation between the number of nodules per plant and nodule fresh weight per plant (Das & Bandyopadhyay, 2011). Shoot length was found to be positively correlated with nodule number and with nodule dry weight in lentil (Anteneh, 2012).
Much of the research output indicated that nodulation status positively correlated with plant tissue nitrogen and shoot biomass (Mnalku *et al.*, 2009).

Conclusion

In general, the genetic correlations for most of the traits in field pea had higher coefficients than the phenotypic correlations. Specifically positive phenotypic correlations were found between aboveground/root mass (weight) and: plant height (r=0.9237) and aboveground/root mass (length) (r=0.5517) as well; between root length and: plant height (r=0.8802), aboveground/root mass (weight) (r=0.7955), stems fresh weight (r=0.7593), root dry weight (g) (r=0.6442). The highest genetic correlations were determined between plant height and: aboveground/root mass (weight) (r=0.9418), root length (r = 0.9439), stems fresh weight (r=0.8808), stems dry weight (r=0.8793), aboveground/root mass (weight) (r=0.8354). The strongest and direct positive effects over aboveground fresh weight (leaves+stems) productivity were found for root dry weight (4804.946), stems dry weight (1218.927) and nodule weight per plant (1153.314). The highest positive indirect contribution were in stems dry weight via root fresh weight (8118.055), stem fresh weight (6350.61) and root length (4558.788). The results of the presented study hopefully represent a contribution to a better knowledge on green mass productivity components in field pea. Collecting data on the mutual relationships among individual productivity components and their effect on forage yield, remains crucial for their optimisation and development of improved field pea genotypes with high, quality and stable yields.

Based on the trait association and the path coefficients for green mass and some its components, it can be concluded that field pea breeders should pay attention to the traits such as stems dry weight, root dry weight and nodule weight per plant when selecting high-yielding genotypes for green mass.

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Table 1: Distinctive features of the investigated genotypes

Traits	Variety (genotype)			
	Shtambovyi	**Pleven 10**	**Rosacrono**	**Pleven 4**
Vine type	short semi-erect	long-prostrate	long semi-erect	long semi-erect
Flower position	terminal (with fasciation – Fa, fac)	axial	terminal (with fasciation– fa, Fac)	axial
Stipule type	double	normal	double	normal
Leaf type	normal	normal	normal	normal
Flower color	white	purple	pink	white

Table 2: Phenotypic correlation coefficients among the characteristics in pea genotypes (r)

Traits	Nodule number per plant	Nodule weight per plant (g)	Specific nodulating ability	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Specific root length	Stems fresh weight (g)	Stems dry weight (g)	Aboveground/ Root mass (length)	Aboveground/ Root mass (weight)	Plant height (cm)
Nodule weight per plant (g)	-0.1789											
Specific nodulating ability	-0.077	0.4364										
Root length (cm)	-0.6012	-0.2413	-0.3129									
Root fresh weight (g)	-0.1106	0.2341	-0.6775	0.1944***								
Root dry weight (g)	-0.3085	0.0336	-0.6595	0.6442***	0.8401							
Specific root length (cm g^{-1})	-0.3755	-0.4152	0.4939	0.3646*	-0.6986	-0.3621						
Stems fresh weight (g)	-0.1443	-0.043	-0.5918***	0.7593**	0.5882	0.8756	-0.2514					
Stems dry weight (g)	-0.1939***	0.0384	-0.5231	0.7525*	0.6071	0.8903	-0.244	0.9871				
Aboveground/Root mass (length)	0.1729	-0.3453***	-0.4794	0.2374***	0.5172	0.6412	-0.1566	0.4724	0.4501			
Aboveground/Root mass (weight)	-0.0892	-0.1927	-0.5244***	0.7955**	0.4747	0.8032	-0.0571	0.9553	0.9263	0.5517***		
Plant height (cm)	-0.2351	-0.3743	-0.4091	0.8802***	0.2547	0.7102	0.2362	0.8417	0.8108	0.5977	0.9237***	
Aboveground fresh weight (leaves+stems)	-0.122	-0.0798	-0.6786***	0.7107	0.7103	0.9275	-0.3082	0.9772	0.9635	0.5667	0.9477	0.8118

