



## Ecological sustainability and stability of quantitative signs in vetch (*Vicia villosa*) varieties

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### ABSTRACT

Ecological sustainability and stability by basic quantitative signs of winter vetch genotypes (*Vicia villosa*) has been assessed. The experiment was conducted in three environments in the central northern Bulgaria during 2014-16 cropping seasons. The following characteristics have been assessed: i) in the beginning of flowering stage – above ground fresh weight (leaves + stems), root length, root fresh weight, nodule number per plant, nodule weight per plant ii) in the technical maturity of seeds stage - seed weight per plant. Analysis of variance showed highly significant differences for the GE (genotype-environment) interaction for most of the signs indicating the possibility of selection for stable entries. Suitable varieties in the direction of both, fresh biomass productivity and ecological plasticity were found. Some of them were considered suitable for growing in a wide range of environmental conditions.

**Key words:** Environment, Genotype, Nodules, Stability, Vetch

The species *Vicia villosa* has been widely used in agro ecosystems as cover crop, and for green fertilization (Chemining'wa and Vessey 2006, Campiglia *et al.* 2010, Mothapo *et al.* 2013). Biological nitrogen fixation is one of the most important sources for production of approximately 65% of nitrogen used in agriculture. Indirect selection in early generations through traits correlated with seed yield and biological nitrogen fixation is important strategy in common bean breeding. In determining the potential of genetically different lines and cultivars, breeders have to observe many different characters that influence yield. Accurate evaluation of these characters is made more difficult by the genotype by environment interaction (Golparvar and Pirbalouti 2011).

Implementation in the production of varieties characterized by high productivity is often accompanied by a loss of sustainability, as they are sometimes environmentally unstable and subject to unfavorable factors. It is, therefore, necessary to look for genotypes that have not only high yield but also resistance to limiting factors as well as environmental stability and on this basis to create new varieties. Productivity breeding is one of the most difficult and complicated tasks, often associated with the need to combine a greater number of valuable traits in one variety (Ermakov *et al.* 2001).

A priority direction in the breeding of winter vetch

is the creation of varieties resistant to the main abiotic factors. Studies in this direction have expanded particularly in relation to the winter resistance of varieties, as well as increasing the realization of seed productivity potential (Tyurin and Zolotaryov 2013).

The aim of this study was to evaluate genotype × environment interactions and stability parameters of eight vetch cultivars for productivity of some main traits for future breeding programmes.

### MATERIALS AND METHODS

The study was conducted in 2014-2016 on the experimental field of the Institute of Forage crops, Pleven, Bulgaria. Sowing was carried out manually in optimal time, according to the technology of cultivation of vetch. Plant material from above ground and root biomass of 8 winter vetch (*Vicia villosa*) varieties originating in the country and abroad, i.e. BGE004222, BGE001847, BGE000637, BGE001076, BGE000639, BGE000643 and BGE001383 was analyzed. The Bulgarian Asko 1 variety was used for control. The following characteristics have been assessed: i) in the beginning of flowering stage - aboveground fresh weight (leaves + stems), root length (cm), root fresh weight (g), nodule number per plant, nodule weight per plant (g); ii) in the technical maturity of seeds stage - seed weight per plant (g). Biometric measurements were made for 15 plants of each variety. The data obtained were processed by two-factor analysis of variance to each trait for determine effects of genotype (G), (E) environment and genotype environment interaction (G×E). The estimation of the ecological stability of the tested cultivars was done through the following

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methods: regression analysis-according to Eberhart and Russell (1966), Tai (1979); analysis of variance-according to Plaisted and Peterson (1959); ecovalence-Wricke (1965) and Annicchiarico (1992); non parametrical analyses using the model of Lin and Binns (1988) and Huehn (1990) and method according Plaisted and Peterson's (1959). GGE biplot model was done, which uses model of Yan (2002). All experimental data were processed statistically by using the software GENES 2009.7.0 for Windows XP (Cruz 2009).

## RESULTS AND DISCUSSION

In modern breeding programs, the question of combining in particular variety, its genetically determined properties of high responsiveness to favorable environment conditions with resistance to negative climatic and biogenic factors of the external environment is of paramount importance (Valekzhanin 2013).

