

Phenology and pomology of almond's cultivars and genotypes using multivariate analysis

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: The present research aimed to study the flower and fruit properties of 60 almond's cultivars and genotypes. All fruit and kernel traits had high heritability (ranged from 20.73 to 92.13%). Double kernel and pistil length showed the most and few genotypic variation, respectively. The most yield belonged to Filippo Ceo, K8-24, Fragiulo and K9-24 (6.9, 6.84, 6.78 and 7.6 kg/tree respectively). Multivariate analysis was the applied technique to determine the relationship among important traits. Kernel weigh caused yield directly and kernel width indirectly. To estimate kernel weight, kernel variables had less R square (0.61) than nut variables (0.94).

1. Introduction

The efficiency of plants breeding program depends on selection of desirable parents and progenies. Almond cultivars differ in the growth and branching pattern, as well as bearing habit (Duval and Grasselly, 1994).

Self-compatibility (Socias i Company and Felipe, 1988; Ortega and Dicenta, 2003), late blooming (Vargas and Romero, 2001), flower density, production rate (Dicenta *et al.*, 1993 a; Gradziel and Kester, 1998), fruit maturity period (Kester and Asay, 1975; Dicenta *et al.*, 1993 b) and kernel properties (Spiegel-Roy and Kochba, 1974, 1981; Kester *et al.*, 1991) are the main almond breeding objectives, to be informed by the relationship among these traits, facilitates the breeding process, and selection of the desirable almond genotypes (Dicenta *et al.*, 1993 a).

Multivariate analysis is the common technique to evaluate almond genotypes according to quantitative and qualitative characteristics (De Giorgio and Polignano, 2001; De Giorgio *et al.*, 2007). Lansari *et al.* (1994) used this method in order to evaluate morphological variation of almonds varieties. Their results indicated that nut and kernel traits, compare to

vegetative characteristics, has important role in genotype distinction. De Giorgio and Polignano (2001) mentioned fruit characteristics are the most effective variables to differentiate almond genotypes. De Giorgio *et al.* (2007) divided 88 almond varieties, into seven separate groups based on morphological traits. Besides, it has been reported that almond nut length and thickness, and kernel length have significant correlations with kernel length, moreover kernel width have most positive effect on kernel weight (Spiegel-Roy and Kochba, 1981; Kester *et al.*, 1977).

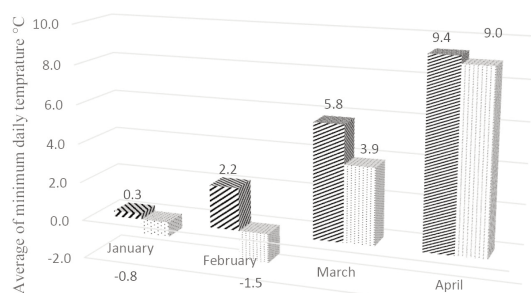
The direct and indirect interaction of the traits which affect the yield can be used as a breeding tool, in this work we analyzed 60 local and foreign almond genotypes and cultivars to understand the casual relation among some important traits.

2. Materials and Methods

Plant materials

This research carried out as randomized complete blocks design, during the years 2013 -2014, in Horticultural sciences research institute, Karaj, Iran (51°35'E, 48°N, 1297 m above sea level, average annual max/min air temperature of 31.3/22.5°C, mean RH of 60%, the average annual precipitation of 148 mm, shallow soil pH=7.5). The average of minimum daily temperature (°C) of January to April (years 2013-2014) is given at figure 1 (Based on Iranian meteorological organization data).

We evaluated 60 almond genotypes (Table 1) using almond descriptors (Gülcan, 1985) (Table 2). All genotypes were 7-year-old and except Tuono, Supernova, Filippo Ceo and Fragiulo, the others were self-incompatible.



	January	February	March	April
█ 2013.0	0.3	2.2	5.8	9.4
: 2014.0	-0.8	-1.5	3.9	9.0

Fig. 1 - Average of minimum daily temperature (°C) from January to April (years 2013-2014).

Table 1 - Almond cultivars and genotypes evaluated in the project

Cultivar and genotypes origin					
Foreign		Local			
Nonpareil	Boty	k1-25	K14-24	K9-2	A-200
Mission	Roby	D124	K13-40	K9-32	K9-20
Perlice	Karmel	K8-24	K9-24	K6-5	K1-16
Padre	Sh-21	Shekofeh	K8-B	K16-23	12-3
Tuono	Sh-6	Mamaie	K11-9	Sahand	8-3
Filippo Ceo	Ne Plus Ultra	Sefid	K4-13	K9-7	D101
Marcona	Sh-17	K8-32	K3-8	K5-6	
Supernova	K1-5	K6-4	K5-17	Rabie	
Frugiulo	Sh-13	K2-22	K5-27	D-99	
Sh-12	Sh-15	K3-12	K3-19	D-8	
A-230		Saba	Talkh asli	Z-3	

Data analysis

Descriptive statistics (minimum, maximum and mean) was used to describe and summarize the data by SPSS 22 and SAS9. Variance components, variance coefficients and Heritability were calculated by Excel 2013.

