Genetic Improvement of Biological Nitrogen Fixation in Common Bean Genotypes (*Phaseolus vulgaris* L.)

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Summary

Fifty common bean genotypes were cultivated in two separately field trials at the research station of Islamic Azad University, Khorasgan Branch during 2008-2009. The experimental design was randomized complete block. Bean seeds were inoculated by *Rhizobium legominosarum* biovar *Phaseoli* isolate L-109 in one of the experiments before sowing. The dose of *Rhizobium* for seed inoculation was 7 miligrams of bacteria for 1 kilogram of seed. The second experiment was control. The second experiment was analyzed in the same way as the first except for biological nitrogen fixation. The results showed definite positive and significant correlation in percentage of nitrogen fixation with some other been characters. Step-wise regression designated that total nitrogen percentage in shoot, number of nodules per plant and biomass yield accounted for 93.8% of variation expect for nitrogen fixation percent. Path analysis indicated that total nitrogen percentage in shoot, number of nodules per plant and biomass yield have direct and positive effect on nitrogen fixation index. Hence, total nitrogen percentage in shoot, number of nodules per plant and biomass yield are promising indirect selection criteria for genetic improvement of nitrogen fixation capability in common bean genotypes.

Key words

Phaseolus vulgaris L., *Rhizobium legominosarum* biovar *Phaseoli*, biological nitrogen fixation, path analysis, cluster analysis

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Introduction

Biological nitrogen fixation is one of the most important resources of about 65% usable nitrogen for agriculture (Thomas et al., 1997). Indirect selection in early generations according to traits correlated with Grain yield and biological nitrogen fixation is an important strategy in common bean breeding.

Correlation coefficient analysis helps researchers to distinguish significant relationship between traits. Step-wise regression can reduce effect of non-important traits in regression model. In this way traits accounted for considerable variations of dependent variable are determined (Agrama, 1996). Path analyses method presented by Li (1956) has been frequently used by researchers to segregate correlation between yield and its components in field crops. Path analysis is used to determine the amount of direct and indirect effects of independent variables on the dependent variable (Ulukan et al., 2003).

Miller et al. (1986) found that nodule weight and nodule number per plant were the best criteria for genetic improvement of biological nitrogen fixation in spotted bean, while pod number per plant has been proposed for this purpose in common bean genotypes (Ali et al., 2000). Attewel and Bliss (1985) reported that higher grain yield in common bean genotypes was related to higher amount of biological nitrogen fixation.

Ghasemi et al. (2005) emphasized on traits: nodule number per plant, nodule weight, pod number per plant and pod weight as the most important criteria for breeding biological nitrogen fixation in common bean cultivars.

Ulukan et al. (2003) reported positive and significant relationships between biological yield with plant height, pod number per plant and grain number per pod in faba bean genotypes. A 63.6% of total coefficient of determination was found in the regression model for biological yield as dependent variable. Direct effects of plant height, pod number per plant and grain number per pod upon biological yield were positive. These traits were determined as selection criteria for genetic improvement of biological yield.

In this research, relationships between biological nitrogen fixation and some metric traits were evaluated to determine which characters directly affected biological nitrogen fixation in Iranian common bean genotypes.

Materials and methods

This experiment was achieved at the research station of Islamic Azad University, Khorasgan Branch during 2008-2009 on fifty common bean genotypes collected from different regions of Iran. The experimental design was randomized complete block with three replications. The farm located at 51° 23′ northern latitude and 32° 32′ eastern longitude. Elevation from sea level is 1590 m. Climate is semi-dry and dry with heat and dry summer based on Köppen method.

Annual mean of precipitation and temperature were 130 mm and 14°C, respectively. Field capacity and wilting point were 38 and 9 percent of weighted humidity. The land was under wheat cultivation in previous year. Soil texture was silty-loam with 1.5% of organic carbon, 0.02% of nitrogen, 20 p.p.m of available phosphorus, 504 p.p.m of available potassium, acidity of 7.8 and 3.5 mmohs/cm electrical conductivity in 0-30 cm depth.

Seeds were sown in four 3 meters length rows in each plot. Distances about 0.3 m and 0.15 m were considered between rows and within rows, respectively. Soil experiment showed the homogeniety of the field in each block. Irrigation was performed every three days until plantlet establishment and every five days after this stage. Weed control was conducted during growing season.

This research involved two separate experiments. In one of them seeds were inoculated with *Rhizobium legominosarum* biovar *phaseoli* isolate L-109, and the other was control (Guler et al., 2001; Katiyar and Singh, 1990). The dose of *Rhizobium* for seed inoculation was 7 miligrams of bacteria for 1 kilogram of common bean seed.

In this way, trait biological nitrogen fixation was estimated using nitrogen-difference method proposed by Tamimi (2002). Isolate L-109 had been recognized as the compatible isolate with Iranian common bean genotypes in this province according to results of previous study (Ghasemi et al., 2005).

Measurements of investigated traits were done on ten normal plants, which have been randomly chosen from the two middle rows of each plot. Grain yield per plant, grain number per pod, pod number per plant, pod yield per plant, 100-grain weight, biological yield, harvest index, nodule number per plant at 50% flowering and total nitrogen percentage in shoot were measured and biological nitrogen fixation was calculated using the formula suggested by Tamimi (2002).

Relationships between traits using simple correlation coefficients were studied. Step-wise regression analysis was done to fit the best model for existent variation in biological nitrogen fixation as dependent variable. Path analysis method presented by Dewey and Lu (1959) was used to calculate the direct and indiret effects of the traits entered to regression model on biological nitrogen fixation. The cluster analysis based on Ward's method was also used to classify the traits and to determine the effective criteria for genetic improvement of biological nitrogen fixation. Data analysis was done using SAS and SPSS programs.