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001

Table 3: Genetic correlation coefficients among the characteristics in pea genotypes (r)

Traits	Nodule number per plant	Nodule weight per plant (g)	Specific nodulating ability	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Specific root length	Stems fresh weight (g)	Stems dry weight (g)	Aboveground/ Root mass (length)	Aboveground/ Root mass (weight)	Plant height (cm)
Nodule weight per plant (g)	-0.7582											
Specific nodulating ability	-0.248	0.292										
Root length (cm)	-0.8185	-0.3805	-0.4471									
Root fresh weight (g)	-0.34	0.1599	-0.8443	0.2742***								
Root dry weight (g)	-0.5746	-0.1107	-0.8135	0.7502***	0.8257							
Specific root length (cm g^{-1})	-0.4211	-0.5009	0.554	0.3677*	-0.7134	-0.3408						
Stems fresh weight (g)	-0.3319	-0.168	-0.7181***	0.8808***	0.5441	0.8711	-0.2307					
Stems dry weight (g)	-0.4095	-0.0771	-0.6498	0.8793**	0.5652	0.8849	-0.2232	0.9874				
Aboveground/Root mass (length)	0.2441***	-0.4743	-0.6465	0.2024***	0.6689	0.7801	-0.2056	0.5809	0.555			
Aboveground/Root mass (weight)	-0.1591	-0.2493***	-0.647***	0.8354	0.5547	0.8989	-0.078	1.00	1.00	0.5653***		
Plant height (cm)	-0.3855	-0.5323	-0.4969	0.9439**	0.2399	0.742	0.2451	0.8602	0.835	0.648	0.9418***	
Aboveground fresh weight (leaves+stems)	-0.2786	-0.2236	-0.8343***	0.8748***	0.6784	0.9375	-0.2905	0.9798	0.964	0.7177	1.00	0.8433

*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001

Table 4: Path coefficients for aboveground fresh weight productivity of pea genotypes

Traits	Nodule number per plant	Nodule weight per plant (g)	Specific nodulating ability	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Specific root length	Stems fresh weight (g)	Stems dry weight (g)	Aboveground/ Root mass (length)	Aboveground/ Root mass (weight)	Plant height (cm)	Total effect
					Indirect effect								
Nodule number per plant	**12.62063**	57.6657	-378.27	203.6541	308.6296	384.3957	422.6626	-940.924	999.5203	114.9877	-69.6071	-1435.56	22
Nodule weight per plant (g)	795.0995	**1153.314**	-953.336	285.1158	171.4609	672.6924	1065.108	-2077.49	2474.422	146.518	-99.576	-2560.93	0.01
Specific nodulating ability	731.9964	92.26512	**-3404.77**	203.6541	336.0633	528.5441	388.1591	-1171.5	914.1954	122.7889	-57.6059	-1532.95	0.02
Root length (cm)	277.6538	11.53314	-109.974	**17.70906**	212.6115	576.5935	906.9741	-3917.41	4558.788	146.5812	-246.105	-2705.21	13
Root fresh weight (g)	315.5157	23.06628	-151.172	283.3449	**685.8436**	768.7914	588.052	-7771.94	8118.055	181.4572	-120.099	-2542.9	0.56
Root dry weight (g)	277.6538	23.06628	-128.36	198.3414	363.4971	**4804.946**	349.5026	-1171.5	1206.738	118.659	-40.5983	-1677.23	0.01
Specific root length (cm)	227.1713	11.53314	-52.4335	230.2177	445.7983	912.9397	**16.53894**	-2801.81	2510.99	290.717	-81.0526	-2001.85	23.21
Stems fresh weight (g)	416.4807	34.59942	-72.8621	221.3632	960.181	1345.385	147.6696	**-232.902**	6350.61	185.6668	-222.447	-2975.73	9.58
Stems dry weight (g)	631.0314	276.7953	-1034.37	177.0906	541.8164	432.4451	209.3532	-926.95	**1218.927**	137.8829	-67.4204	-1496.88	2.05
Aboveground/Root mass (length)	252.4125	11.53314	-261.827	256.7813	89.15966	384.3957	1844.729	-2552.61	2706.018	**33.2248**	-174.738	-2723.24	5.61
Aboveground/Root mass (weight)	113.5856	11.53314	-212.798	150.527	109.735	192.1978	878.6313	-815.157	950.7632	146.5812	**-3.84039**	-1352.6	33.35
Plant height (cm)	429.1013	11.53314	-131.084	286.8867	178.3193	624.643	1030.503	-2259.15	2681.64	160.3827	-122.006	**-36.0694**	71

direct effect – underlined bolded numbers

Table 5: Regression analysis (ANOVA) of the aboveground fresh weight productivity per plant in regard to the other traits

