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Genotype Environment Interaction and Stability of Quantitative Traits in Pea (*Pisum sativum* L.) Varieties

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ABSTRACT

Purpose: *The purpose of the study was to evaluate genotype × environment interactions and stability parameters of seven pea varieties by basic quantitative traits and to determine their suitability as initial material for future breeding programmes.*

Research Method: *Both, above ground and root biomass of plants were analysed. Following characteristics were assessed in the beginning of flowering stage: plant height (cm), leaf fresh weight (g), stem fresh weight (g), root fresh weight (g), nodule number per plant, nodule weight per plant (g). Biometric measurements were made to 10 plants of each variety. Regression analysis, analysis of variance, non-parametrical analyses were used. All experimental data were processed statistically using the computer software GENES 2009.7.0 for Windows XP.*

Findings: *Wt6803 variety could be selected as the most promising selection material in terms of plant height and number of nodules, but in terms of leaf fresh weight and stem fresh weight NDPO80138-B-2 and Mir varieties, respectively.*

Value: *Pea varieties of breeding interest with regard to the both, genotype environment interaction and stability of quantitative traits were shown. They could be actively used in the creation of new varieties with increased general adaptability.*

Keywords: *environment, genotype, Pisum, stability methods*

INTRODUCTION

Increasing the yield potential has always been in existence and remains of fundamental importance in breeding programs. However, modern varieties should not only be of high yielding standard and of high quality but should be resistant to adverse environmental factors (Potanin *et al.,* 2014). Most modern varieties of *Pisum sativum* L. have high potential for yield, the realization of which is limited due to their low homeostatistics and sensitivity to adverse environmental factors (Lakhanov, 1999). As a legume crop it plays an important role as a source of vegetable protein in solving the protein problem of livestock production.

Modern varieties of pea under unfavorable conditions such as severe drought or excessive moisture, or damage by pests were found to decrease seed weight form by 55-72% less seed weight than under favorable conditions. In the breeding process, there is even a tendency to degrade the adaptive properties of plants to environmental stressors, which in the near future may become a major reason for limiting further progress in the production of this crop (Amelin, 2012).

The target of selection for resistance to adverse environmental factors requires a comprehensive assessment of breeding material from the early stages of breeding (Zykin *et al.,* 2011). The main direction in realizing the possible yield of crops is the selection of adaptive varieties that combine

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sufficient ecological plasticity with high stability over the years (Goncharenko, 2005; Eliseev, 2014). In this regard, one of the main tasks facing the breeders of a given crop is the optimal genotype capable of realizing its potential and responding appropriately to environmental changes (Buhayov *et al.,* 2013).

In breeding genetic studies, the Eberhart and Russell method (1966) is widely used. A number of scientists indicate that the use of this method is effective for assessing the stability parameters of yield elements of early stages of pea's selection. Assessment of the parameters of adaptability allows to fully revealing their ability to withstand the influence of negative factors of the environment (Fadeev, 2014). The aim of this study was (i) to evaluate genotype × environment interactions and stability parameters of seven pea varieties by basic quantitative traits and (ii) to determine their suitability as initial material for future breeding programmes.

MATERIALS AND METHODS

Plant material from aboveground and root biomass of 7 pea (*Pisum sativum* L.) varieties originating in the country and abroad, and perspective ones, i.e., X07P54, X06PWY, NDPO80138-B-2, CA1P, L020140, Wt6803 (foreign) and Mir (Bulgarian) was analysed. The study was conducted during 2014-2016 in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria (43.4067° N, 24.6065° E). Sowing was carried out manually during early March. The following characteristics have been assessed at the beginning of the flowering stage: plant height (cm), leaf fresh weight (g), stem fresh weight (g), root fresh weight (g), nodule number per plant and nodule weight per plant (g). Biometric measurements were made in 10 plants of each variety.

