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Genetic Characterization of Agronomic, Physiochemical, and Quality Parameters of Dry Bean Landraces under Low-Input Farming

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ABSTRACT

Dry bean landraces could be cultivated under Low-Input (LI) farming conditions because of their yield stability and quality traits. The objective of this research was to evaluate and identify landraces with high yield and stable performance under LI environment and study the relationships among agronomical, physiochemical, and quality traits. Seven landraces of common bean (*Phaseolus vulgaris* L.) were evaluated in field trials under certified organic management during three consecutive growing seasons (2008-2010) at two different areas located in northern Greece in a RCBD with four replicates. Site per year was considered as one environment. A ranking of landraces according to seed yield potential indicated a group of five high yielding landraces, while Genetic Coefficient of Variation (GCV) for seed yield (9.80%) and number of pods/plant (9.57%) indicated useful genetic variability within landraces, combined with high heritability values (H^2= 0.71 and 0.95, respectively). GGE biplot analysis for yield performance and stability indicated that landrace Kastoria fell within the scope of an ideal genotype, followed by three other promising landraces. Significant positive correlation was detected between cooking time and Ash (0.94). High *GCV* values for hydration increase (16.77%) and cooking time (15.65%) combined with their high heritability (H^2= 0.98 and 0.89, respectively) are of great interest for further genetic advancement. These results indicate that dry bean landraces may provide the appropriate differentiation in several important traits when cultivated under LI conditions, so, effort should be directed to exploit this variability for the development of new varieties suitable for LI agriculture.**

Keywords: GGE biplot, Low-input agriculture, *Phaseolus vulgaris* L., Yield stability.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most widely cultivated legume crop, representing near 50% of grain legumes for human consumption (McClean *et al*., 2004; Acosta-Gallegos *et al*., 2007). Common beans are essential in Mediterranean diet schemes and they are recognized as a significant source of high quality and low cost protein. In addition, amino-acids contained in common beans are of high diet value (Reyes-Bastidas *et al*., 2010). Moreover, as nutrition is nowadays health oriented, common beans appear to help reduce the risks of serious human diseases (Rondini *et al*., 2012).

In Greece, common bean cultivation dates back to 16th century AD (Zeven, 1998). The long-term cultivation and selection in distinct microenvironments, combined with the extensive genetic heterogeneity, led to various

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landraces with particular genetic, morphological, and sensory traits (Papoutsi-Costopoulou and Gouli-Vavdinoudi, 2001; Mavromatis *et al*., 2007; Papadopoulos *et al*., 2012). This genetic material is of great value for breeders (Beebe *et al*., 2001; Gomez *et al*., 2004; Mavromatis *et al*., 2012) and the exploitation of bean genetic resources, particularly of large-seeded white races from European countries, including Greece, has been suggested (Singh, 2001; Angioi *et al*., 2010). Dry bean local varieties or populations are recommended for cultivation under low-input culture systems because they can guarantee the production of beans with traits that are in agreement with the consumers' quality standards and exploit the potential of niche markets (Ayeh, 1988; Camacho Villa *et al*., 2005).

Landraces are variable populations, morphologically identifiable with certain genetic integrity that is characterized by a specific adaptation to the environmental conditions of the area of cultivation (Zeven, 1998; Veteläinen *et al*., 2009; Angioi *et al*., 2011). A significant trait of landraces is yield stability across years that is mainly attributed to their genetic heterogeneity and tolerance to biotic and abiotic stress (Singh *et al*., 2003; Angioi *et al*., 2010). According to Ceccarelli (1989), adaptation in crop plants approximates to yield stability over environments. Therefore, landraces are expected to overcome genotype by environment interactions more easily than genetically uniform modern varieties (Erskine *et al*., 1994) and could perform better in low-input farming systems. In addition, high quality and sensory traits are frequently met in many landraces. This observation is attributed to the selection and maintenance that was conducted by farmers during the long-term cultivation history of landraces (Piergiovanni and Lioi, 2010; Karaköy *et al*., 2014).

The objectives of this research were to discriminate dry bean landraces according to their agronomic, physicochemical, and quality traits, and identify genotypes with high yield and stable performance across low-input environments, keeping seed quality traits that are significant for plant breeders and consumers.