Results and discussion

Correlation analysis (Table 1) revealed that all the traits except grain number per pod had positive and significant relationship with biological nitrogen fixation. Step-wise regression analysis for biological nitrogen fixation as dependent variable and the other traits as independent variables (Table 2) indicated that traits: total nitrogen of shoot, nodule number per plant and biological yield accounted for 93.8% of variation that existed in biological nitrogen fixation. Amongst, trait total nitrogen percentage in shoot accounted for 65.1% of variation of biological nitrogen fixation.

Path analysis for biological nitrogen fixation based on traits entered to regression model (Table 3) revealed considerable positive and direct effect of these traits on biological nitrogen fixation, while their indirect effects were smaller than direct effects. Because of that, total nitrogen of shoot, nodule number per plant and biological yield are suggested as the best indirect selection criteria for genetic improvement of biological nitrogen fixation in common bean genotypes. These traits have the largest direct effect on biological nitrogen fixation.

Table 1. Correlation coefficients for traits studied in common bean genotypes (n=50)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
(1) Grain yield (gr)	1									
(2) Grain number per pod	0.322 **	1								
(3) Pod number per plant	0.597 **	0.118	1							
(4) Pod yield per plant (gr)	0.709 **	0.073	0.417 **	1						
(5) 100-grain weight (gr)	0.356 **	0.073	0.403 **	0.372 **	1					
(6) Biological yield (gr)	0.626 **	0.125	0.295 **	0.837 **	0.331 **	1				
(7) Harvest index (%)	0.427 **	0.265 **	0.288 **	-0.142	0.044	-0.406 **	1			
(8) Nodule number per plant	0.388 **	0.007	0.454 **	0.064	0.208 **	-0.203 **	0.584 **	1		
(9) Total nitrogen percentage in shoot (%)	0.529 **	0.212 **	0.547 **	0.272 **	0.407 **	0.216 **	0.339 **	0.466 **	1	
(10) Biological nitrogen fixation (%)	0.524 **	0.078	0.464 **	0.296 **	0.299 **	0.272 **	0.226 **	0.517 **	0.671 **	1

*, **: Significant at 0.05 and 0.01 probability levels, respectively

Table 2. Step-wise regression for biological nitrogen fixation (dependent variable) in common bean genotypes

Variable	$b_{(1)}$	S.E.	r^2	t	Prob
Total nitrogen percentage in shoot (%)	0.466	1.091	0.651	4.395	0.000
Nodule number per plant	0.342	0.535	0.787	3.236	0.000
Biological yield (gr)	0.209	4.944	0.938	2.180	0.000
Intercept	-50.181	8.463		-5.929	0.033

(1): b values have been tested relative to zero.

Table 3. Path analysis for biological nitrogen fixation in common bean genotypes

Variable	(1)	(2)	(3)	Sum of effects
(1) Total nitrogen percentage in shoot(%)	0.551	0.167	0.053	0.772
(2) Nodule number per plant	0.210	0.457	-0.051	0.618
(3) Biological yield (gr)	0.097	-0.073	0.449	0.475
Residual effects	0.667			

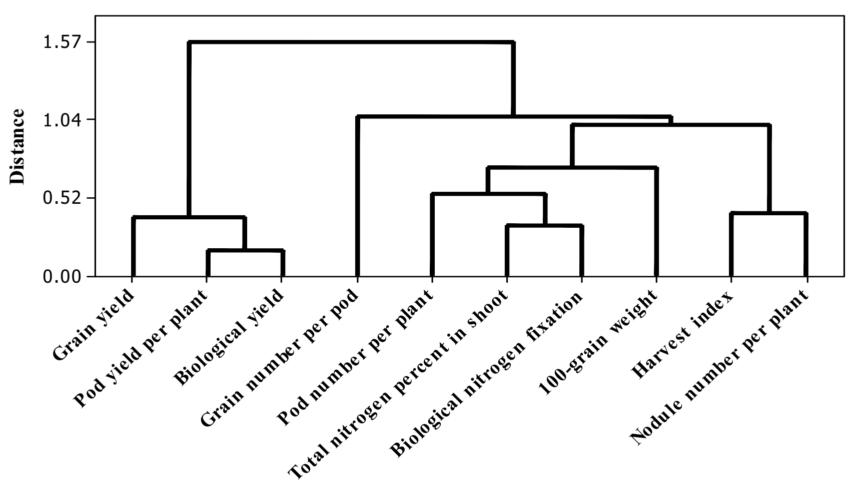

Figure 1.
Dendrogram of cluster analysis to classify variables based on Ward's method

Ghasemi et al. (2005), Yadegari (2003) and Amini et al. (2004) reported similar results for these traits. Also, the findings given by Miller et al. (1986) in spotted bean are in aggrement with results of this research. On the other hands, pod number per plant has been proposed for this purpose in common bean genotypes by Ali et al. (2000) that is inconsistent with results of present study. Attewel and Bliss (1985) also proposed higher grain yield in common bean genotypes as the criteria to obtain the higher amounts of biological nitrogen fixation.

The dendrogram obtained from cluster analysis (Fig 1) showed relative consistency between the result of traits classification given by using two multivariate methods. Some other researchers (Abdollahi et al., 2009; Barron et al., 1999; Ghasemi et al., 2005; Tadesse and Bekele, 2001) reported similar results.

On the whole, we can suggest indirect selection in early generations according to traits that have the highest direct effect on dependent variables. These traits are usually determined by means of statistical procedure like correlation, regression and path analysis. This study showed that traits: total nitrogen of shoot, nodule number per plant and biological yield are the best indirect selection criteria for genetic improvement of biological nitrogen fixation in common bean genotypes.

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