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Pomological, phenotypical diversity and biochemical characterization of fortheen almond morphotypes from Morocco

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Abstract. This study was focused on almond germplasm characterization of 14 genotypes from the experimental Ain Taoujadate collection based on morphological, pomological and biochemical traits. Results revealed that Commercial and local (*Prunus dulcis* L.) cultivars vary considerably in their fruit and kernel characteristics. The majority of important correlations were determined among the fruit traits (length, width, thickness, and weight) and the kernel traits (length, width, thickness, and weight). Morphological dendrogram clustered the genotypes into two main groups. The first group consists of only local accessions. The second group includes, in addition to local genotypes, a mixture of foreign genotypes. The heterogeneous cluster obtained, maybe the cause of an exchange of plant material as seeds between Morocco and other countries.

Keywords: Almond – *Prunus dulcis* – Phenotypic characters – Correlation.

Diversité pomologique, phénotypique et caractérisation biochimique de quatorze morphotypes d'amandier du Maroc

Résumé. *Cette étude s'est centrée sur la caractérisation du germoplasme de 14 génotypes d'amandier provenant de la collection expérimentale d'Ain Taoujadate basée sur des caractères morphologiques, pomologiques et biochimiques. Les résultats ont révélé que les cultivars commerciaux et locaux* (Prunus dulcis *L.) variaient considérablement pour les caractéristiques du fruit et de l'amandon. La majorité des corrélations importantes furent trouvées parmi les caractères du fruit (longueur, largeur, épaisseur, et poids) et les caractères de l'amandon (longueur, largeur, épaisseur, et poids). Le dendrogramme morphologique séparait les génotypes en deux groupes principaux. Le premier groupe consistait seulement en accessions locales. Le deuxième groupe comportait, en plus de génotypes locaux, un mélange de génotypes étrangers. Le cluster hétérogène obtenu pouvait être causé par un échange de matériel végétal comme des semences, entre le Maroc et d'autres pays.*

Mots-clés. *Amandier* – Prunus dulcis – *Caractères phénotypiques – Corrélation.*

I – Introduction

Almond is one of the oldest nut crops [*Prunus dulcis* (Mill.) D.A.Webb; syn. *P. amygdalus* Batsch]. It is mentioned far back in history, even in the Bible. The almond tree is thought to have originated in the mountainous regions of Central Asia (Grasselly, 1976). It was spread from its origin through the Mediterranean by the Phoenicians, Greeks and Romans. World almond production is concentrated in Asia, Mediterranean basin, California (Kester and Horel, 1980). Morocco ranks fifth among the nations of the world in almond production, with many zones where climatic conditions are ideal for this species. The variability in the environment and climate has led to an extensive diversity of almond genotypes in each productive region (Lansari *et al.,* 1994). In previous works, phomological and productive traits in some Moroccan almond cultivars have been already reported

(Lansari *et al*., 1994). The extent of the diversity of the Moroccan almond collection has not been sufficiently described, so there is a need to identify the best cultivars for almond cultivation development (Lansari *et al*., 1994). To maintain and exploit crop germoplasm efficiently, analysis of phenological and pomological traits is required.

Recent research findings are associated with characterization and identification of almond hull, shell and skin phenolic compounds to use them as natural antioxidants and antiradicals in foods and oxidative damage (Esfahlan *et al*., 2012, Jahanban *et al*., 2012; Hanine *et al*., 2014, 2015).

The objective of this study was to evaluate the morphological and pomological characteristics, determine the correlation among traits, and the physicochemical composition of 14 almond genotypes from the experimental Ain Taoujadate collection. Furthermore, an evaluation of economically valuable traits was performed to identify useful genotypes for almond producers and breeding programs.

II – Material and methods

The plant material examined was collected from the experimental Ain Taoujadate of the National Agronomic Research Institute (Meknes, Morocco). The name and geographic origins of cultivars are reported in Table 1. A total of 14 samples were included in this study. The fourteen cultivars included eight major commercial cultivars and six local Moroccan cultivars. Quantitative traits were investigated using the IPGRI almond (Gulcan, 1985). The pomological research was conducted on 30 fruits per cultivar. Data were recorded on fruit and kernel weight (g), width (mm), length (mm), and thickness (mm), and on percentage of kernel (%). The percentages of empty fruits and double kernels were also recorded. Almond blanching and skin removal was carried out according to Bolling *et al*. (2009). Total polyphenols analysis was performed colorimetrically by the Folin-Ciocalteu method as modified by Singleton *et al*. (1965). Total flavonoid content was determined spectrophotometrically according to Zhishen *et al*. (1999) with some modifications. Oil content was measured on dried kernels (30-40 g) using nuclear magnetic resonance (NMR) (Oxford 4000, Oxford Analytical Instruments Ltd.) according to AOCS (1998). All the assays were carried out in triplicate. Significant variables were calculated, subjecting results to a linear regression, using SPSS statistical program version 10.0 (SPSS Inc., Chicago, Illinois). Only variables with a confidence level superior to 95% ($P<0.05$) were considered as significant.