*Analyses of variance:* The results of dispersal analysis of the signs (Table 1) confirm the differences in terms of years. For the aboveground fresh weight, root length, nodule per plant and seed weight per plant, the genotype (variety) and environment (year) factors were reliable. This is an indication that the tested patterns differ in their genetic nature with respect to these signs. The genotype-environment interaction as the most important factor was also true, although for the root length, nodule number per plant and seed weight per plant its influence was the least. This is an objective prerequisite for assessing the ecological stability of the tested patterns by the above ground fresh weight, root length, nodule per plant and seed weight per plant. The factor environment has the most influence, so patterns showed a significant variation in the numerical expression of these signs by years. By root fresh weight, the difference was significant only by the factor year, and no significant differences were found for the nodule weight

per plant. Results of this study are in agreement with the previous report by Farid (2015) and Namayanja *et al.* (2014) for common bean, according which highlighted the relative advantage of the genotypes in terms of nodule number and weight.

Genotype-environment interactions were found to be highly significant not only for dry matter yield but also for seed yield. Similarly, Nizam *et al.* (2011) and Sayar *et al.* (2013) found significant genotype-environment interactions in some vetch species in terms of dry matter yield. Farshadfar *et al.* (2013) highly significant differences for the GE (genotype-environment) interaction for chickpea genotypes indicating the possibility of selection for stable entries. Similar were the findings of Dimova and Petrovska (2010) for maize populations.

*Stability analysis of variants:* Varieties in which "bi" is considerably less than one ( $bi < 1$ ), regardless of the value of the  $Si^2$  variation, can be considered as little promising. They are devoid of such important biological and economic qualities as responsiveness in improving the conditions of the environment. In terms of the stability of the studied traits, those varieties in which the  $Si^2$  dispersion was close to zero are the best.

On average for the period of study the highest aboveground fresh weight (Fig. 1) was found to form BGE000643, followed by BGE001847 and BGE000637. Asko 1 ranks fourth. As a result of the calculation of ecological plasticity parameters using different mathematical approaches (Table 2), they are characterized by a different spectrum of the adaptive responses. The most productive varieties of the aboveground fresh weight had a wide ecological response, the value of their regression coefficient being significantly above one, indicating their responsiveness to a positive change in the environment. Stability of the trait was reported in the comparatively

Table 1 Analysis of variance for stability for seed yield and yield components in vetch cultivars (2014-2016)

| Source of variation | Df | Mean sum of squares for the traits studied |                  |                       |                   |                             |                           |
|---------------------|----|--------------------------------------------|------------------|-----------------------|-------------------|-----------------------------|---------------------------|
|                     |    | Aboveground fresh weight (leaves + stems)  | Root length (cm) | Root fresh weight (g) | Nodules per plant | Nodule weight per plant (g) | Seed weight per plant (g) |
| Environment (E)     | 2  | 5835.24**                                  | 69.47**          | 6.45*                 | 10845.74**        | 1.80                        | 31.85**                   |
| Genotype (G)        | 7  | 916.70**                                   | 25.32**          | 0.54                  | 704.07**          | 0.21                        | 4.02**                    |
| G x E Interaction   | 12 | 1314.40**                                  | 16.64**          | 1.55                  | 688.13**          | 0.26                        | 2.32*                     |
| Env/Genotype        | 16 | 1879.50**                                  | 23.24**          | 2.16*                 | 1957.83**         | 0.46                        | 6.01**                    |
| Env/BGE004222       | 2  | 1130.17**                                  | 39.72**          | 0.04                  | 4309.27**         | 0.21                        | 4.42*                     |
| Env/BGE001847       | 2  | 2223.85**                                  | 13.25**          | 0.30                  | 267.38**          | 0.02                        | 20.06**                   |
| Env/BGE000637       | 2  | 1819.68**                                  | 33.49**          | 2.52                  | 2024.37**         | 0.12                        | 7.03*                     |
| Env/BGE001076       | 2  | 1460.67**                                  | 1.53             | 0.48                  | 147.42**          | 0.02                        | 9.55**                    |
| Env/BGE000639       | 2  | 595.58**                                   | 93.48**          | 0.71                  | 908.87**          | 0.05                        | 0.00                      |
| Env/BGE000643       | 2  | 6230.96**                                  | 2.27             | 2.47                  | 1231.20**         | 2.80                        | 1.96                      |
| Env/BGE001383       | 2  | 207.76**                                   | 0.15             | 0.36                  | 6201.67**         | 0.36                        | 0.66                      |
| Env/Asko 1          | 2  | 1367.34**                                  | 2.05             | 10.43**               | 572.50**          | 0.07                        | 4.41*                     |
| Total               | 24 |                                            |                  |                       |                   |                             |                           |

