



Inter-annual and genotypic variation of morphological and physicochemical characters in Moroccan loquat (*Eriobotrya Japonica* Lindl.)

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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: Plant development is constantly affected by biotic and abiotic factors, which influence their morphology and chemical composition. In this context, the evaluation of morphological and physicochemical variation of 35 loquat genotypes during two consecutive years, 2015 and 2016, were carried out. The results revealed a significant difference of the morphological and the physicochemical traits between the two years. Indeed, 2016 showed high values for fruit and leaf traits as well as the physicochemical parameters, while 2015 recorded the highest values for seeds traits. In addition, the ANOVA results showed a significant effect of genotype on the physicochemical parameters and the morphological characters, excluding the geometric diameter and spherical index of seeds. Regarding the effect of year, it was also significant on physicochemical parameters and morphological traits except the size and shape of fruit and the seed shape. For the genotype x year interaction effect, it was significant on all traits studied, with the exception of the traits relating to geometric diameter of fruit and seed plus the sphericity index of the seed. Thus, size and shape of fruit remained stable over these two consecutive years. The identification of stable traits presents a result that could be beneficial for breeding programs.

1. Introduction

Loquat (*Eriobotrya japonica* Lindl.), belonging to *Rosaceae* family, is an evergreen tree, native to China (Gariglio *et al.*, 2002). The world production of loquat is about 314,384 tons, 64% of this amount exhibited by China (Caballero and Fernández, 2003). In the Mediterranean region, loquat crops are highly developed, particularly in Spain and Turkey (Del

Mar Romero Escudero *et al.*, 2011). Loquat cultivation is considered a commercial crop in some countries, while in others it is grown only in family orchards (Caballero and Fernández, 2003). In Morocco, this tree, with its yellow fruits, is planted as a commercial consumable crop as well as an ornamental crop (Hussain, 2011) in the regions of Fez-Meknes, Khemisset, Tetouan and in the region of Marrakech but it is localized especially in Berkane with an area representing 85% of the national surface (Skiredj and El Macane, 2003). The Berkane region is mostly covered by loquat tree due to its mild and sunny microclimate and well-drained fertile soils (Rhomari, 2013). In 2021, the production of loquat in Berkane crossed 10,000 tons, with an improvement in the size of the fruits and the gustative quality of this excellent local product (Chellay, 2022).

The estimates of trait heritability were found to be relatively low to moderate (Jiwuba *et al.*, 2020). Ezenwaka *et al.* (2018) suggests that the combination of genotype and environmental effects greatly influenced trait expression. Indeed, phenotypic expression as well as observed variation in plant growth and development depend on both genetic background (G), environment (E) and their interaction (G × E) (Falconer and Mackay, 1996). A result, a clear understanding of G×E will provide a solid basis for identifying superior and stable genotypes in different environments (Zhang *et al.*, 2010). The study of the effect of these factors on the variation of morphological and chemical parameters of plants, including loquat, is limited, but with climate change it has become an obligation. In the region of Berkane, the main producer of this fruit in Morocco, the temperature recorded a decrease, while the rainfall increased during 2015 and 2016 (Meteobleu, 2024). These observations encouraged to explore the effect of these changes on the morphological and physicochemical characteristics of 35 loquat genotypes during these two consecutive years. Indeed, high trait stability present one of the main challenges of plant breeding programs.

2. Materials and Methods

Plant material

In April 2015, a prospection was carried out in loquat plantations of Zegzel, Takerboust, Taghsrout and Tazaghin, belonging to the Berkane region, to identify the genotypes that will be involved in this

study (Fig. 1). The choice of the genotypes based on a numerous agronomic and economic criteria, such as tardiness and earliness, shape, size and color of fruit, shape of leaves as well as the good physical condition of tree. Indeed, a total of 10 mature, healthy fruits and 10 well-developed leaves were collected randomly during April and May of 2015 and 2016 from 35 adult and young trees.

