

Genetic Variability, Correlation and Path Analyses for Agronomic Traits in Lentil Genotypes

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ABSTRACT

Lentil (*Lens culinaris* Medik) is a diploid, autogamous species and is one of the oldest crops in the world. In Argentina, one of the most common problems is the narrow genetic base which must be broadened to provide greater production stability. The objectives were to study the genetic variation for different agronomic traits, the phenotypic and genotypic correlation coefficients among these traits and the direct and indirect effects of these traits on seed yield and to characterize the germplasm for use as parents in a breeding program. Thirty genotypes were evaluated. The collected data were analyzed to determine significant varietal differences by employing Principal Component Analysis (PCA). Genotypic and phenotypic correlation coefficients were estimated and Path coefficient analysis was calculated. Highly significant differences among genotypes for all traits recorded were found indicating the presence of genetic variability; thus broad-sense heritability estimates were high for all traits under study indicating little environmental influence. Correlation analysis indicated that the values of genotypic correlations were slightly higher, in general, than the phenotypic correlations. Different morphological traits showed significant direct and indirect effects on number of pods per plant. Number of branches had the greatest direct effect on number of pods per plant ($p = 0.40$) followed by total number of nodes ($p = 0.27$); height of first pod showed a highly negative direct effect on pod number per plant ($p = -0.37$) and the highest moderate indirect negative effects via number of nodes at the first pod ($p = -0.24$). PCA allowed the discrimination of four groups of cultivars with higher similarity. Our results provide better insight regarding the relationship between various characters determining either timing of flowering or productivity, and provide information on the likely interest of a particular accession as a parent in initial crosses for breeding stable and high-yielding varieties.

Keywords: genetic correlation, *Lens culinaris*, path coefficient, yield components

INTRODUCTION

The genus *Lens* is a member of the legume tribe *Vicieae* which includes the major legume crops of the classical Mediterranean civilizations, faba bean, pea along with lentil. *Lens* is a small Mediterranean genus which contains the cultivated lentil (*L. culinaris* Medikus subsp. *culinaris*) and six related taxa (Ferguson *et al.* 2000).

Lentil (*Lens culinaris* Medik.) is a diploid ($2n=2x=14$), autogamous species and is one of the oldest crops in the world, which originated in the Near East (Zohary 1972). Its ancestry is supposed to be the result of a single domestication event (Zohary 1999). Nowadays, lentil is still traditionally cultivated in the Mediterranean Basin, Asia and has certain diffusion in the Americas. Although not a major crop, it provides a substantial portion of Dietary Energy Supply on a regional basis (FAO 2008). Pulses are, therefore considered as the main source of protein for livestock feed and inland fish production. Moreover, these crops have the unique ability to improve soil fertility by fixing atmospheric nitrogen symbiotically.

Two cultivated varietal groups are distinguished in lentil (Barulina 1930), based on seed shape and dimension: small seeded (less than 6 mm in diameter) var. *microsperma* and large seeded (over 6 mm) var. *macrosperma*. In Argentina, most cultivated types are actually improved varieties. Morphological variation within each varietal group is quite appreciable (Sonnante and Pignone 2001; Martino *et al.* 2004; Bermejo *et al.* 2009).

One of the problems in Argentina is the narrow genetic base of lentil (4 varieties) which must be broadened through introgression of new genes from exotic germplasm. Gene-

rally, breeders narrow the genetic diversity in their breeding populations in the process of selecting the required trait combinations for outputs of improved varieties (Maxted *et al.* 2000). The maintenance of diversity in agriculture is essential to protect plant genetic resources, maintain the genotype-environment interaction, and to provide greater production stability.

Lentil breeding has a relatively short history compared to many of the major crops such as cereals (Materne and McNeil 2007). In our breeding program the methodology is based on a bulk population method with single plant selection at F_5 . In this generation the lines are evaluated for morpho-agronomic traits to develop cultivars capable of producing high seed and straw yields. The simultaneous improvement of seed yield and others agronomic traits appears to depend on the availability of genetic variation for these traits. An assessment of crop developmental traits is needed to determine appropriate selection criteria for simultaneous improvement of agronomic traits and seed yield.

The objectives of this study were: (i) to assess lentil genetic variation in germplasm for different agronomic traits, (ii) to assess the phenotypic and genotypic correlation coefficients among these traits and the direct and indirect effects of these traits on the seed yield, and (iii) to characterize the germplasm for use as parents in a breeding program.

MATERIALS AND METHODS

The present investigation for correlation, and path analysis studies of morphological traits of lentil was conducted at the research station of the Faculty of Agriculture of Rosario University, Argentina ($33^{\circ} 1' S$ and $60^{\circ} 53' W$), in 2008. The experimental material

Table 1 Experimental lines used in this experiment.