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

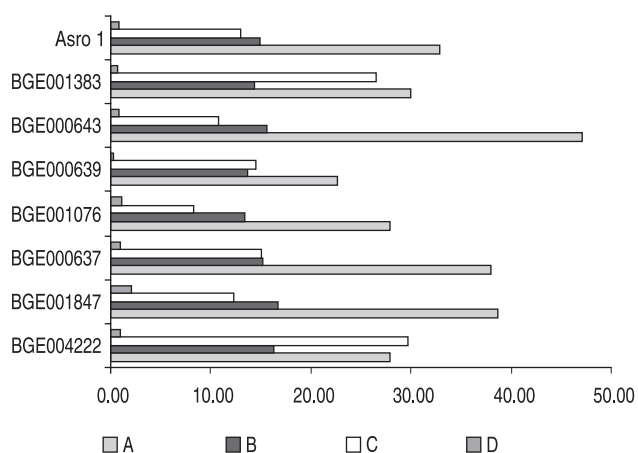


Fig 1. Productivity of the varieties according to the studied traits A -Aboveground fresh weight (leaves + stems) (g); B - Root length (cm); C -Nodule number per plant; D - Seed weight per plant (g).

less productive varieties such as BGE001383, BGE000639 and Asko 1. The most favorable combination of the value of the tested characteristic and its stability was found in BGE004222 on all three types of characteristics (regression, variance and nonparametric).

With respect to root fresh weight, varieties that accumulated significant biomass (BGE000643, BGE001847 and BGE000637) formed longer root system as well. Ecologically unstable can be characterized varieties BGE000639, BGE004222 and BGE000637. The reason for such an agro ecological classification gives us the magnitudes of the regression coefficients that are significantly above one ( $b = 2.13-2.69$ ).

Varieties BGE001076 and BGE001383 were distinguished by high ecological stability but also with low values of the root length trait. The phenotypic stability of this trait, assessed on the parameters of the variance and nonparametric analysis (Table 3), identifies the varieties BGE001847 and the Bulgarian variety Asko1 (control) as most suitable for future breeding work.

The best grade for number of nodules per plant stability based on the regression coefficient and the "PP" parameter of Plaisted and Peterson were found in BGE000643 and BGE000639. However, they failed to form a large number of nodules per plant and therefore, it is inappropriate to use them in the breeding process. Variety BGE000637 combines a good manifestation of the trait number of nodules, having a relatively high ecological stability as well and the highest breeding value.

Namayanja *et al.* (2014) are observed significant correlation between shoot dry weight in the adult plant nodulation assay with nodule number. Appiah *et al.* (2015) reported a positive correlation between symbiotic nitrogen fixation (SNF) and seed yield in chickpeas and cowpea. However, Farid (2015) does not point to any significant correlation between yield and percent nitrogen derived from the atmospheric air (%Ndfa). Mothapo (2011) reported

that plant biomass and tissue N were linearly correlated to nodule mass, while correlation to nodule number was low for biomass and N respectively, indicating nodule mass to be a better indicator of symbiotic efficiency.

The patterns BGE001383 and BGE004222 showed the highest number of nodules per plant, but due to the high regression coefficient ( $b_i = 2.11$  and  $b_i = 1.77$ ) they can be assigned to the group of ecologically unstable varieties. They represent a certain breeding interest and can be used in the combinative selection.

According to the obtained data on the seed productivity (Fig. 1) from the tested varieties vetch as the most promising were found BGE001847, BGE001076 and BGE000637. The significantly lower value of the genotype-interaction factor implies greater stability of the patterns studied by the trait weight of the seeds per plant. This was confirmed by the low values of the parameters " $S_i^2$ " and " $\lambda_i$ ", which are very close for the different varieties and are close to zero.