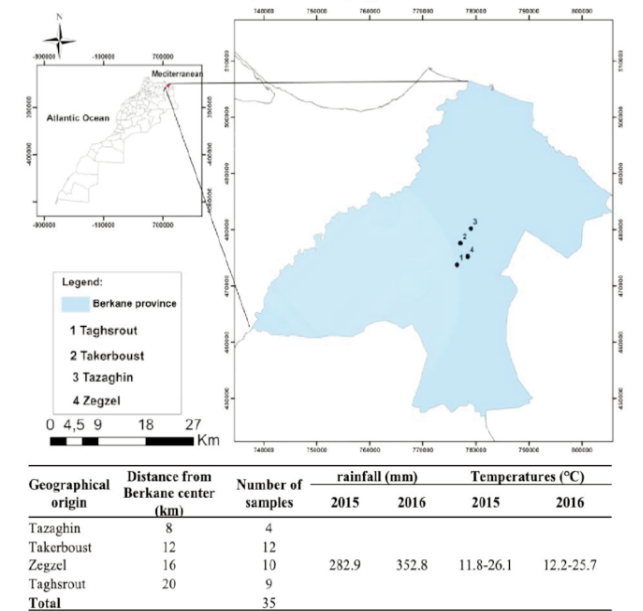


Fig. 1 - Sampling sites of loquat genotypes studied and their geographic and climatic parameters.

Morphological analysis

A total of 17 characteristics related to leaves, fruits and seeds, listed in International Union for Plant Protection descriptor (UPOV, 1998), were investigated (Table 1). The weights were determined using an electronic balance with a sensitivity of 0.01 g, while the dimensions were measured using a digital caliper (Stainless Hardned) with a sensitivity of 0.01 mm. Leaf length measured as the distance from the apex to the base of the leaf, including the blade and petiole. Regarding the blade width, it is measured at the widest part of the leaf.

Physicochemical analysis

Soluble solid content, determined by a refractometer (ATAGO C.O. Ltd; Model PR-1). Briefly, a few drops of the juice of each genotype were placed on the prism of the equipment surface and the soluble solid content expressed in °Brix. The titratable acidity was measured by potentiometric titration using a standardized alkaline solution (Serrano *et al.*, 2003).

Table 1 - Fruit, seeds, leaf traits and physicochemical parameters analyzed

Traits	Code
<i>Fruits</i>	
Weight of the fruit (g)	FW
Geometric diameter of the fruit (mm)	GDF
Sphericity index of the fruit	SIF
Surface of the fruit (mm ²)	SF
Volume of the fruit (cm ³)	VF
Weight of flesh (g)	WF
Flesh ratio	FR
<i>Seeds</i>	
Average weight of the seed (g)	AWS
Geometric diameter of the seed (mm)	GDS
Sphericity index of the seed	SIS
Surface of the seed (mm ²)	SS
Volume of the seed (cm ³)	VS
<i>Leaf</i>	
Leaf length (cm)	LL
Blade length (cm)	BL
Blade width (cm)	BW
Petiole length (cm)	PL
Number of veins	NV
<i>Physicochemical parameters</i>	
Soluble solids content (°Brix)	
Titrate acidity (g/l malic acid)	
pH	

GDF= (Fruit length x Fruit width x Fruit thickness)^{0.333};

SIF= GDF/Fruit length;

SF= $\pi \times \text{GDF}^2$;

VF= $(\pi/6) \times \text{GDF}^3$;

WF= FW-AWS;

RF= FW-AWS/FW;

GDS= (Seed length x Seed width x Seed thickness)^{0.333};

SIS= GDS/Seed length;

SS= $\pi \times \text{GDS}^2$;

VS= $(\pi/6) \times \text{GDS}^3$;

NV were counted.

An amount of 10 ml of fruit juice was diluted in 50 ml of distilled water and then titrated with a 0.1 NaOH solution until pH 8.2 was reached. Titratable acidity is expressed per g malic acid L⁻¹. Concerning the pH values of the juice, it was measured using an electronic pH meter (PH211R, HANNA®) with three replicates for each sample.