The data obtained were processed by two-factor analysis of variance for each trait to determine the effects of genotype (G), environment (E) and genotype-environment (G x E) interaction. The next methods were used: regression analysis (Eberhart and Russell, 1966); Tai (1979), (ai; λi); analysis of variance - mean variance component (PP) according to Plaisted and Peterson (1959); ecovalence (W^2), Wricke (1965) and Annicchiarico (1992); non parametrical analyses through P_i parameter using and rank (R) on the model of Lin and Binns (1988). Plaisted and Peterson's (1959) mean variance component (PP) was a measure of a variety's contribution to the G x E interaction and was computed from a total of pair-wise analysis. According to superiority measure (Pi) of Lin and Binns (1988), the distance mean square between the cultivar's response and the maximum response over locations were the major parameters in identifying superior cultivars. Dispersion and regression analysis were used for quantitative assessment of ecological stability and adaptability of varieties. The dispersion analysis (ANOVA) mainly reflects the additive effects of the trait and the regression gives information about both, the additive effects and some of its interaction with the environment (Zobel *et al.,* 1988). All experimental data were processed statistically using the computer software GENES 2009.7.0 for Windows XP (Cruz, 2009).

RESULTS AND DISCUSSION

The unfavorable conditions during vegetation were specifically reflected on the individual genotypes. Pea is a crop with great compensation capabilities, which is a prerequisite for greater stability of yield and its components (Acikgoz *et al.,* 2009).

The study period covers three consecutive years which had different climatic conditions. Table 01. presents the data on average monthly temperatures and the amount of precipitated rainfall by months during vegetation. The vegetation 2014 was the most favorable for the study period with average monthly air temperatures (April 12.3 °C, May 16.7 °C, June 20.6 °C) and rainfall 139.8 mm, 83.0 mm and 54.3 mm, respectively. As a result of the balanced combination of air temperature and optimum rainfall a favorable ground for plant development occurred. The second year (2015) had relatively higher temperatures of 18.8 °C in May and an uneven precipitation distribution, characterized by a drought in April (43.6 mm) and May (30.6 mm), and a larger quantity in

June (95.7 mm). The third year (2016) occupied an intermediate position over the other two years with temperatures in the months of April and May, close to normal (15.3-16.4 °C) and rainfall between 73.1 and 76.5 mm.

The performance of a given genotype depends on its genetic potential and the environment upon which it is grown (Mangistu *et al.,* 2011). The result of the study shows that there were significant (P = 0.05) difference among genotype, locations and GxE, indicating the need to assess the stability of genotypes. The GxE was significant showing variable performance of the genotypes in various environments.

The varieties studied form their productivity potential depending on the resistance to the stressful climatic conditions and the degree of adaptability to them under the specific conditions of the area. At the height of the plant, the X07P54, X06PWY, NDPO80138-B-2 and CA1P varieties significantly (P = 0.05) retreated to Mir variety, the plants reaching a height of 92.06 cm, followed by the Wt6803 (89.63 cm) variety, which is very close to the standard and L020140 with plant height of 82.06 cm (Figure 01.).

The Wt6803 variety reported the highest average productivity in terms of the leaf fresh weight (12.04 g) exceeding both, the Mir standard (10.34 g) and X06PWY (9.58 g). Mir and Wt6803 have close leaf fresh weights. The same varieties form a larger number of nodules per plant (45-54). In other varieties, the leaf fresh weight was lower and almost equal and the number of nodules per plant varied from 10.56 (X07P54) to 34.54 (L020140).

Dispersion analysis

Through the dispersion analysis, the sum of the squares of the investigated traits is divided by the effects of genotype, environment, and genotype - environment interaction (Table 02.). The results of the analysis show that the gradations of all three factors were statistically reliable for the plant height, leaf fresh weight, stem fresh weight and the number of nodules per plant.

The effect of the environment factor was found unreliable for the root fresh weight (i.e. the conditions were the same and the experiment was incorrect). The genotype-environment interaction factor was also unreliable, suggesting that the entire variability of the trait was genetically determined and there was no point in analyzing its environmental stability. Genotypic differences and the influence of the environment were insignificant to the nodule weight per plant. The environment (year) had the largest part in the total variability in both, leaf fresh weight and stem fresh weight, and the smaller was found the part of the genotype and the GxE. The part of the environmental factor (year) in the overall dispersion of these traits was related to the significant variability of climatic conditions in the years of study period. The portion of the variation due to the genotypes for the plant height sign was very significant and exceeds the portion due to the genotype-environment interaction. Therefore, an effective selection can be done regardless the environment.