MATERIALS AND METHODS

Genetic Material and Experimental Design

Seven landraces of common bean (*Phaseolus vulgaris* L.) were collected between 1998-2003 in areas traditionally cultivated with beans in Northern Greece and Former Yugoslav Republic of Macedonia (FYROM) and were studied. The landraces were named as following according to their origin: Agios Germanos (Agri), Chrisoupoli (Chr), Florina (Flo), Kastoria (Kas), Laimos (Lem), Nakolets (Nak) and Plati (Plt). Each landrace, cultivated for over 30 years from each farmer avoiding seed mixing, was originally collected (500 g sample) from farmers' stocks. Field experiments were established under low-input farming (certified organic management according to the EU Regulation No. 834/2007) during three consecutive growing seasons (2008-2010) at two different areas: Pili, Kastoria (40^o 46´ N 21^o 02´ E latitude, 858 m altitude, Environments 1, 2, 3) and Agios Germanos, Florina (40^o 50´ N 21^o 09´ E latitude, 960 m altitude, Environments 4, 5, 6) (Table 1). Pili was considered a well-irrigated and fully fertilized site, whereas Agios Germanos was considered intermediated area according to irrigation and fertilization due to scarcity of irrigation water during the period of bean crop seed maturation. Soil characteristics and climatic conditions for each environment are shown in Table 1. Common bean landraces were harvested at the physiological maturity stage. The usual low-input farming practices (deep field plowing, row cultivation, manual weed removal, etc.) were applied. The field trials in each environment were arranged as randomized complete blocks with four replications. Individual plots consisted of four rows of 3 m length spaced 0.70 m apart, and within-row spacing of 0.60 m.

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Table 1. Climatic conditions and soil characteristics of the field trials sites.

Environment	Mean temperature (°C)	Rainfall (mm)	Soil type	pH	$CaCO_3$ (%)	EC^a ($mS\ cm^{-1}$)	Organic matter (%)
Env 1	11.8	650	L [b]	7.56	4.8	0.264	1.15
Env 2	11.5	640	L	7.56	4.8	0.380	2.37
Env 3	11.9	660	L	7.56	4.8	0.380	2.37
Env 4	11.3	700	SL [c]	6.32	0.1	0.253	1.37
Env 5	11.1	690	SL	6.32	0.3	0.253	1.37
Env 6	11.5	680	L	6.75	0.1	0.237	1.11

[a] Soil Electrical Conductivity; [b] Loam, [c] Sandy Loam.

A two-way ANOVA was applied to Genotype×Environment (GE) model using the mixed procedure, considering genotypes as fixed and environment as random effect (Annicchiarico, 2002). Analysis of variance across Environments (E) was performed with the statistical software JMP 5.1 (SAS Institute, 2004). Broad-sense Heritability (H^2) was estimated to assess the effectiveness of the testing program in differentiating among cultivars, as follows: $H^2 = \sigma^2_g/\sigma^2_p$, in which phenotypic variance (σ^2_p is estimated as $\sigma^2_p = \sigma^2_g + (\sigma^2_{ge}/e) + (\sigma^2_\varepsilon/re)$, where σ^2_g is the genotypic variation, σ^2_ε is the error variation, σ^2_{ge} is genotype×environment variation, e and r are the numbers of environments and replications per environment, respectively.

The Genotypic Coefficient of Variation (GCV)

$GCV\% = \left(\sqrt{\sigma^2_g}/X\right) * 100$ was also calculated.

Landraces with high *GCV* for a particular character were assumed as the most responsive to breeding selection and genetic gains. Comparison of mean values was carried out using Student *t*-test. To determine stability across environments, a Genotype plus Genotype×Environment (GGE) biplot analysis was conducted using the GGE biplot pattern explorer software (Yan 2002; Yan and Kang, 2003).

Measurements and Observations

Agronomical Characteristics: Seed Yield (SY) (g $plant^{-1}$), number of Pods/Plant (PP) and seed Weight (W100) were recorded for each replication.

Morphological Characteristics*:* Seed Length (LEN), seed Width (WID) and Thickness (THI) were determined on 20 seeds per plot.