Experimental lines	Code	Type	Origin
I29BK	1	Macrosperma	Argentina
A101	2	Macrosperma	Argentina
A102	3	Macrosperma	Argentina
A103	4	Macrosperma	Argentina
A104	5	Macrosperma	Argentina
A105	6	Macrosperma	Argentina
A106	7	Macrosperma	Argentina
A108	8	Macrosperma	Argentina
A109	9	Macrosperma	Argentina
A110	10	Macrosperma	Argentina
A111	11	Macrosperma	Argentina
A112	12	Macrosperma	Argentina
A113	13	Macrosperma	Argentina
A114	14	Macrosperma	Argentina
A116	15	Macrosperma	Argentina
A119	16	Macrosperma	Argentina
B105	17	Macrosperma	Argentina
B113	18	Macrosperma	Argentina
B115	19	Macrosperma	Argentina
B116	20	Macrosperma	Argentina
B119	21	Macrosperma	Argentina
B101	22	Microsperma	Argentina
B103	23	Microsperma	Argentina
B108	24	Microsperma	Argentina
B110	25	Microsperma	Argentina
B112	26	Microsperma	Argentina
B114	27	Microsperma	Argentina
B117	28	Microsperma	Argentina
B118	29	Microsperma	Argentina
B121	30	Microsperma	Argentina

consisted of 30 genotypes of lentil from our breeding program (**Table 1**). Plants grown in a greenhouse were exposed to natural light under a 14/10 h regime of 25/20°C. The genotypes were planted in 5 dm³ pots containing as substrate a mixture of sterile soil, peat and perlite (1: 1: 1) and in a randomized block design with 20 replications. Observations were recorded on number of branch (NB), length (LF) and width (WL) of folioles, length (LP) and width (WP) of pods, number of pods per plant (NP), number of folioles (NF), plant height (PH), height of the first pod (HFP), number of nodes at the first pod (NNFP), days to flowering (DF), days to maturity (DM) and number of total nodes (NTN).

The collected data were analyzed by analysis of variance to determine significant varietal differences among the 30 genotypes using SAS (SAS Guide 1982) and was also analyzed by employing PCA (Principal Component Analysis) using InfoGen software (Balzarini and Di Renzo 2003). Genotypic and phenotypic correlations coefficients were estimated using the software GENES (Cruz 2001) and the Path coefficient analysis was calculated using the software InfoGen. Thus, path analysis plays an important role in determining the degree of relationship between yield and yield components.

Table 2 Genetic parameters for different traits in lentil.

Traits	Mean values	Mean square	Error	F-value	PCV (%)	GVC (%)	H ²
NB	2.61	7.99	0.90	8.8***	36.3	22.7	0.88
NF	10.20	51.05	3.19	15.9***	17.6	15.2	0.94
DF	103.50	5703.01	352.32	16.2***	18.1	15.8	0.93
HP	34.60	867.34	52.51	16.5***	20.9	18.4	0.94
NTN	17.40	133.58	12.45	10.7***	20.2	14.1	0.90
DM	110.80	4928.64	432.21	11.4***	18.7	13.5	0.91
HFP	18.90	457.94	37.17	12.3***	32.2	24.2	0.92
NNFP	9.12	47.37	7.26	6.5***	29.5	15.5	0.84
WP	0.60	0.16	0.01	13.6***	18.2	14.4	0.93
LP	1.07	0.59	0.04	14.7***	18.6	15.4	0.93
NP	14.9	1820.05	74.99	24.3***	57.9	62.3	0.96
WF	0.28	0.08	0.006	13.8***	27.4	21.8	0.93
LF	0.94	0.51	0.04	12.5***	21.4	16.2	0.91

NB: number of branch, NF: number of folioles, DF: days to flowering, PH: plant height, NTN: number of total nodes, DM: days to maturity, HFP: height of the first pod, NNFP: number of nodes at the first pod, width (WP) and length (LP) of pods, NP: number of pods per plant, width (WL) and length (LF) of folioles

RESULTS AND DISCUSSION

Highly significant differences among genotypes for all the traits recorded were found (**Table 2**) indicating the presence of genetic variability which is necessary for an effective breeding program. This polygenic variation may be phenotypic, genotypic or environmental (Biçer and Şakar 2008) thus its relative values give an idea about magnitude of variability.