III – Results and discussion

Physical characteristics of the almond fruit and kernels differed significantly among cultivars (Table 1). Kernel length and width ranged from 16.74mm to 27.49mm and 10.72 mm to 16.1 mm, respectively. The kernels weights was found to be from 0.55 g for Tahala to 2.12 g for Tizin'addi 2, and fruit weight 1.41 g for Rizlane 1 to 4.66 g for Texas and Ferragnès.

Regarding width and length, Ferragnes and Fournat de Breznoud showed the longest fruits and kernel (over 36 and 26 mm, respectively) while Tahala produced the smallest (23 mm and 16 mm).The percentage of empty fruits of all genotypes was null. Only Toundout, Amekdouch, Rizlane1, Lauranne, and Texas showed double kernels. The mean values of the double kernels varied from 3 to 27%. Oil content varied significantly among genotypes, ranging from 35% to 57% of the kernel dry mass (Table 1). The oil content of Rizlane 2 and Fournat de Brezenaud was consistently low (between 35.2 and 41.74%), while that of Toundout and Texas was consistently high 56% with the remaining genotype being intermediate. The ranges of variability for this parameter were similar to those already reported (Abdallah *et al.* 1998; Askin *et al*., 2007; Kodad *et al*., 2014).

However the values obtained were remarkably lower than those registered in some Spanish, Italian, French and North American commercial cultivars (Sathe *et al*., 2008). Almond oil content and com-

Table 1. Mean values of examined quantitative traits observed in 14 almond cultivars.