According to the stability of grain productivity, the studied winter vetch patterns can be evaluated as follows:

- highly productive, with a coefficient of regression ( $b_i > 1$ ). These are the varieties BGE001847, BGE001076 and BGE000637 with a highly predictable response to environmental conditions characterized by very good responsiveness. They are suitable for growing under favorable conditions;
- a mean high value of productivity, with a regression coefficient  $b_i \approx 1$ . These are varieties close to the ideal type, responding well to improved conditions, with high adaptive ability. These varieties can be assigned BGE004222 and Asko1 - low productive, with regression coefficient ( $b_i < 1$ ). This group includes the varieties BGE000643, BGE001383 and BGE000639. These patterns according to the environmental plasticity indicators are characterized by increased resistance to stress factors.

**GGE biplot analysis:** The graphical representation (Fig. 2) divides the plane of distraction of the hypothetical environments and allows to visualize the advantages of each of the patterns. Only two major components (PC1 and PC2) are represented in the model used, because in this way the regularities are best represented and unnecessary data were eliminated.

By the aboveground fresh weight trait, the GGE biplot analysis showed that the first two major PC1 and PC2 components account for 94.3% of the total variability of the trait caused by the interaction of the genotype - environment. In Fig. 2, according to the axis of the abscissa (productivity of the fresh biomass), the varieties BGE000643, BGE001847 and BGE000637 had the highest values and BGE000639 was found the least productive. The position of BGE000637 and Asko 1 relative to the ordinate axis and the origin of the coordinate system define them as highly variable, and BGE001847 and BGE000639 are characterized by high stability.

The graph showing the variance of the varieties and

Table 2 Estimates of the adaptability and stability parameters for the investigated traits of the vetch varieties