The highest phenotypic and genotypic coefficient of variation (PCV and GCV) were for number of pods per plant (57.9 and 62.3%, respectively) and the lowest PCV for days to flowering and maturity (18%) and for length and width of pods (18.2%) (**Table 2**). Heritability is an important parameter in the genetic studies of quantitative characters. It is considered as an index of transmissibility of the character from parents to their off-spring. Heritability in a broad-sense is the ratio of genetic variance to the total (phenotypic) variance. Thus, a population expressing a larger proportion of genetic variability for a particular character or group of characters will be more amenable to selection (Mishra *et al.* 2007). Broad-sense heritability estimates were high for all traits under study indicating few environmental influences. These values and the additive genetic variance components were identical in the family variance components. Therefore, if the additive genetic variance is the predominant component of genetic variance in the selection populations, then the heritability estimator based on the variance component due to lines should be adequate to predict the permanent response to selection (Holland *et al.* 2003). Similar results were found by Dixit and Dubey (1985) who reported the highest heritability estimate for days to flowering, however, moderate heritability (59.7%) was observed for seed yield. Erskine *et al.* (1985) reported highest heritability estimate for average seed weight (98%) and Lakshmi *et al.* (1986) and Rathi *et al.* (2002) recorded higher heritability for 100-seed weight and Dayachand (2007) reported higher estimates of broad-sense heritability for days to maturity.

Correlation coefficient study

Adequate knowledge about the degree and direction of the association of characters is a pre-requisite for operating an efficient selection program. Exhaustive studies on the inter-relationship of characters among themselves and also between yield and yield components have been carried out. In this study, the genotypic and phenotypic correlations coefficients calculated among examined traits are given in **Table 3**.

Significant and positive phenotypic and genotypic correlation coefficients were found between NB and NP ($r = 0.61$ and $r = 0.63$, respectively). Similar results were obtained by Sarwar *et al.* (1984) and Zaman *et al.* (1989) who reported a positive correlation of seed yield with NP, number of primary and secondary branches per plant in indige-

Table 3 Genotypic (rg) (below diagonal) and phenotypic (rp) correlation coefficients (above diagonal).

	NB	NF	DF	HP	NN	DM	HFP	NNFP	WP	LP	NP	WF	LF
NB		.26	.36	.17	.38	.35	-.15	-.00	-.10	-.46	.60	-.26	-.01
NF	.25		.50	.22	.15	.52	.36	.43	.45	.49	-.05	.39	-.24
DF	.36	.49		.60	.25	.97	.56	.47	.18	.08	-.14	.11	.16
HP	.16	.20	.60		.38	.56	.59	.34	.31	.16	-.08	-.04	.21
NN	.4	.12	.22	.36		.24	-.15	.34	.16	.15	.61	-.07	.11
DM	.35	.51	.98	.56	.22		.53	.43	.21	.11	-.12	.14	.20
HFP	-.18	.35	.57	.60	-.19	.54		.68	.44	.26	-.61	.26	.31
NNFP	-.02	.44	.48	.34	.34	.44	.67		.41	.28	-.19	.19	.25
WP	-.15	.44	.15	.30	.13	.17	.44	.43		.89	-.18	.70	.75
LP	-.08	.49	.04	.14	.13	.07	.26	.29	.91		-.04	.79	.78
NP	.62	-.06	-.15	-.10	.64	-.14	-.64	-.20	-.20	-.06		-.21	-.06
WF	-.30	.39	.08	.02	-.10	.12	.26	.19	.73	.83	-.22		.79
LF	-.04	.21	.13	.19	.08	.17	.31	.25	.78	.81	-.07	.81	

NB: number of branch, NF: number of folioles, DF: days to flowering, PH: plant height, NTN: number of total nodes, DM: days to maturity, HFP: height of the first pod, NNFP: number of nodes at the first pod, width (WP) and length (LP) of pods, NP: number of pods per plant, width (WL) and length (LF) of folioles

Table 4 The direct and indirect contribution of different traits on number of pods in lentil.