Table 01: Climatic characterization of the experimental period

Months	2014			2015			2016		
	T	rainfall	humidity	T	rainfall	humidity	T	rainfall	humidity
	°C	mm	%	°C	mm	%	°C	mm	%
I	0.8	41.8	82.0	1.9	12.4	80.0	-0.5	98.0	78.0
II	2.3	3.4	82.0	2.3	39.2	80.0	8.7	46.0	75.0
III	9.7	76.9	68.0	6.7	68.4	71.0	8.5	76.6	73.0
IV	12.3	139.8	76.0	12.2	43.6	54.0	15.3	73.1	66.0
V	16.7	83.0	70.0	18.8	30.6	66.0	16.4	76.5	71.0
VI	20.6	54.3	67.0	20.7	95.7	64.0	23.0	45.8	67.0
VII	23.1	71.8	67.0	25.8	21.5	54.0	24.6	7.8	57.0

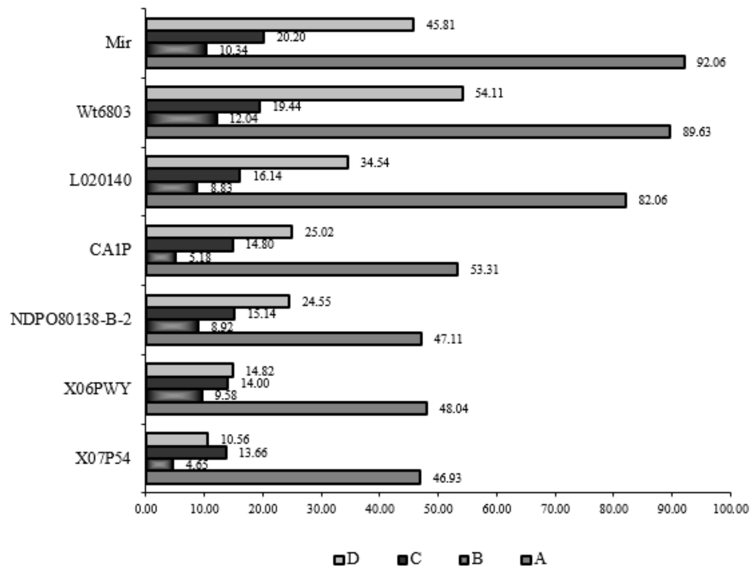


Figure 01: Productivity of the varieties according to the studied traits A - plant height (cm) ($LSD_{0.05}$=20.55); B - leaf fresh weight (g) ($LSD_{0.05}$=12.73); C - stem fresh weight (g) ($LSD_{0.05}$=19.60); D - nodule number per plant ($LSD_{0.05}$=28.15)

Table 02. Analysis of variance for stability parameters in pea cultivars for the period 2014-2016

Source of variation	Df	Mean sum of squares for the traits studied					
		Aboveground mass			Root mass	Nodules	
		plant height (cm)	leaf fresh weight (g)	stem fresh weight (g)	fresh weight (g)	number	weight (g)
Environments (E)	2	885.11**	1160.22**	3245.14**	0.306	5235.10**	0.08
Genotypes (G)	6	6745.87**	108.09**	102.24**	2.04	3787.16**	0.18
G x E Interactions	12	350.03**	47.53**	86.59**	0.87	1453.81**	0.23
Env/Gen	14	426.47**	206.48**	537.81**	0.79	1993.99**	0.21
Env/X07P54	2	273.92**	60.36**	487.50**	0.09	230.27**	0.05
Env/ X06PWY	2	1258.51**	485.20**	1015.72**	0.28	782.00**	0.01
Env/NDPO80138-B-2	2	5.7727*	246.74**	622.67**	0.16	1267.28**	0.14
Env/CA1P	2	264.29**	19.20**	196.67**	0.35	710.67**	0.06
Env/L020140	2	35.30**	99.55**	75.88**	0.1	2076.86*	0.23
Env/ Wt6803	2	226.29**	411.68**	683.57**	2.89	815.70**	0.15
Env/ Wt6803	2	921.25**	122.65**	682.65**	1.68	8075.17**	0.83
Total	20						

*Significant at P = 0.05 (*), ** P = 0.01(**)*

Ecological stability assessment

Plant height

The X06PWY, Mir and X07P54 varieties, whose linear regression coefficients were bi = 2.70, bi = 2.41 and bi = 1.33 (Table 03.), have been found to be ecologically plastic by the height of the plant. Such a genotype's response to the environment conditions is inherent in intensive type varieties. The varieties NDPO80138-B-2 (bi = 0.21), CA1P (bi = 0.28) and L020140 (bi = 0.41) were characterized by a poor response to the improvement in conditions for cultivation, which is typical for extensive type varieties. According to the index (PP) Plaisted and Peterson (1959) and (W^2), Wricke (1965), the varieties Wt6803 and L020140 showed the best combination of stability and the plant height traits.