| Variety                                              | Eberhart and Russell<br>(1966) |                 | Tai<br>(1979) |          | Lin and Binns<br>(1988) | Plaisted and<br>Peterson (1959) | Wricke<br>(1965) | Annic-chiarico<br>(1992) |
|------------------------------------------------------|--------------------------------|-----------------|---------------|----------|-------------------------|---------------------------------|------------------|--------------------------|
|                                                      | bi                             | Si <sup>2</sup> | ai            | li       | Pi                      | PP                              | W <sup>2</sup>   | W <sub>i</sub>           |
| <i>Aboveground fresh weight (leaves + stems) (g)</i> |                                |                 |               |          |                         |                                 |                  |                          |
| BGE004222                                            | 1.20**                         | 32.55**         | 1.199         | 93.565   | 486.01                  | 143.89                          | 221.40           | 71.386                   |
| BGE001847                                            | 1.72**                         | 21.35**         | 1.725         | 61.546   | 188.50                  | 181.19                          | 874.16           | 98.245                   |
| BGE000637                                            | 1.41**                         | 145.63**        | 1.412         | 416.643  | 263.20                  | 187.08                          | 977.28           | 86.861                   |
| BGE001076                                            | 1.40**                         | 8.60**          | 1.404         | 25.136   | 470.03                  | 147.39                          | 282.59           | 65.226                   |
| BGE000639                                            | 0.01**                         | 18.99**         | 0.107         | 54.223   | 1064.69                 | 427.12                          | 5177.99          | 38.296                   |
| BGE000643                                            | 2.88**                         | 71.25**         | 2.881         | 203.733  | 80.58                   | 446.46                          | 5516.35          | 93.558                   |
| BGE001383                                            | 0.05**                         | 82.05**         | 0.054         | 234.818  | 614.75                  | 229.32                          | 1716.37          | 66.926                   |
| Asko 1                                               | 0.19**                         | 536.06**        | 0.191         | 1532.032 | 617.68                  | 338.98                          | 3635.39          | 68.872                   |
| <i>Root length (cm)</i>                              |                                |                 |               |          |                         |                                 |                  |                          |
| BGE004222                                            | 2.13*                          | 0.03            | 2.144         | 0.308    | 0.375                   | 2.77                            | 22.92            | 103.174                  |
| BGE001847                                            | 1.22                           | 0.08            | 1.224         | 0.339    | 0.182                   | 1.55                            | 1.45             | 110.585                  |
| BGE000637                                            | 1.71                           | 3.04**          | 1.721         | 9.169    | 2.149                   | 2.89                            | 24.94            | 94.104                   |
| BGE001076                                            | 0.01*                          | 0.41            | 0.029         | 1.593    | 8.489                   | 2.66                            | 20.92            | 84.462                   |
| BGE000639                                            | 2.69**                         | 12.05**         | 2.715         | 34.573   | 8.892                   | 7.80                            | 110.87           | 75.366                   |
| BGE000643                                            | 0.07*                          | 0.69*           | 0.087         | 2.381    | 2.899                   | 2.86                            | 24.40            | 98.035                   |
| BGE001383                                            | 0.05*                          | 0.15            | 0.066         | 0.013    | 8.288                   | 2.57                            | 19.42            | 85.084                   |
| Asko 1                                               | 0.39                           | 0.10            | 0.378         | 0.805    | 3.189                   | 1.92                            | 8.03             | 96.649                   |
| <i>Nodule number per plant</i>                       |                                |                 |               |          |                         |                                 |                  |                          |
| BGE004222                                            | 1.77**                         | 31.53**         | 1.766         | 90.642   | 1.50                    | 0.064                           | 1.907            | 150.334                  |
| BGE001847                                            | 0.42**                         | 12.72**         | 0.416         | 36.933   | 317.74                  | -0.026                          | 0.337            | 80.405                   |
| BGE000637                                            | 1.22**                         | 8.84**          | 1.215         | 25.824   | 152.70                  | 0.014                           | 1.024            | 52.700                   |
| BGE001076                                            | 0.31**                         | 7.15**          | 0.308         | 20.848   | 433.68                  | -0.024                          | 0.361            | 36.159                   |
| BGE000639                                            | 0.79**                         | 20.73**         | 0.795         | 59.792   | 217.71                  | 0.173                           | 3.810            | 66.479                   |
| BGE000643                                            | 0.95                           | 1.60**          | 0.951         | 5.150    | 253.50                  | 0.114                           | 2.784            | 12.785                   |
| BGE001383                                            | 2.11**                         | 75.42**         | 2.106         | 215.886  | 85.02                   | -0.029                          | 0.286            | 10.940                   |
| Asko 1                                               | 0.44**                         | 123.08**        | 0.442         | 352.250  | 310.11                  | 0.595                           | 11.193           | 26.742                   |
| <i>Seed weight per plant (g)</i>                     |                                |                 |               |          |                         |                                 |                  |                          |
| BGE004222                                            | 1.05                           | 0.197           | 1.054         | 0.010    | 1.249                   | 0.034                           | 0.040            | 63.776                   |
| BGE001847                                            | 2.19                           | 0.197           | 2.227         | 0.927    | 0.118                   | 0.788                           | 13.232           | 118.001                  |
| BGE000637                                            | 1.33                           | 0.198           | 1.339         | 0.011    | 0.990                   | 0.082                           | 0.868            | 61.163                   |
| BGE001076                                            | 1.55                           | 0.199           | 1.566         | 0.041    | 0.843                   | 0.169                           | 2.400            | 45.544                   |
| BGE000639                                            | 0.10                           | 0.199           | 0.034         | 0.146    | 3.177                   | 0.489                           | 7.990            | 19.424                   |
| BGE000643                                            | 0.70                           | 0.200           | 0.692         | 0.013    | 1.490                   | 0.072                           | 0.708            | 83.881                   |
| BGE001383                                            | 0.13                           | 0.036           | 0.104         | 0.562    | 1.973                   | 0.442                           | 7.172            | 45.725                   |
| Asko 1                                               | 1.05                           | 0.191           | 1.051         | 0.025    | 1.410                   | 0.036                           | 0.064            | 52.373                   |

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

their distribution on the quadrants thus formed on the root length trait indicates that the varieties BGE001076 and Asko 1 are grouped closer to the origin of the coordinate system, which identifies them as the most stable. Variety BGE004222 has positive value for PC1 but negative for PC2, indicating that it is highly environmental sensitive and cannot be expected to perform well. The position of BGE001383 and BGE000639 along the ordinate and their

remote location from the beginning indicates that they have a slight general adaptation but a very well-defined specific adaptation.