	NB	NF	DF	HP	NTN	DM	HFP	NNFP	WP	LP	WF	LF
NB	0.40	0.00	-0.04	0.00	0.02	0.01	0.05	0.00	0.00	0.00	0.00	0.00
NF	0.03	0.01	-0.05	0.00	-0.03	0.02	-0.06	0.00	0.00	0.00	0.00	0.00
DF	0.07	0.00	-0.22	0.02	-0.01	0.06	-0.12	0.00	0.00	-0.01	0.00	0.00
HP	0.03	0.00	-0.07	0.05	0.09	0.02	-0.15	0.00	0.00	0.00	0.00	0.00
NTN	0.02	0.00	0.01	0.02	0.27	-0.01	0.05	0.00	0.00	-0.01	0.01	0.00
DM	0.07	0.00	-0.20	0.02	-0.02	0.07	-0.10	0.00	0.00	-0.01	0.00	0.00
HFP	-0.06	0.00	-0.07	0.02	-0.04	0.02	-0.37	0.02	0.00	0.00	0.00	0.00
NNFP	-0.04	0.00	-0.02	0.01	0.02	-0.02	-0.24	0.03	0.00	0.00	0.00	0.00
WP	-0.05	0.00	0.01	0.00	-0.07	0.00	-0.05	0.00	-0.02	0.02	-.01	0.00
LP	-0.04	0.00	0.04	0.00	-0.06	-0.01	0.00	0.00	-0.01	0.04	-0.01	0.00
WF	-0.06	0.00	0.00	0.00	-0.06	0.00	-0.03	0.00	0.00	0.01	-0.03	0.01
LF	-0.02	0.00	0.00	0.00	-0.04	0.00	-0.04	0.00	0.00	0.01	-0.02	0.01

NB: number of branch, NF: number of folioles, DF: days to flowering, PH: plant height, NTN: number of total nodes, DM: days to maturity, HFP: height of the first pod, NNFP: number of nodes at the first pod, width (WP) and length (LP) of pods, NP: number of pods per plant, width (WL) and length (LF) of folioles

nous as well as in exotic lentil germplasm. On the other hand, a positive correlation of seed yield per plant with NB, PH, number of seeds per plant and 100-seed weight were observed by Murari *et al.* (1988) in a lentil collection. Significantly positive genotypic and phenotypic correlations between number of folioles and days to flowering ($r = 0.50$) and days to maturity ($r = 0.52$) were observed.

A positive and highly significant correlation coefficient was found between NTN and NP ($r = 0.61$) but NTN is correlated with PH ($r = 0.57$). Singh and Singh (1991) observed that plant height was always positively correlated with seed yield per plant in both *microsperma* and *macrosperma* lentils. Interesting correlation coefficients were found between length and width pod with length and width folioles ($r_{wp-WF} = 0.70$, $r_{wp-LF} = 0.76$, $r_{lp-WF} = 0.80$ and $r_{lp-LF} = 0.79$). This fact is important due to the ease of the measurement of the leaflets. Also, a positive association between seed size and pod size, which was reported by Sharma and Sharma (1978) working with *macrosperma* and *microsperma* accessions of lentil, can be useful in selecting variability for seed size indicating that the selection of size of leaflets affects the size of the pods.

The analysis indicated that that the values of genotypic correlations were slightly higher, in general, than the phenotypic correlations.

Path coefficient analysis

NP is an indicator of performance in seeds (Kumar *et al.* 1995; Rakesh *et al.* 2005). The direct and indirect effects of 13 examined traits on NP were estimated by path coefficient (Table 4). Any of the morphological traits showed significant direct or indirect effect on NP. NB had the greatest direct effect on NP ($p = 0.40$) followed by NTN ($p = 0.27$) while HFP showed a high negative direct effect on NP ($p = -0.37$) and the highest moderate indirect negative effects via NNFP ($p = -0.24$). Also DF had high moderate indirect negative effects on NP via DM ($p = -0.20$). Pandey *et al.*

(1992) reported that NP had a highly positive direct effect on seed yield per plant based on analysis of 1300 germplasm accessions of indigenous origin. However, DF, PH and NB had highly positive indirect effects via NP. PH, NB and NP could emerge as direct yield contributors while number of secondary branches per plant, NP, and number of seeds per pod influenced the seed yield indirectly via NB (Kumar *et al.* 1995).

A pre-requisite for the efficient selection of cultivars in all plant breeding programs is a detailed understanding of the extent and distribution of the genetic variation available. This analysis involves a mathematical procedure that trans-

Table 5 Principal component analysis: eigenvalues and percent of variation accounted for the first four principal components (PCs).

	PC1	PC2	PC3	PC4
Eigenvalue	4.38	3.19	1.57	1.18
Variance (%)	34	25	12	10
Cumulative. (%)	34	58	70	80
Character	Eigenvector			
NB	0.14	0.22	0.54	0.19
NF	-0.16	0.15	-0.02	0.74
DF	-0.16	0.45	0.27	0.03
HP	-0.11	0.33	0.08	-0.52
NN	0.39	0.05	0.03	-0.19
DM	-0.16	0.43	0.29	0.01
HFP	-0.32	0.32	-0.23	-0.12
NNFP	-0.11	0.27	-0.41	0.17
WP	-0.38	-0.22	0.04	-0.11
LP	-0.34	-0.32	0.12	0.07
NP	0.34	-0.13	0.39	0.10
WF	-0.36	-0.24	0.20	0.06
LF	-0.34	-0.16	0.34	-0.18