Multivariate regression analysis was performed to assess the relationship among variables by adjusted R square (ADJRSQ) procedure. Briefly, the subsets of

Table 2 - Quantitative traits using in 60 almond cultivars and genotypes

Trait	Measuring unit	Measuring method
<i>Flower characteristics</i>		
Flower size	cm	Caliper
Petal length	cm	Caliper
Petal width	cm	Caliper
Stamen number	number	Counting
Pistil length	cm	Caliper
Pistil Diameter	cm	Caliper
<i>Nut characteristics</i>		
Nut Length	mm	Caliper
Nut Width	mm	Caliper
Nut Thickness	mm	Caliper
Nut Weight	g	Digital balance
<i>Kernel characteristics</i>		
Kernel Length	mm	Caliper
Kernel Thickness	mm	Caliper
Kernel Width	mm	Caliper
Kernel Weight	g	Digital balance
Kernel Percentage	%	Calculating
Double kernels	%	Counting
<i>Yield</i>	Kg/tree	Digital balance

the independent variables, which have the best estimation of some important dependent variables (like flower size, double kernel percentage) were selected. Then Path analysis was used to describe the direct relation among variables focusing on causality.

3. Results

Table 3 represents the statistical descriptive and variance component. Based on the results, the most genotypic variance (126.37 and 99.08 respectively) belonged to the double kernel and the kernel percentage, while pistil length and thickness had the least (0.01).

The most heritability belonged to kernel percentage and double kernel (92.13% and 89.74% respectively). The rest of the variables had heritability over 20.73%.

Various researches reported different almond kernel weigh heritability such as 64% (Kester *et al.*, 1977), 45% (Spiegel-Roy and Kochba, 1981) and 78% (Dicenta *et al.*, 1993 b); so to have high kernel weight (5.1 grams) progenies, the parents with high kernel weight should be chosen. In the present study kernel weigh heritability was 70.15%.

Filippo Ceo (9.6 kg/tree), k8-24 (6.84 kg/tree), Fragiulo (6.78 kg/tree) and k9-24 (6.7 kg/tree) had

the most yield and Talkh asli (2.33 kg/tree), D101 (2.44 kg/tree), 12-3 (2.45 kg/tree), 8-3 (2.8 kg kg/tree) and Sefid (2.82 kg/tree) had the lowest.

In some environmental conditions (e.g. low temperature before flowering) almond progenies have been produced double kernel nuts even if the parents did not appearance the trait (Egea and Burgos, 1995; Sánchez-Pérez *et al.*, 2007). It has been found that complexity and dominant inheritance of the mentioned trait is affected by genotype and phenotype, which have been reported by several researchers (Dicenta *et al.*, 1993 a, b; Arteaga and Socias i Company, 2001; Sánchez-Pérez *et al.*, 2007). In our study, the double kernel varied from 0 to 57%, suggests it have been influenced by the genotypes differences.

Food industries seeks for almond progenies with the medium thickness and smooth surface nuts (Sánchez-Pérez *et al.*, 2007). We found nut thickness between 9.08-20 mm. Kester *et al.* (1977) reported nut thickness heritability about 0.71, whereas we estimated it about 0.41.

In the present investigation, stepwise regression was used to identify the yield causal system which separates independent variables into direct and indirect ones (Table 4 and Fig. 2). According the data, nut weight had the most standardized beta (0.944). As well as the nut related traits (length, width, thickness

Table 3 - Descriptive statistic, heritability, phenotypic and genotypic coefficients of almond traits

Traits	Max	Mean	Min	Variance components			Heritability	Coefficient of variance	
				Phenotypic	Environmental	Genotypic		Genotypic	Phenotypic
Flower size	5	3.88	2.9	0.39	0.11	0.28	71.04	13.57	16.10
Petal length	2.5	1.65	1	0.13	0.05	0.08	59.08	16.81	21.87
Petal width	1.8	1.35	0.98	0.08	0.04	0.04	52.38	15.54	21.47
Stamen number	37	26.83	15	25.57	4.00	21.57	84.35	17.31	18.84
Pistil length	1.72	1.46	1.1	0.03	0.02	0.01	45.65	8.10	11.98
Pistil thickness	0.52	0.26	0.1	0.04	0.03	0.01	23.08	38.21	79.54
Nut length	47.17	33.35	26.59	24.05	4.67	19.38	80.60	13.20	14.70
Nut width	28.82	20.08	13.28	15.66	4.00	11.66	74.45	17.00	19.70
Nut thickness	20	14.07	9.08	9.64	5.67	3.98	41.23	14.17	22.06
Nut weight	4.76	2.72	0.97	5.47	4.33	1.13	20.73	38.99	85.65
Kernel length	30.89	24.29	18	8.50	1.00	7.50	88.23	11.27	12.00
Kernel width	16.78	12.10	9.19	4.94	1.63	3.31	66.97	15.03	18.37
Kernel thickness	9.12	6.98	4.61	1.38	0.63	0.74	54.02	12.35	16.81
Kernel weight	1.67	1.06	0.56	0.13	0.04	0.09	70.15	28.82	34.41
Kernel percentage	68.88	42.02	23.52	137.17	10.80	126.37	92.13	26.75	27.87
Double Kernel	57	9.95	1	110.41	11.33	99.08	89.74	100.00	105.56
Yield	7.4	5.21	2.62	2.69	0.95	1.74	64.72	25.31	31.46

and weight); the kernel variables (length, width and percentage) significantly influenced the yield (Table 4). The most direct effect related to kernel weight, nut weight, nut thickness, nut width and nut length, respectively (Fig. 2). It is recommended that these traits can be assumed as the selection criteria to improve almond commercial yield.