Certain differences showed the varieties according to the trait number of nodules per plant. In the quadrant formed by the positive values of PC1 and PC2, only variety BGE001847 has been found, forming a relatively large number of nodules per plant and distinguishing with

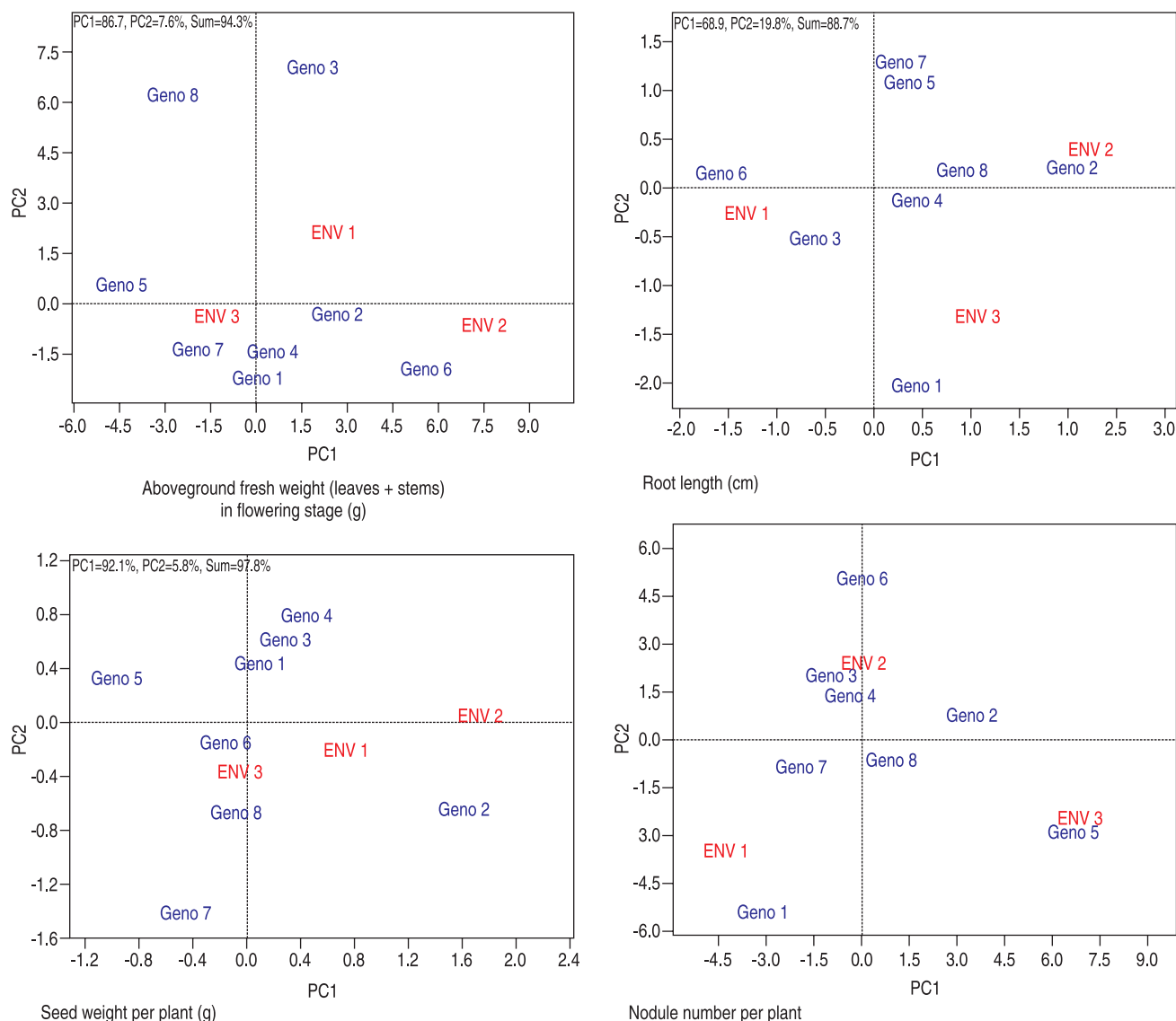


Fig 2. GGE biplot analysis of the studied traits. Aboveground fresh weight (leaves + stems) in flowering stage (g), root length (cm), seed weight per plant (g) and nodule number per plant. Geno 1 - BGE004222; Geno 2 - BGE001847; Geno 3 - BGE000637; Geno 4 - BGE001076; Geno 5 - BGE000639; Geno 6 - BGE000643; Geno 7 - BGE001383; Geno 8 - Asko 1.