Table 03: Estimates of the adaptability and stability parameters for the investigated traits of the pea varieties

Cultivar	Eberhart and Russell (1966)		Tai (1979)		Lin and Binns (1988)	Plaisted and Peterson (1959)	Wricke (1965)	Annicchiarico (1992)
	bi	Si^2	ai	λi	Pi	PP	W^2	W_i
plant height (cm)								
X07P54	1.33*	18.90**	1.34	55.68	1105.8	42.05	124.3	72.91
X06PWY	2.70**	132.58**	2.71	386.77	1113.5	116.52	1400.8	61.44
NDPO80138-B-2	0.21**	0.20	0.22	0.25	1137.2	56.53	372.5	65.67
CA1P	0.28**	71.20**	0.38	207.71	921.6	104.69	1198.1	69.71
L020140	0.41**	5.33**	0.41	16.08	90.42	41.51	115.0	118.09
Wt6803	1.17	21.00**	1.17	61.82	13.63	41.42	113.3	132.46
Mir	2.41**	74.55**	2.41	217.68	5.01	85.93	876.5	141.57
leaf fresh weight (g)								
X07P54	0.60**	0.20	0.60	-0.02	39.83	7.595	52.15	50.65
X06PWY	1.71**	0.14	1.71	0.10	5.01	14.338	167.74	71.35
NDPO80138-B-2	1.21	0.07	1.22	0.77	7.33	5.554	17.15	91.79
CA1P	0.27**	2.45**	0.27	7.65	44.20	15.480	187.32	49.96
L020140	0.77	0.32	0.77	1.50	12.89	5.727	20.13	98.42
Wt6803	1.56**	2.49**	1.56	7.78	0.36	11.468	118.54	121.46
Mir	0.86	0.05	0.86	0.43	6.83	4.981	7.34	116.03
stem fresh weight (g)								
X07P54	1.02	0.68*	1.02	2.57	29.44	8.745	4.90	71.84
X06PWY	1.47**	2.41**	1.48	7.58	31.83	21.446	222.62	51.02
NDPO80138-B-2	1.15*	3.13**	1.15	9.70	19.88	10.666	37.83	78.70
CA1P	0.56**	19.13**	0.57	56.34	33.52	24.298	271.52	74.37
L020140	0.39**	1.13*	0.40	3.82	28.49	28.606	345.36	90.48
Wt6803	1.18**	12.19**	1.19	36.12	3.65	13.953	94.17	104.10
Mir	1.20**	4.74**	1.20	14.42	1.55	12.118	62.71	115.47
nodule number								
X07P54	0.51**	13.51**	0.51	39.97	1522.2	0.166	424.83	21.67
X06PWY;	1.01	9.05**	1.01	26.97	1245.1	0.173	46.32	31.47
NDPO80138-B-2	1.15**	107.18**	1.16	313.19	825.04	0.165	573.16	45.92
CA1P	0.01	284.03**	0.01	828.85	1000.1	0.166	2950.58	49.93
L020140	0.01**	830.44**	0.02	2422.54	729.84	0.147	5593.60	85.09
Wt6803	1.04	0.18	1.04	0.06	87.27	0.158	3.05	195.07
Mir	3.27**	24.76**	3.27	71.81	250.57	0.100	7854.23	60.30

*Significant at P = 0.05 (*), ** P = 0.01(**)*

Leaf fresh weight

Mir (Si2= 0.05) and NDPO80138-B-2 (Si2=0.07) were found the most stable in the ability to form more leaf fresh mass, followed by X06PWY (Si^2=0.14) and X07P54 (Si^2=0.20) (Table 03.). More significant variations in these traits were found in Wt6803 and CA1P. The most responsive to leaf fresh weight changes were X06PWY (bi=1.71) (P = 0.01) and Wt6803 (bi=1.56) (P = 0.05). At a high level of agro technology, they could form a maximum amount of fresh mass but also abruptly reduce it under limiting environmental conditions. The varieties CA1P (bi = 0.27) and X07P54 (bi = 0.60) were characterized with a poor response to improved growing conditions and they increased the level of the sign very poorly under favorable conditions. The best score of both, stability and the level of the leaf fresh weight based on the Annicchiarico Wi parameter

(1992) received the varieties L020140 and the standard Mir.