ANOVA					
	df	SS	MS	F	Significance F
Regression	12	20176.15	1681.346	128.0519	3.26E-25
Residual	35	459.5567	13.13019		
Total	47	20635.71			

Table 6: Regression coefficients of the aboveground fresh weight per plant in regard to the other traits

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	2.318772	3.946394	0.587567	0.560595	-5.69283	10.33038
X1	0.05551	0.080523	0.689364	0.495139	-0.10796	0.218981
X2	-53.4903	42.83698	-1.2487	0.220065	-140.454	33.47336
X3	16.84518	30.03086	0.560929	0.57842	-44.1207	77.81107
X4	-0.37158	0.314328	-1.18214	0.24512	-1.0097	0.266541
X5	10.0761	5.897506	1.708536	0.096392	-1.89647	22.04867
X6	-0.84037	31.12876	-0.027	0.978616	-64.0351	62.35436
X7	0.035376	0.03249	1.08884	0.283665	-0.03058	0.101333
X8	1.786819	0.308086	5.79974	1.42E-06	1.161371	2.412267
X9	-0.14293	1.549133	-0.09227	0.927012	-3.28784	3.001975
X10	0.22818	0.641412	0.355746	0.724166	-1.07396	1.530316
X11	-0.0079	0.060921	-0.1296	0.897627	-0.13157	0.11578
X12	-0.0832	0.099988	-0.83208	0.411007	-0.28618	0.119788

Where: Y – aboveground fresh weight (leaves + stems) (g); X1 - Nodule number per plant; X2 - Nodule weight per plant (g); X3 - Specific nodulating ability; X4 - Root length (cm); X5 - Root fresh weight (g); X6 - Root dry weight (g); X7 - Specific root length (cm g^{-1}); X8 - Stem fresh weight (g); X9 - Stems dry weight (g); X10 - Aboveground/Root mass (length) (cm); X11 - Aboveground/Root mass (weight) (g); X12 - Plant height (cm)
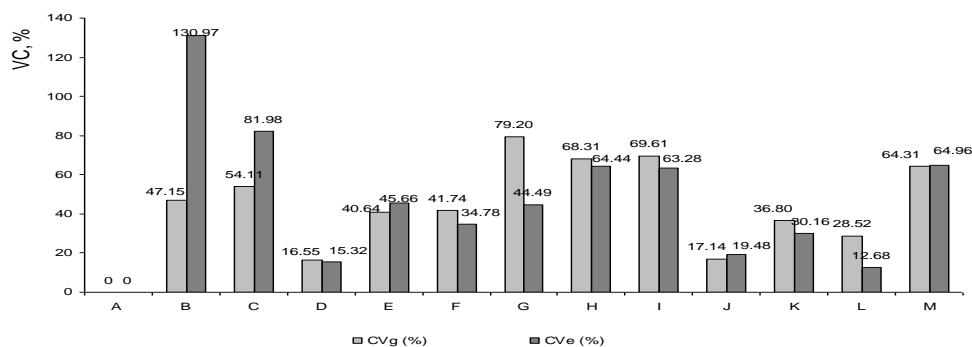

Fig. 1: Genotypic coefficient of variation (CVg%) and phenotypic coefficient of variation (GVe%)
A - Nodule number per plant; B - Nodule weight per plant (g); C- Specific nodulating ability; D - Root length (cm); E - Root fresh weight (g); F - Root dry weight (g); G - Specific root length (cm g^{-1}); H - Stem fresh weight (g); I - Stems dry weight (g); J - Aboveground/root mass (length) (cm); K - Aboveground/root mass (weight) (g); L - Plant height (cm); M - Aboveground fresh weight (leaves + stems) (g)