Physicochemical Characteristics: Hydration Increase (HI) of bean seeds was calculated as the percentage of increase in mass of beans soaked in distilled water for 12 h; Hydration Capacity (HC) expressed as hydration capacity per seed was determined by dividing the mass gained by the seeds in 12 h by the number of seeds present in the sample by the method of Bishnoi and Khetarpaul (1993); Seed Coat Proportion (SCP%) was determined on 10 seeds per plot, as the ratio in weight between coat and cotyledon expressed in percentage, after removing the seed coat from the cotyledons, both after soaking and keeping them for 24 hours at 105°C; Cooking Time (CT) was recorded according to the method described by Iliadis (2001). The nutritional quality traits, determined in the finely grounded samples obtained from all plots for each landrace and location, were protein content P (%) (measured with the Kjeldhal method; N×6.25) and mineral ash percentage A (%) (AOAC, 2000) calculated on a dry weight basis.

RESULTS AND DISCUSSION

Yield and Stability

Seed yield and number of pods/plant were significantly ($P< 0.01$) affected by Genotype (G) and Environment (E) main effects and their interaction GEI (Table 2). Sum of

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squares accounted for by G, E and GEI was used as an indicator of the total variation attributable to each component. Environment was the main contributor for SY and PP, with 80.0 and 81.1%, respectively. On the other hand, genotype contributed 7.6 and 7.8%, whereas GEI was 12.4 and 11.1%, respectively. Interaction was low in comparison to E, however, the ratio of GE/G was 1.6 and 1.4 fold for SY and PP, respectively. This might be due to significant crossover interactions of landrace performance across the testing environments. The Heritability in a broad sense (H^2) was also moderate to high for seed yield (0.67) and PP (0.71), whereas the relatively high *GCV*% for yield (GCV= 9.80%) and PP (GCV= 9.57%) indicated the useful genetic variability within landraces and the potential for yield improvements through appropriate breeding schemes.

The mean SY indicated a group of five high yielding landraces with no significant differences (Table 3). Thus, landraces Kas, Lem, Nak, Plt and Flo constituted the high yielding group, whereas landraces Chr and Agi formed the low yielding group. The GGE biplot analysis revealed that there were landraces × year crossover interactions (Figure 1). Landrace Kas was the best in Env1 (Pili08) and Env4 (Ger08), landrace Flo was the best performing in Env2 (Pili09), landrace Lem in Env3 (Pili10) and Env5 (Ger09) whereas, landraces Kas and Flo in Env6 (Ger10). According to the former results, there was no repeatable yield pattern for a single landrace and the choice of the best performing landrace should be based on yield potential and stability (Mekbib, 2003). The GGE biplot analysis for the "ideal" landrace based on yield and stability values revealed the following ranking Kas> Lem, Plt, Nak> Flo> Chr> Agi (Figure 2). Given that Env1-Env3 were considered optimum low-input environments, while Env4-Env6 were considered moderate stress low-input environments, landrace Kas followed by Lem, Plt, and Nak indicated the more stable and high SY performance (Figure 2). Such landraces may constitute valuable genetic resources from which to derive commercial cultivars through appropriate progeny evaluation and selection (Tokatlidis *et al*., 2010; Kargiotidou *et al*., 2014). Breeding strategies need to exploit the existing variation within bean landraces, and such germplasms could broaden the genetic base of commercial beans for developing high yield and stable cultivars (Galván *et al*., 2006; Raggi *et al*., 2013).

Seed Parameters

The W100, LEN, THI and WID were allsignificantly (P< 0.01) influenced by the

Table 2. Analysis of genetic parameters for seed yield, number of pods per plant, and seed characteristics for seven dry bean landraces.

Source of variance	df	SY (g plant^{-1})	PP	W100 (g)	LEN (mm)	THI (mm)	WID (mm)
Environment (E)	5	55616.04**	13807.94**	938.25**	4.73**	1.97**	3.38**
Genotypes (G)	6	4410.87**	1105.69**	805.26**	32.51**	2.45**	4.99**
GEI	30	1441.69**	316.22**	43.35**	0.90*	0.29 **	0.12**
Polledерror	108	506.9	116.5	19.0	0.49	0.10	0.06
SS_G%		7.6	7.8	44.6	17.1	79.4	31.3
SS_E%		80.0	81.1	43.3	50.8	9.6	53.0
SS_{GE}%		12.4	11.1	12.0	32.1	11.0	157
GCV %		9.80	9.57	5.58	4.61	6.87	5.75
H^2		0.67	0.71	0.95	0.63	0.97	0.88

* Significant at 0.05 statistical level, ** significant at 0.01 statistical level.