Also, direct and indirect variables which effect kernel weight (Table 5), double kernel and flower size have been evaluated via multivariate linear regression. Nut characteristics (nut weight and width) had more regression coefficients. Kernel weight estimation entering three independent variables (for example, kernel length, width and thickness) had more R² than two variables (like kernel length and width).

Double kernel estimation using nut traits did not have acceptable R², neither kernel traits. Although the flower size was influenced by the number of stamens, petal length, and width significantly, but the R² was very low (0.353).

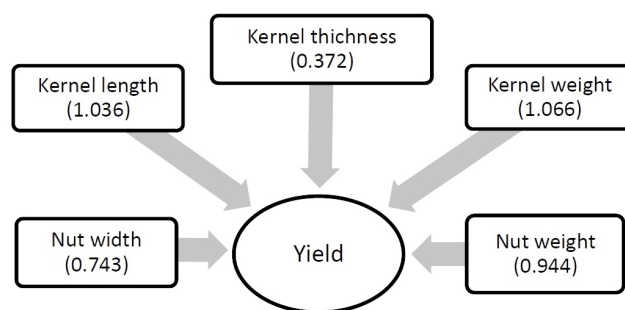


Fig. 2 - Path analysis for traits that affect almonds yield (beta coefficients have given at parenthesis).

4. Discussion and Conclusions

Yield improvement is the most important objective of the almond breeding programs. The commercial yield of almond fruit trees (kg kernel per tree) results the interaction of self (in)compatibility, flower

Table 4 - Yield causality analysis and stepwise correlation coefficients

	Yield	Nut weight	Kernel weight	Kernel thickness	Kernel width	Nut width
Nut weight	0.488**	1				
Kernel weight	0.655**	0.780*	1			
Kernel thickness	0.565**	0.630**	0.697**	1		
Kernel width	0.612**	0.625**	0.752**	0.778**	1	
Nut width	0.466**	0.568**	0.587**	0.594**	0.832**	1
Kernel length	0.266*	0.608**	0.524**	0.281*	0.516**	0.381**
Nut length	0.266*	0.580**	0.489**	0.203		
Kernel percentage	-0.570**	-0.176				
Flower size	0					
Pistil length	-0.088					
Petal width	-0.053					
Stamen number	-0.153					
Pistil length	-0.110					
Nut thickness	-0.022					
Double Kernel	0.140					

*, **, denote statistical significance at 5 and 1% levels, respectively.

Table 5 - Kernel weight estimation based on different independent variables

Dependent variable	Independent variables entering to equation				R ²
Kernel weight	Nut weight	Nut width	Kernel thickness	Kernel percentage	0.9461
Kernel weight	Nut length	Nut weight	Nut width	Double Kernel	0.9455
Kernel weight	Nut weight	Nut width	Kernel percentage		0.9454
Kernel weight	Kernel length	Kernel width	Kernel thickness		0.6193
Kernel weight	Kernel length	Kernel thickness			0.5888
Kernel weight	Kernel width	Kernel thickness			0.5827
Kernel weight	Kernel length	Kernel width			0.5759

buds density, fruit abscission, kernel dry weight and Kernel size (Garcia *et al.*, 1996).

Although some small kernel cultivar (such as 'Felisia') are ideal for chocolate bars and almond drops (Socias i Company and Felipe, 1999), large kernel almond are valued almost. The trait, as well as its correlated trait, varies each year.

High correlation between almond in shell weight and kernel weight (-0.82) (Sánchez-Pérez *et al.*, 2007), in shell kernel ratio and in shell weight (-0.72) and in shell kernel ratio and kernel weight (0.7) (Dicenta and García, 1993) have been reported previously.

Based on our result nut length significantly correlated with nut width (0.59) and nut weight (0.71). Moreover kernel weight showed high correlation with kernel length (0.64) and nut weight (0.69). the kernel weight influenced the yield directly and nut weight indirectly. While nut width, weight and diameter influenced nut length.

Based on the results K8-24 had relatively high nut length (33.5 mm), nut width (28.82 mm), kernel length (25.01 mm) and kernel width (7.48 mm). Fragiulo, K8-24 and Filippo Ceo had relatively high kernel weight (1.486, 1.378 and 1.373 gr respectively). K8-24 and Fragiulo had relatively high nut and kernel weight.

According to our findings, Kernel weight can be used as a selection criterion for almond breeding programs. Regression models for kernel weight estimation revealed that nut characteristics like length, width and weight had more R^2 (0.94) than the kernel characteristic such as weight, width and percentage ($R^2=0.61$).

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