Cultivar	Origin	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit thickness (mm)	Kernel weight (g)	Kernel length (mm)	Kernel width (mm)	Kernel thickness (mm)	Empty fruit %	Double kernels %	Kernel %	Flavonoids (mg Eq Catechin/ g DW)	Oil cointent %	Total phenol cont. of oil (mg Eq gallic/g oil)	Phenols content of oil (mg GAE/g DW)
'Toundout/1R'	Morocco (Errachidia)	2.46^{f}	27.84^{e}	23.04^{dc}	16.47^{cd}	1.16^{c}	21.46^{c}	14.35^{b}	8.74^{b}	0	3.3	47.1^{a}	16.4^{ed}	56.8^{a}	1.5^{bc}	16.1±0.47
'Amekchoud/3J'	Morocco (Errachidia)	3.35^{ed}	33.3^{c}	22.69^{ed}	17.19^{abc}	1.39^{ab}	24.02^{b}	13.83^{b}	9.63^{a}	0	26.7	41.5^{b}	14.5^{e}	53.2^{d}	0.3^{f}	15±0.76
'Rizlane 1'	Morocco (Oujda)	1.41^{g}	24.07^{g}	14.42^{j}	11.28^{g}	0.63^{e}	17.9^{e}	15.24^{a}	6.67^{g}	0	3.3	47.4^{a}	17.9^{cd}	46.9^{i}	1.6^{b}	7.8±0.65
'Rizlane 2'	Morocco (Oujda)	3.37^{ed}	25.75^{f}	20.84^{f}	16.1^{d}	0.63^{ef}	19.43^{d}	11.37^{d}	5.78^{h}	0	0	18.7^{f}	18.0^{cd}	35.2^{m}	1.3^{bc}	20.3±1.32
'Tizin'addi 2'	Morocco (Tiznit)	2.36^{f}	2411^{g}	15.65^{j}	11.27^{g}	2.12^{f}	17.91^{e}	10.72^{d}	3.74^{i}	0	0	22.8^{ef}	18.0^{cd}	45.7^{k}	1.5^{bc}	12.7±0.96
'Tahala'	Morocco (Tiznit)	2.42^{f}	23.16^{g}	17.96^{h}	12.68^{f}	0.55^{ef}	16.74^{f}	15.98^{a}	6.26^{gh}	0	0	23.1^{ef}	18.1^{cd}	46.4^{j}	0.8^{e}	7.5±0.50
'Marcona'	Spain	4.28^{b}	29.23^{d}	25.03^{a}	17.62^{a}	1.05^{d}	19.97^{d}	15.59^{a}	7.45^{ef}	0	0	24.5^{ef}	20.3^{bcd}	50.3^{h}	2.0^{a}	8.2±0.03
'Ferraduel'	France	4.43^{ab}	35.02^{b}	24.11^{b}	16.28^{d}	1.21^{c}	24.6^{b}	14.38^{b}	7.54^{def}	0	0	27.4^{cde}	17.7^{cd}	50.8^{g}	0.9^{de}	11.6±0.19
'Tuono'	Italy	3.16^{e}	28.46^{ed}	22.01^{e}	17.27^{ab}	0.97^{d}	19.93^{d}	12.97^{c}	8.55^{bc}	0	0	30.8^{cd}	18.0^{cd}	51.7^{e}	0.8^{e}	13.5±0.57
'Ferragnès'	France	4.6^{a}	37.74^{a}	23.68^{cb}	16.63^{bcd}	1.43^{a}	27.49^{a}	14.39^{b}	8.17^{bcd}	0	0	31.1^{cd}	22.1^{b}	52.4^{d}	0.9^{de}	16.1±0.85
'Fournat de Brezenaud'	France	4.48^{ab}	36.69^{a}	25.42^{a}	15.07^{e}	1.48^{a}	26.69^{a}	16.1^{a}	6.94^{fg}	0	0	33.2^{c}	14.2^{e}	41.7^{i}	1.2^{cde}	7,1±0,81
'Desmayo Rojo'	Spain	3.71^{c}	29.16^{d}	23.24^{cbd}	16.73^{bcd}	0.95^{d}	20^{d}	12.95^{c}	8.37^{bc}	0	0	25.8^{de}	21.5^{bc}	51.1^{f}	1.2^{cd}	5.2±0.84
'Lauranne'	France	3.47^{cd}	35.15^{b}	25.8^{a}	17.51^{a}	1.3^{b}	24.97^{b}	15.36^{a}	7.98^{cde}	0	6.9	42.3^{ab}	21.1^{bc}	46.5^{j}	1.5^{bc}	16.1±0.44
'Texas'	USA	4.66^{a}	27.37^{e}	19.84^{g}	17.25^{abc}	1.18^{c}	20.14^{d}	12.79^{c}	9.94^{a}	0	3.3	25.7^{de}	19.2^{cd}	56.4^{b}	1.3^{bc}	22.9±2.00

Values with different letters show significant differences according to Duncan's Multiple Range Test (95%).

position depend primarily on the genotype but may be affected by factors such as the year and the specific environmental conditions of the growi*ng* region (Yada *et al.,* 2011). The high oil content, comparable to commonly used oil seeds like rapeseed or sunflower seeds, makes kernels from genus *Prunus* highly suitable for commercial oil production.

Total flavonoids levels were significantly determined by cultivar. Rizlane-2 and Ferragnès exhibited the highest contents of these compounds (25.7 and 22.1 mg/g), while Fournat de Breznaud, Toundout and Amekchoud/3J showed the lowest concentrations (between 14.1 and 16.3 mg/g) (Table 1).

The most significant content of phenolic compounds is observed in the variety Marcona, followed by the genotype Rizlane1, the values fluctuate between 20 and 16 mg / g. These values are much higher than those obtained by the cultivars Amekchoud/3d and Tahala which recorded the lowest values and whose contents are respectively 3.1 and 8.1 mg / g, lowest oil content (3.1 and 8.1 mg/g, respectively).

The morphological dendrogram (Fig. 1) clustered the genotypes into two main groups. The first group consists of only local accessions. The second group includes, in addition to local genotypes, a mixture of foreign genotypes. The heterogeneous cluster obtained, maybe the cause of an exchange of plant material as seeds between Morocco and other countries.