Stem fresh weight

Low variability in the degree of stability of stem fresh weight was found in X07P54 X07P54 (Si^2=0.68), L020140 (Si^2=1.13) and X06PWY (Si^2=2.41) (Table 03.). The PP parameters of Plaisted and Peterson (1959) and W^2 of Wricke (1965) also ranked X07P54 in the first position and the second NDPO80138-B-2. To the group of varieties with the highest variations can be assigned CA1P and Wt6803, whose stability indexes were Si^2=19.13 and Si^2=12.19, respectively. The X06PWY, Mir and Wt6803 varieties exhibit a high degree of responsiveness to changing environment and the coefficient of linear regression is within the range of 1.18 to 1.47.

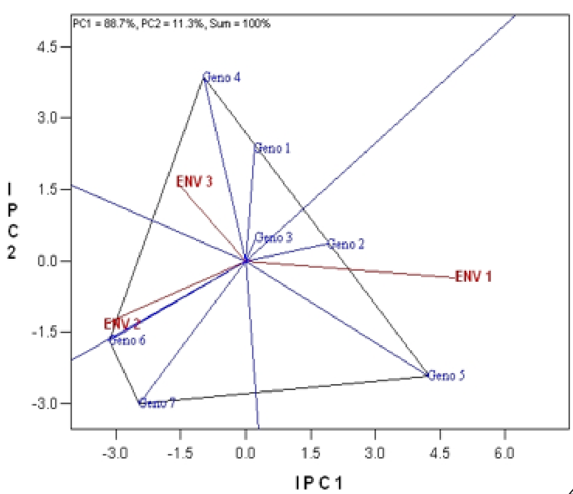
Number of nodules per plant

Ecological plasticity in this sign shows the varieties Mir (bi=3.27) and NDPO80138-B-2 (bi=1.15) (Table 03.) and they can be referred to the intensive types of varieties requiring a high level of agro-technology. The coefficient of regression gives reason to believe that the CA1P, L020140 and X07P54 varieties were poorly responsive and will react insignificantly to changes in environmental conditions.

This suggests that these varieties can find better use in extensive farming where the highest returns will be at minimum cost. The regression coefficient of the X06PWY (bi = 1.01) and Wt6803 (bi = 1.01) varieties was very close to one, and therefore, there is a complete correlation between the variation in the number of nodules and the changes in environmental conditions.

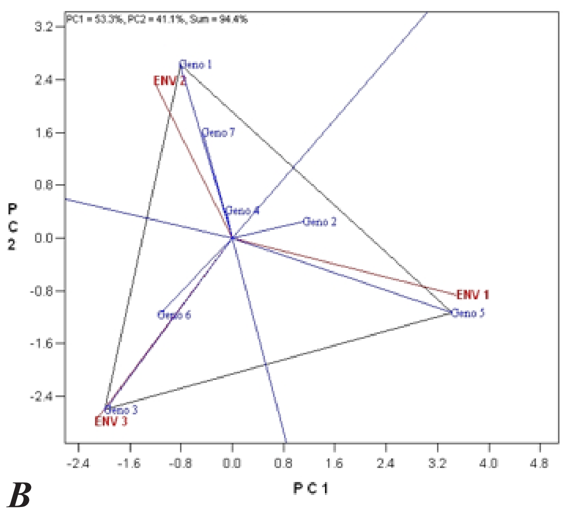
GGE biplot analysis

Genotypic environment interaction (GEI) is further divided using the principal components method (Figure 02.). The model retains only two major components (PC1 and PC2) as this model is best suited to highlighting the regularities and eliminating unnecessary data. The main components (PC1 and PC2) can be easily depicted on 2D graphics so that the interaction between each genotype and a particular environment to be visualized. GGE biplot analysis has shown that the first two major components (PC1 and PC2) account for 100% of the total variability of the plant height sign caused by genotype - environment interaction.