very good stability of the trait. Asko 1, which is a control, although it is in the same quadrant of BGE000639 variety, differs in its stability, which is also evident from its very close position to the abscissa axis. The biological features of BGE000639 place it in the first position on this trait, but also with a variation higher than the average for the studied group of varieties.

The location of the varieties on the basis of the seed weight per plant confirms the assessment of the patterns based on the stability parameters. The two major principal components account for 97.9% of the total variability of the trait. The variety BGE001847 showed the highest value on PC1. Its negative value in terms of PC2 puts it in the group of unstable and variable, but with good responsiveness to growing in good environment. The positive value of BGE004222, BGE000637 and BGE001076 PC1 and the closest to zero value of PC2, indicate better environmental

stability and productivity of three varieties.

As a result of the complex assessment of the initial set of varieties of winter vetch 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. In the breeding programs in the direction of fresh biomass productivity and ecological plasticity, the varieties BGE00063 and BGE001847 were found suitable. BGE001847 and Asko 1 were found as potential sources for root length. As a promising initial material in the selection of varieties with a larger number of nodules per plant it is advisable to use the varieties BGE001847, BGE001076 and BGE000643. Varieties BGE004222, BGE000637 and BGE001076 showed high performance grains of high ecological stability and were suitable for growing in a wide range of

Table 3 Classification of genotypes based on nonparametric analysis of Huehn (1990)

| Variety   | 2014                                                             | 2015 | 2016 | Average | 2014                      | 2015 | 2016 | Average |
|-----------|------------------------------------------------------------------|------|------|---------|---------------------------|------|------|---------|
|           | Aboveground fresh weight (leaves + stems) in flowering stage (g) |      |      |         | Root length (cm)          |      |      |         |
| BGE004222 | 4                                                                | 2    | 1    | 2       | 3                         | 6    | 6    | 5       |
| BGE001847 | 3                                                                | 5    | 4    | 4       | 2                         | 3    | 1    | 2       |
| BGE000637 | 6                                                                | 1    | 5    | 4       | 6                         | 8    | 2    | 5       |
| BGE001076 | 2                                                                | 3    | 3    | 3       | 1                         | 7    | 4    | 4       |
| BGE000639 | 1                                                                | 7    | 8    | 5       | 8                         | 4    | 8    | 7       |
| BGE000643 | 7                                                                | 8    | 7    | 7       | 7                         | 2    | 7    | 5       |
| BGE001383 | 5                                                                | 4    | 6    | 5       | 5                         | 5    | 5    | 5       |
| Asko 1    | 8                                                                | 6    | 2    | 5       | 4                         | 1    | 3    | 3       |
|           | Nodule number per plant                                          |      |      |         | Seed weight per plant (g) |      |      |         |
| BGE004222 | 7                                                                | 8    | 1    | 5       | 1                         | 1    | 2    | 1       |
| BGE001847 | 4                                                                | 4    | 6    | 5       | 4                         | 8    | 8    | 7       |
| BGE000637 | 3                                                                | 2    | 5    | 3       | 6                         | 4    | 3    | 4       |
| BGE001076 | 6                                                                | 7    | 4    | 6       | 7                         | 5    | 5    | 6       |
| BGE000639 | 2                                                                | 5    | 3    | 3       | 8                         | 7    | 6    | 7       |
| BGE000643 | 1                                                                | 3    | 2    | 2       | 5                         | 3    | 4    | 4       |
| BGE001383 | 8                                                                | 6    | 8    | 7       | 4                         | 6    | 7    | 6       |
| Asko 1    | 5                                                                | 1    | 7    | 4       | 4                         | 2    | 1    | 2       |

(1)- Better performance (8)-Worst performance

environmental conditions.

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