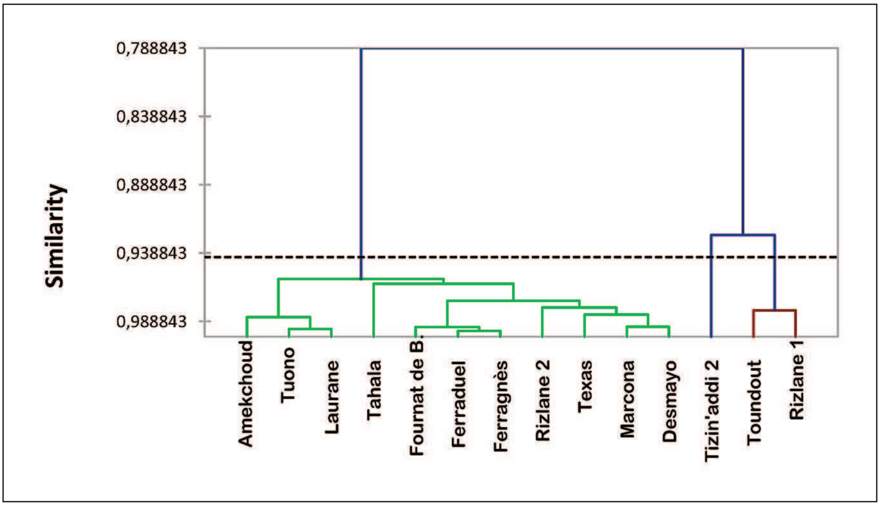

Fig. 1. Dendrogram of similarity with 14 almonds using morphological descriptors.

All examined genotypes are highly adapted to the environmental conditions in Morocco and could be a very interesting source of genetic diversity as indicated in the correlation of some characters of kernel (Table 1). High levels of variation in fruit and kernels were reported by other authors (Čolić, 2012). These results can be explained by self-incompatibilty of almond trees. This high phenotypic variability corresponds with previous reports on molecular characterization using different markers as nuclear and chloroplast simple sequence repeats (Zeinalabedini, 2012) or amplified fragment length polymorphisms (Sorkheh *et al.*, 2007). The results of the empty kernels agree with the previous ones generated by other authors (Dicenta and García,1993; Sánchez-Pérez *et al.*, 2007). In addition, the influence of the environment on the production of double kernels is also well known (Kester and Asay, 1975).

Correlation coefficients between the fruit traits show a strong correlations ($p<0.01$) were observed between most of the studied traits. In addition to the high correlations between fruit traits (length, width, thickness, and weight) (r = 0.48-0.77), kernel traits (length, width, thickness, and weight) were also correlated with each other (r = 0.16-0.70) and with fruit traits. However, no correlation (r = 0.086; p>0.05) was found between the fruit weight and kernel width. Similar findings were reported in 32 almond cultivars investigated by Zeinalabedini (2012). Fruit weight had significant positive correlation with kernel weight (r = 0.53; p<0.01), fruit length (r = 0.60; p<0.01) and the fruit width (r = 0.58; p<0.01).Talhouk *et al*. (2000), Ledbetter (2008), Tavassolian (2008) and Sorkheh *et al*. (2010) established significant correlations between fruit weight and kernel weight. A negative correlation was determined between the fruit weight and the kernel percentage (r = -0.37; p<0.01). These findings are in harmony with the results obtained on almond (Sánchez-Pérez *et al*., 2007).

The results of correlation analysis between polyphenolic and flavonoids content exhibited significant correlations among the total phenolic content, the flavonoid content. Statistically significant (P<0.05) correlation was found between the total phenolics in both almond skin and blanched almonds (r = 0.29 and r = 0.33, P<0.05).

IV – Conclusion

In this study, morphological, pomological and biochimical traits of 14 cultivar from morocco were evaluated. According to our research, genetic diversity was observed regarding morphological traits, indicating that there are rich and valuable plant materials. The research will be continued with the aim of improving production rates for further selection programmes in almond culture breeding. A potential source of markers is available for characterizing almond and for testing species differentiation at the molecular level.

References

Abdallah A., Ahumada M.H. and Gradziel T.M., 1998. Oil content and fatty acid composition of almond kernels from different genotypes and California production regions. In: *Journal of the American Society for Horticultural Science,* 123, p. 1029-1033.

AOCS, 1998. Official methods and recommended practices of the American Oil Chemists' Society (5th ed.). Champaign: AOCS Press.

Askin M.A., Balta M.F., Tekintas F.E., Kazankaya A. and Balta F., 2007. Fatty acid composition affected by kernel weight in almond [Prunusdulcis (Mill.) D.A. Webb.] genetic resources. In: *Journal of Food Composition and Analysis*, 20, p. 7-12.

Bolling B.W., Dolnikowski G., Blumberg J.B. and Chen C.-Y.O., 2009. Quantification of almond skin polyphenols by liquid chromatographylyphenols by liquid. In: *Journal of Food Science*, C326quidBl.