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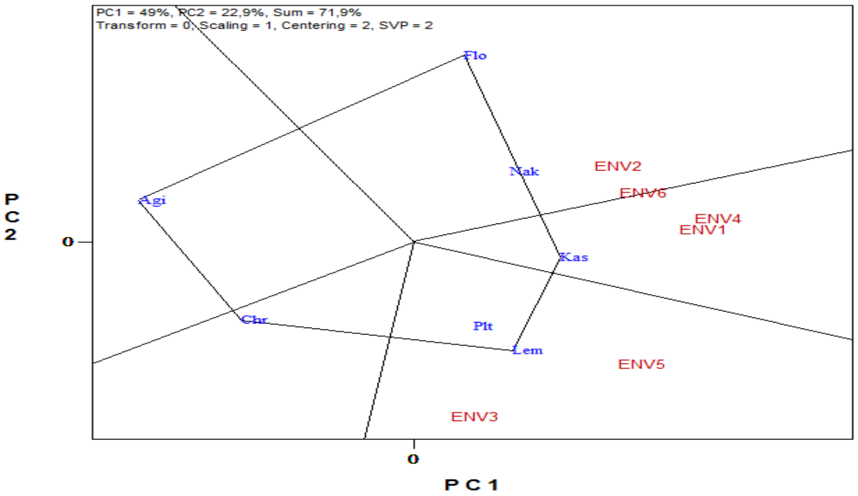

Figure 1. GGE biplot analysis for grouping based on seed yield of seven dry bean landraces grown in six environments under low-input cultivation.

Table 3. Mean values for seed yield, number of pods per plant, and seed character for seven dry bean landraces.

Landrace	SY (g plant^{-1})	PP	W100 (g)	LEN (mm)	THI (mm)	WID(mm)
Agi	64.5^{B}	36.1^{C}	79.7^{a}	19.3^{a}	10.0^{a}	5.3^{d}
Chr	76.7^{B}	40.3^{BC}	68.2c	17.3^{c}	9.4^{d}	5.5^{bc}
Flo	93.2^{A}	42.8^{B}	74.6b	18.1^{b}	9.8^{ab}	5.5^{b}
Kas	97.1^{A}	53.0^{A}	62.3d	16.5^{d}	9.1^{e}	5.3^{cd}
Lem	101.43^{A}	49.8^{A}	69.1c	15.8^{e}	9.3^{d}	6.6^{a}
Nak	98.0^{A}	49.3^{A}	70.4c	18.0^{e}	9.6^{c}	5.3^{d}
Plt	94.6^{A}	53.9^{A}	76.2b	18.3^{b}	9.7^{bc}	5.3^{cd}
CV%	6.1	23.0	6.0	3.9	3.3	4.1
Probability	*	*	*	*	*	*

* Significant at 0.05 level

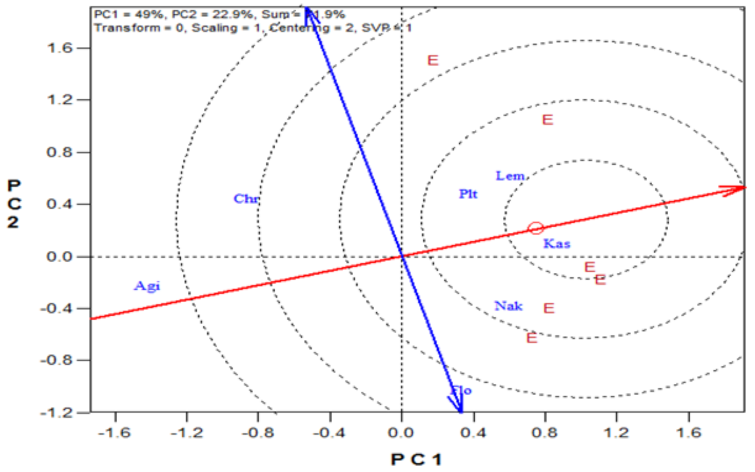

Figure 2. GGE biplot analysis for ranking dry bean land races for stability and seed yield for seven dry bean landraces grown in six environments under low-input cultivation.