A

B



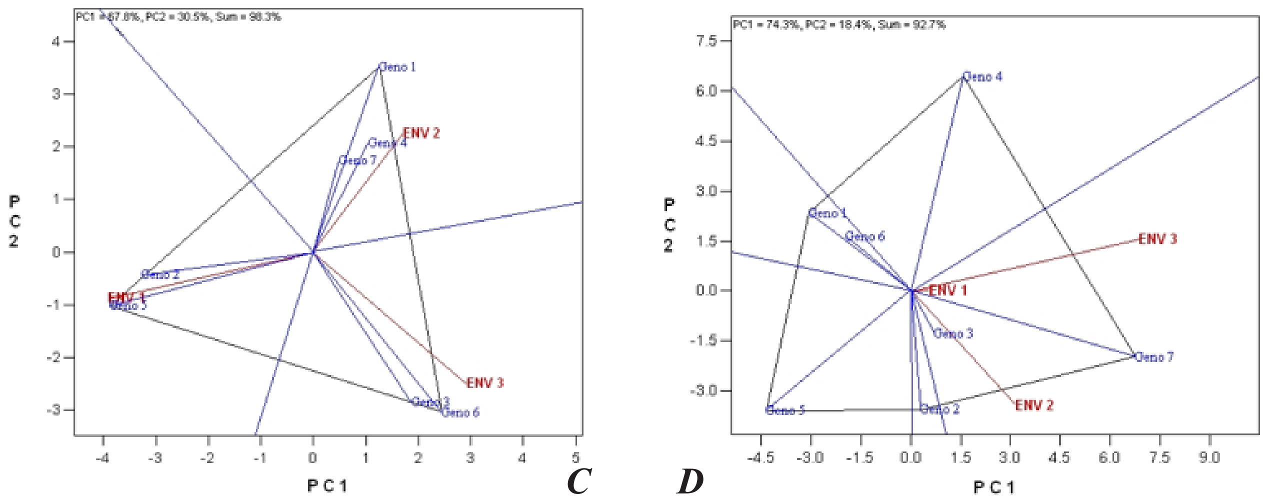


Figure 02: The GGE biplot based on the traits tested

The cultivars were designated with the symbol GENO and the respective number from 1 to 7, as the following, GENO 1 - X07P54, GENO 2 - X06PWY, GENO 3 - NDPO80138-B-2, GENO 4 - CA1P, GENO 5 - L020140, GENO 6 - Wt6803, GENO 7 - Mir. The years were designated with the letters ENV and numbers 1, 2 and 3 for years 2014, 2015 and 2016.

A - plant height (cm), B - leaf fresh weight (g), C - stem fresh weight (g), D - nodule number per plant

The genotype that forms the corner of the polygon for each sector that divides the biplot is the one that gives the maximum value of a given attribute in the environments that fall into that sector. At the height of the plant, the vertices of the polygon corners in the study were genotypes CA1P, L020140, Wt6803 and Mir.

The GGE biplot analysis by the leaf fresh weight shows that the first two major components PC1 and PC2 account for 94.4% of the total variability of the sign caused by the genotype - environment interaction. The CA1P is located close to the beginning of the coordinate system. Positive values of PC1 and PC2 define this genotype as the most stable in this sign, confirming the Eberhart and Russell stability analysis (1966). The remoteness of the X07P54, NDPO80138-B-2, Wt6803 and Mir varieties from the abscissa and their displacement along the ordinate indicates that they were more dependent on environmental conditions and showed a specific adaptation.

According to the GGE biplot analysis of stem fresh weight, the varieties were arranged to form a triangle on whose peaks were situated X07P54 having the best development in 2015 (ENV2), NDPO80138-B-2 with which was favorable to it 2016, and L020140 in the sector defined by 2014 (ENV1). According to the arrangement with respect to the abscissa line, the Wt6803 and Mir varieties the most productive on the stem fresh weight and the X06PWY variety was found highly variable and least productive. Growing varieties with a different reaction to environmental conditions would provide the farmer with more stable yields and yields per area and year.

In the GGE biplot analysis for the number of nodules, the two principal components comprise 92.70% of the dispersion of experiment. At the top of the polygon the varieties were located along with the larger nodule number (Mir, CA1P and L020140) and fewer nodule number (X06PWY and X07P54). The position of CA1P and L020140 relative to the ordinate axis and the beginning of the coordinate system designate them as high-variable, and NDPO80138-B-2 is characterized by high stability. Stability, however, is not the only parameter for evaluating genotypes, as the stable genotypes were in many cases low productive.