Čolić S., Rakonjac V., Zec G. Nikolić D. and Fotirić Akšić M., 2012. Morphological and Biochemical Evaluation Of Selected Almond [PrunusDulcis (Mill.) D.A.Webb] Genotypes In Northern Serbia. In: *Turk J Agric For*, 36, p. 429-438.

Dicenta D. and García J.E., 1993. Phenotypical Correlations Among Some Traits. In: Almond. *J Genet. Breed.* 46, p. 241-246.

Esfahlan A.J. and Jamei R., 2012. Properties of biological activity of ten wild almond (*Prunusamygdalus* L.) species. In: *Turk J Biol.*, 36, p. 201-209.

Grasselly C., 1976. Origine Et Evolution De L'amandier Cultive. In: *Options Mediterr.*, 32, p. 45-49.

Gulcan R., 1985. Descriptor List For Almond (PrunusAmygdalus). (Revised Ed.). International Board For Plant Genetic Resources, Rome, Italy.

Hanine H., Zinelabidine L.H., Hssaini H., Nablousi A., Ennahli S., Latrache H. and Zahir H., 2014. Pomological and biochemical characterization of almond cultivars in Morocco. In: *Turkish Journal of Agricultural and Natural Sciences*, 1, p. 743-753.

Hanine H., Zinelabidine L.H., Hssaini H., Ennahli S., Latrache H. and Hmid I., 2015. Phenotypic, Morphological Diversity and Biochemical Characterization Of 14 Almond cultivars from Morocco. In: *Mor. J. Chem.*, 3, p. 190-201.

Jahanban Esfahlan A. and Jamei R., 2012. Properties of biological activity of ten wild almond (*Prunus amygdalus* L.) species. In: *Turk J Biol.,* 36, p. 201-209.

Kester D.E and Asay R.N., 1975. Bud failure in almonds: Selection for freedom from bud failure. In: *Calif, agric.,* 29, p. 13.
Kodad O., Estopanan G., Juan T., Alonso J.M. and Espiau M.T., 2014. Oil content, fatty acid composition and tocopherol concentration in the Spanish almond genebank collection. In: *Scientia Horticulturae*, 2, p. 99-107.
Lansari A., Lezzoni F. and Kester D.E., 1994. Morphological variation within collections of Moroccan almond clones and Mediterranean and North American cultivars. In: *Euphytica*, 78, p. 27-41.
Ledbetter C.A., 2008. Shell cracking strength in almond (*Prunus dulcis* [Mill.] D.A. Webb) and its implication in uses as a value-added product. In: *BioresourceTechnol.,* 99, p. 5567-5573.
Sánchez-Pérez R., Howad W., Dicenta F., Arús P. and Martínez-Gómez P., 2007. Mapping major genes and Quantitative Trait Loci controlling agronomic traits in almond. In: *Plant Breed.,* 126, p. 310-318.
Singleton V.L. and Rossi J.A., 1965. Colorimetry of total phenolics with phosphomolibdic-phosphotungstic acid reagents. In: *American Journal of Enology & Viticulture*, 16, p. 144-158.
Sathe S.K., Seeram N.P., Kshirsagar H.H., Heber D. and Lapsley K., 2008. Fatty acid composition of California grown almonds. In: *Journal of Food Science*, 73, p. C607-C614.
Sorkheh K., Shiran B., Khodambashi M., Moradi H., Gradziel T.M. and Martínez-Gómez P., 2010. Correlations between quantitative tree and fruit almond traits and their implications for breeding. In: *SciHortic,* 125, p. 323-331.
Tavassolian I., 2008. Construction of a Microsatellite Based Genetic Linkage Map of almond. Doctoral Thesis. The University of Adelaide. School of Agriculture, 179 p.
Yada S., Lapsley K. and Huang G., 2011. A review of composition studies of cultivated almonds: Macronutrients and micronutrients. In: *Journal of Food Composition and Analysis,* 24, p. 469-480.
Zeinalabedini M., Majourhat Khayam-Nekoui K.M., Grigorian V., Torchi M., Dicenta F. and Martínez-Gómez P., 2012. Comparison of the use of morphological protein and DNA markers in the genetic characterization of Iranian wild *Prunus* species. In: *SciHortic*, 116, p. 80-88.
Zhishen J., Mengcheng T. and Jianming W., 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. In: *Food Chemistry*, 64, 4, March 1999, p. 555-559.