NB: number of branch, NF: number of folioles, DF: days to flowering, PH: plant height, NTN: number of total nodes, DM: days to maturity, HFP: height of the first pod, NNFP: number of nodes at the first pod, width (WP) and length (LP) of pods, NP: number of pods per plant, width (WL) and length (LF) of folioles

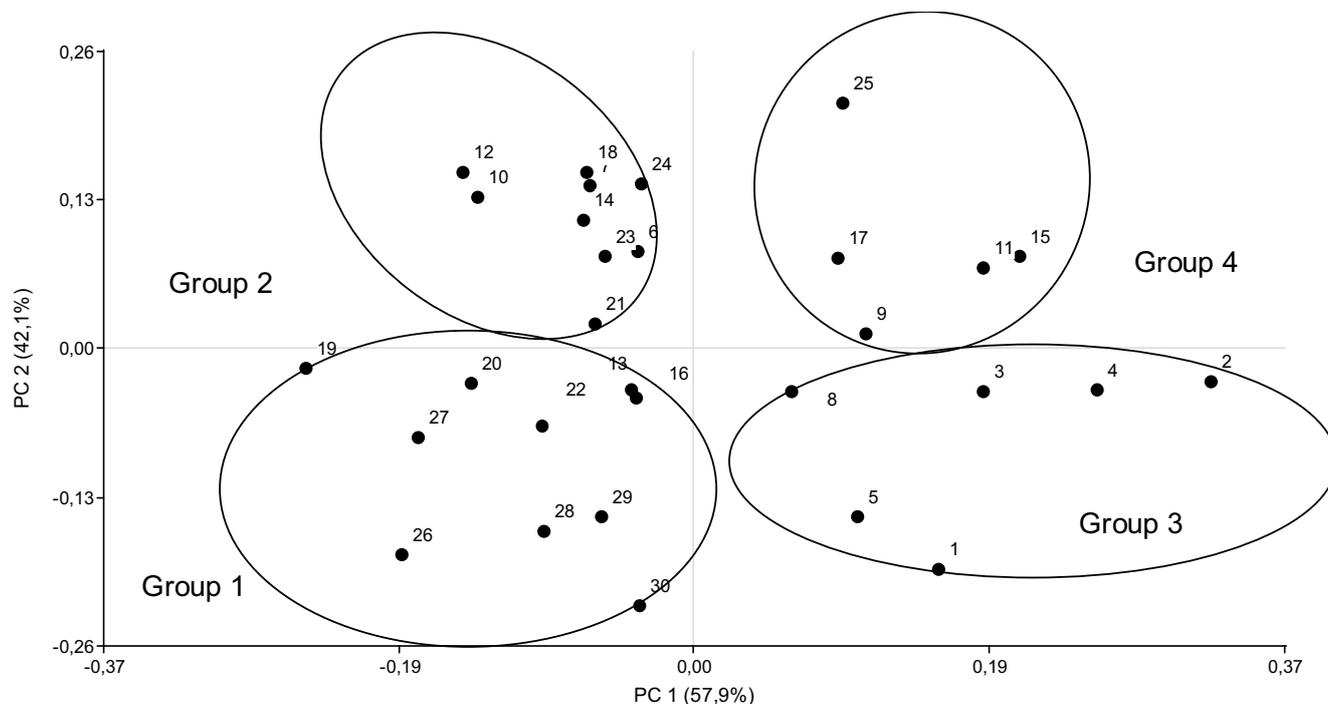


Fig. 1 Graphic representation of the behavior of 30 lines of lentil according the first two principal components and identification of clusters.

forms a number of possibly correlated variables into a smaller number of uncorrelated variables. The first principal component accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. The first principal component (the eigenvector with the largest eigenvalue) explained 34% of total variance. The second principal component explained 25% and the third and fourth 12 and 9%, respectively (Table 5).

PCA allowed the discrimination of four groups of cultivars with higher similarity (Fig. 1). Group 1 (10 lines) presented the fewest nodes and pods and also the largest pods and folioles and short flowering cycle; group 2 (9 materials) with the same characteristics but long flowering cycle. Groups 3 and 4 had most nodes and pods and also the smallest pods and folioles but group 3 presented a short cycle to flowering.

In summary, our results suggest a better insight regarding the relationship between various characters determining either timing of flowering or productivity, and provide information on the likely interest of a particular accession as a parent in initial crosses for breeding stable and high-yielding varieties.

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