Table 04: Estimates of Spearman correlations between leaf fresh weight (under the diagonal), stem fresh weight (above the diagonal) and the methods of stability and adaptability for the analysis of the effectiveness of different algorithms to identify genotypes of pea

	Leaf fresh weight	bi	Si^2	ai	λi	Pi	PP	W^2	W_i	
		0.132	0.198	0.132	0.198	-0.93**	-0.188	-0.188	0.927**	Stem fresh weight
bi	0.771*		-0.262	0.980**	-0.262	-0.388	-0.618	-0.618	-0.128	bi
Si^2	0.067	0.119		-0.257	0.990**	-0.064	0.23	0.23	0.141	Si^2
ai	0.771*	0.99**	-0.12		-0.257	-0.386	-0.609	-0.609	-0.132	ai
λi	0.067	-0.122	0.980**	-0.123		-0.065	0.229	0.229	0.141	λi
Pi	-0.95**	-0.83*	0.174	-0.838*	0.174		0.507	0.507	-0.855*	Pi
PP	0.163	0.116	0.589	0.113	0.583	0.271		0.987**	-0.225	PP
W^2	-0.163	0.116	0.589	0.113	0.583	0.271	0.980**		-0.225	W^2
W_i	0.909**	0.483	0.064	0.483	0.067	-0.84*	-0.46	-0.46		W_i

**, ** Significant at ($P < 0.05$) and ($P < 0.01$), respectively*

Correlation analysis

The quantitative trait of leaf fresh weight strongly correlated with the stability parameters of Eberhart and Russell (1966), Tai (1979) and Annicchiarico (1992) (r = + 0.771; r = + 0.067; r = + 0.909) and negatively with the Pi of Lin and Binns (1988) (r = - 0.95) (Table 04.). The two types of statistical data for stability (bi, ai) of Eberhart and Russell (1966) and Tai (1979) show significant correspondence, both between them and with the W_i of Annicchiarico (1992) (r = + 0.483) although it is not statistically significant. The correlations between the stability parameters of Eberhart and Russell and Wricle were in agreement with the results of Mulusew *et al.* (2009) and Goa and Mohammed (2013). Our result was also in agreement with the work of Alberts (2004) for maize, Molla (2010) for millet, Abate (2011) for wheat and Kefelegn (2012) for beans. Lin and Binns's (Pi) method showed a highly significant negative rank correlation (r = - 0.95) (P = 0.05) with fresh leaf weight. This indicates that high yielding and responsive varieties like Wt6803 leaned to have lower Pi value.

The sign of stem fresh weight interacts positively with the parameter Wi (r = + 0.927) and strongly negative with P_i (Lin and Binns, 1988) and W_i (r = - 0.930). Positive but statistically insignificant were the correlations of this sign with the stability parameters defined by Eberhart and Russell (1966) and Tai (1979). The correlation between the parameters PP and W^2 is significant (r = + 0.987) at a high probability level. The regression coefficient (bi) has a negative correlation with the stem fresh weight and with almost all parameters, except the coefficients ai and λi of Tai (1979). Pi has a positive correlation with PP and W^2 (r = + 0.507).

Acikgöz *et al*. (2009), Orak and Nizam (2009) and Nizam *et al.* (2011) note that varieties of high stability show predominantly average and low yields and react poorly in improving the growing conditions. The same authors reported significant differences between genotype - environment interaction and were determined for the elements of productivity and seed yield of pea genotypes.

CONCLUSIONS

As a result of the complex assessment of the initial set of pea varieties on the parameters of ecological stability and plasticity, promising forms with a different spectrum of ecological reaction have been established and they can be actively used in the creation of new varieties with increased general adaptability.

ANOVA test showed the main effects due to genotype, environment and the genotype x environment were highly significant for plant height, leaf fresh weight, stem fresh weight and the number of nodules per plant.

According to the results of the study the Wt6803 variety can be selected as the most promising breeding material in terms of plant height and number of nodules, and NDPO80138-B-2 and Mir varieties in terms of fresh leaf weight and stem fresh weight.

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