G, E, and their interaction, except GEI for LEN ($P< 0.05$) (Table 2). W100, THI, and WID were all strongly influenced by genetic factors presented by high H^2 (0.88%), whereas LEN was influenced mainly by GEI (1.8 fold higher than G) and, for this reason, its heritability was moderate (63%). Genetic coefficient of variation of seed parameters was low to moderate in the rage of 4.61-6.87 (Table 2). Landrace Agi, which was the lowest yielding one, gave the highest values for W100, LEN, and THI (Table 3), suggesting a negative correlation between W100 and SY and W100 and PP (Table 6). This finding is in agreement with other references of a negative association between SY and W100 (White and Gonzalez, 1990; White and Montes, 2005), however, other researchers (Gonzalez *et al*., 2006) underline that this correlation is significantly affected by the environment.

Cooking and Quality Parameters

Cooking parameters were significantly influenced by G, E, and their interaction, except GEI for P% which proved to be insignificant (Table 4). HI%, HC, SCP%, CT, and A% were highly heritable (H^2> 0.80), whereas P% was medium high heritable (0.74%). The parameters with the highest GCV% values were HI% (16.77%) and CT (15.65%), both parameters with high H^2, indicating the high potential for genetic advancement. Regarding *HI*%, landraces Chr, Agi, and Lem were the best, and for HC landrace Agi (Table 5). Elia *et al*. (1997) found high negative phenotypic correlation between CT and HC (-0.87*) and suggested the HC trait as an indirect selection method for cooking time, while Shellie and Hosfield (1991) reported a lower phenotypic correlation (-0.37*) between CT and HC. However, our results indicated a very low and insignificant phenotypic correlation that

Table 4. Analysis of genetic parameters and partitioning of sum of squares for cooking and quality parameters for seven dry bean landraces.

Source	HI%	HC	SCP%	CT (Min)	P%	A%
Environment(E)	2476.23**	0.27**	3.96**	63.12*	36.57**	1.30**
Genotypes (G)	695.5**	0.05**	2.68**	319.39**	6.95**	0.33**
GEI	260.47**	0.01**	0.26*	36.60**	1.77ns	0.04**
Pollederror	79.30	0.00	0.16	9.39	1.36	0.03
SS_G%	36.0	41.1	39.2	45.9	40.8	41.7
SS_E %	47.3	40.3	43.9	38.2	42.1	41.1
SS_{GE}%	5.09	6.64	2.84	6.45	1.37	1.82
GCV%	16.77	0.19	0.64	15.65	0.45	0.14
H	0.98	0.80	0.90	0.89	0.74	0.88

Table 5. Mean values for cooking and quality parameters for seven dry bean landraces.

Landrace	HI%	HC	SCP%	CT (min)	P%	A%
Agi	68.9^{AB}	0.51^{A}	7.8^{CD}	41.9^{A}	24.0^{BC}	4.5^{A}
Chr	69.7^{A}	0.47^{BC}	8.1^{B}	31.9^{E}	24.5^{AB}	4.2^{E}
Flo	67.2^{AB}	0.49^{AB}	7.7^{D}	35.8^{CD}	23.9^{BCD}	4.3^{CD}
Kas	64.6^{BC}	0.39^{E}	8.0^{BC}	35.2^{D}	23.7^{CD}	4.2^{DE}
Lem	67.9^{AB}	0.44^{CD}	7.4^{E}	41.5^{AB}	24.9^{A}	4.4^{AB}
Nak	60.2^{C}	0.41^{DE}	8.2^{AB}	39.8^{B}	24.1^{BC}	4.3^{C}
Plt	55.0^{D}	0.40^{E}	8.4^{A}	37.1^{C}	23.2^{D}	4.4^{BC}
CV%	13.7	15.8	4.9	8.1	4.8	4.2
Probability	**	**	**	**	**	**

**Significance at $P< 0.01$

Table 6. Correlation coefficients and seed yield, number of pods/plant, seed characteristics and quality parameters for seven dry bean landraces.

	PP	SY	W100	HI%	HC	CT	SCP%	P%
SY	0.86**							
W100	-0.48	-0.50						
HI%	-0.72*	-0.48	-0.12					
HC	-0.94**	-0.74*	0.61	0.71*				
CT	-0.02	0.01	0.44	-0.06	0.16			
SCP%	0.31	-0.03	-0.01	-0.71*	-0.50	-0.43		
P%	-0.28	0.01	-0.32	0.68	0.28	0.16	-0.65	
A%	-0.10	-0.11	0.66	-0.09	0.29	0.94**	-0.39	0.09

*Significant at $P< 0.05$, **Significant at $P< 0.01$.

was -0.06 between CT and *HI*% and 0.16 between CT and HC (Table 6). The aforementioned results cannot support the aspect that hydration capacity is an indirect selection criterion for short cooking time. Genotype contribution was high (> 45.9%) for CT, environment contribution was moderate (38.2-47.3%) for all other traits, and GEI was way smaller than G (Table 4). Different CTs were observed among landraces. Landrace Chr (31.9 minutes) followed by Kas (35.2 minutes) showed the shortest cooking time (Table 5). Short CT is a significant advantage especially for landrace Kas, which is the more stable and high-yielding landrace under low-input environment (Figure 2). It is well known that beans with good agronomic performance but extended CT are less acceptable for the consumers (Gonzalez *et al*., 2006; Chiorato *et al*., 2015). In addition, GGE biplot analysis indicated that CT varied between environments (Figure 3), which is in agreement with other researchers (Proctor and Watts, 1987; Carbonell *et al*., 2003; Garcia *et al*., 2012; Papadopoulos *et al*., 2012). When landraces were cultivated in Env1-3, longer cooking time was recorded in comparison with those observed when the same genotypes were grown in Env4-5. This could be attributed to the different $CaCO_3$ level in the soil of the two experimental fields. Paredes-Lopez *et al*. (1989) reported that high Ca soil concentration results in higher cooking time in common bean seeds. In particular, soils in Env1-3 were characterized by $CaCO_3$ concentration over 4%, whereas soils in Env4-6 were near 0% (Table 1). Similarly, increased cooking times were reported in bean cultivars with higher seed Ca concentrations (Shimelis and Rakshit, 2005).

A highly positive (0.94, $P< 0.01$) correlation between CT and A% was observed (Table 6). Since CT is a trait of great importance, further investigation is needed to clarify if selection for low A% could serve as an easy and fast method for indirect selection of genotypes with short CT. Seed coat proportion percent was negatively correlated with HI (-0.71, $P< 0.01$). Similar results were reported by Elia *et al*. (1997) and Gonzalez (2006) who concluded that the highest thick seed coat genotypes were the slowest to absorb water. Non existing association between SY and P% was detected. However, it seems that a stable pattern of the correlation of these two traits does not exist, as there are contradictory reports about positive, negative, or non-existing association between SY and protein concentration (Leleji *et al*., 1972; Kelly and Bliss, 1975; Polignano, 1982; Osborn and Brown, 1988; Gonzalez *et al*., 2006). Finally, a negative association pattern was observed between both SY and PP with seed hydration traits. Particularly, PP was negatively correlated with *HI*% (-0.72, $P< 0.05$) and HC (-0.94, $P< 0.01$), while HC was negatively correlated with SY (-0.74, $P< 0.01$). The former observations suggest that selection

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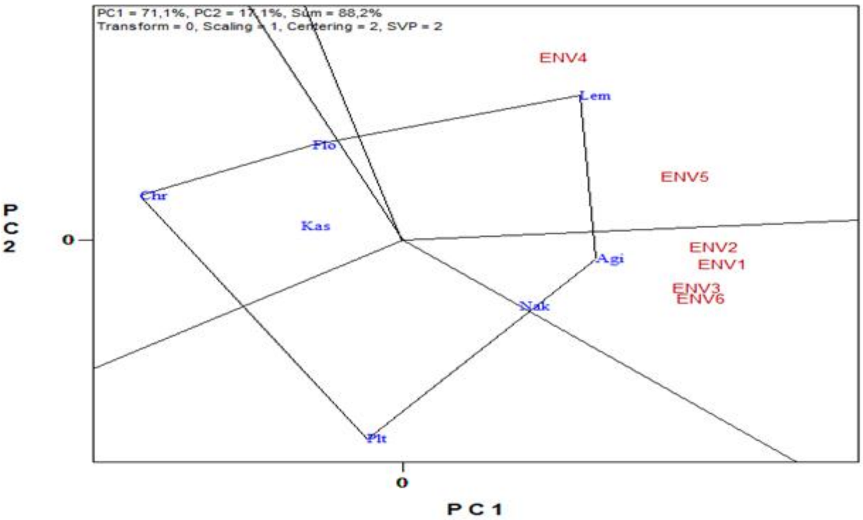

Figure 3. GGE biplot analysis for grouping based on cooking time for seven dry bean landraces grown in six environments under low-input cultivation.

for high yielding varieties is difficult to be associated with high levels of seed hydration.

In conclusion, dry bean landraces may provide the required variability for several important traits for low-input production systems. Landraces were discriminated for mean yield performance and stability, however, crossover interactions existed for SY and CT. Seed yield potential of the landraces could be improved, however, the breeder should consider critical quality parameters. Notably, a valuable genetic variability was observed for CT. Further research should be directed to exploit this variation for the development of new varieties suitable for low-input agriculture.

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شناسایی ژنتیکی پارامترهای اگرونومیکی، فیزیکوشیمیایی و کیفی ارقام بومی لوبیای خشک در شرایط کشت با نهاده کم

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چکیده

به علت پایداری عملکرد و صفات کیفی ارقام بومی لوبیای خشک می توان آنها را در شرایط کم-نهاده کشت کرد. هدف پژوهش حاضر ارزیابی و شناسایی ارقام بومی با عملکرد بالا و با ثبات در شرایط محیطی کم-نهاده و مطالعه رابطه بین صفات اگرونومیکی، فیزیکوشیمیایی و صفات کیفی بود. به این منظور، در یک ازمایش صحرایی در شرایط گواهی شده مدیریت ارگانیک و با استفاده از طرح آماری بلوک های کامل تصادفی، هفت رقم بومی لوبیا (*Phaseolus vulgaris* L.)در طی سه فصل رشد پی در پی (2008-2010)در دو منطقه متفاوت واقع در شمال یونان ارزیابی شدند. در این آزمایش هر قطعه آزمایش در سال به عنوان یک محیط در نظر گرفته شد. رده بندی ارقام بومی متناسب با عملکرد پتانسیل دانه حاکی از یک گروه شامل ۵ رقم بومی با عملکرد بالا بود، در حالیکه ضریب تغییرات ژنتیکی (GCV)برای عملکرد دانه (۹/۸٪) و برای تعداد کپسول در بوته (۹/۵۷٪) به وجود تغییرات سودمندی در ارقام مزبور همراه با وراثت پذیری بالا(به ترتیب $H^2=$ ۰/۷ و $H^2=$۰/۹۵) اشاره داشت. تجزیه بای پلات GGE برای عملکرد و پایداری آن نشان داد که رقم بومی Kastoriaیک ژنوتیپ مطلوب بود و به دنبال آن سه رقم بومی دیگر نوید بخش قرار داشتند. همچنین، همبستگی مثبت معناداری بین زمان پخت و مقدار Ash (۰/۹۴**) به دست آمد. نیز، بالا بودن GCV برای افزایش هیدراسیون (hydration increase) (۱۶/۷۷٪) و زمان پخت (۱۵/۶۵٪) در این رقم ها همراه با وراثت پذیری بالای آنها (به ترتیب $H^2=$ ۰/۹۸ و $H^2=$۰/۸۹) در بهبود ژنتیکی بیشتر ارزشمند هستند. این نتایج حاکی از آن است که ارقام بومی لوبیای خشک که در شرایط کم-نهاده کشت شوند ممکن است زمینه را برای تمایزیابی مناسب در چند صفت مهم فراهم نمایند. بنابراین باید تلاش کرد از این تغییرات برای ایجاد رقم های جدید مناسب کشاورزی در شرایط کم-نهاده سود جست.

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