Statistical analysis

Data obtained were subjected to statistical analysis using SAS software (SAS Institute Inc., 1988).

Indeed, the two-way analysis of variance (ANOVA) tests was performed to determine the effect of genotype, year and genotype x year interaction on the morphological traits as well as the physicochemical parameters of genotypes. The comparison of means was performed by Duncan's test.

3. Results

The assessment of morphological and physicochemical characters of 35 loquat genotypes, from the Berkane region, revealed a significant difference between the two consecutive years (Table 2). In addition, a significant effects of genotype, year and their interaction on the most of the traits studied, were recorded (Table 3). In fact, the comparison of fruit values indicated a significant difference of fruits

Table 2 - Averages comparison of fruit, seed, leaf and physicochemical characteristics during 2015 and 2016

Traits	Years	
	2015	2016
<i>Fruit</i>		
Fruit weight	41.02 b	42.73 a
Geometric diameter of the fruit	42.28 a	41.58 a
Sphericity index of the fruit	0.86 a	0.87 a
Fruit surface	53.86 a	54.98 a
Fruit volume	37.96 a	39.03 a
Weight of the flesh	34.80 a	35.55 a
Flesh ratio	0.81 b	0.83 a
<i>Seed</i>		
Average weight of the seed	2.58 a	2.36 b
Geometric diameter of the seed	15.11 a	14.43 a
Sphericity index of the seed	0.71 a	0.72 a
Surface of the seed	7.23 a	6.36 b
Volume of the seed	1.85 a	1.54 b
<i>Leaf</i>		
Leaf length	22.47 b	23.71 a
Blade length	21.16 b	22.51 a
Blade width	6.46 b	7.34 a
Petiole length	1.31 a	1.20 b
Number of veins	0.41 b	0.43 a
<i>Physicochemical characteristics</i>		
Soluble solids content	7.17 b	9.77 a
Titrate acidity	5.69 b	8.09 a
pH	3.18 b	4.52 a

Table 3 - Genotype, year and their interaction effect on fruit, seed, leaf and physicochemical characters

Source of variation	ddl	Mean square	F-Value	Pr>F
<u>Fruit</u>				
<u>Fruit weight</u>				
Genotype	34	1746.70	22.03	<.0001
Year	1	512.50	6.47	0.0112
Genotype × year	34	472.97	5.97	<.0001
Error	630	79.27		
<u>Geometric diameter of the fruit</u>				
Genotype	34	495.17	2	0.0008
Year	1	84.38	0.34	0.5593
Genotype × year	34	308.47	1.25	0.1608
Error	630	247.26		
<u>Sphericity index of the fruit</u>				
Genotype	34	0.06	17.09	<.0001
Year	1	0.0006	0.17	0.6763
Genotype × year	34	0.01	3.09	<.0001
Error	630	0.003		
<u>Fruit surface</u>				
Genotype	34	1573.73	24.28	<.0001
Year	1	218.49	3.73	0.0668
Genotype × year	34	13125.09	5.96	<.0001
Error	630	64.81		
<u>Fruit Volume</u>				
Genotype	34	1682.73	22.39	<.0001
Year	1	198.99	2.65	0.1042
Genotype × year	34	421.05	5.60	<.0001
Error	630	75.14		
<u>Weight of the flesh</u>				
Genotype	34	877.43	19.18	<.0001
Year	1	65.57	1.43	0.2318
Genotype × year	34	218.82	4.78	<.0001
Error	452	45.74		
<u>Flesh ratio</u>				
Genotype	34	0.01	6.81	<.0001
Year	1	0.02	14.20	0.0002
Genotype × year	34	0.008	5.03	<.0001
Error	452	0.001		
<u>Seed</u>				
<u>Average weight of the seed</u>				
Genotype	34	2.13	5.95	<.0001
Year	1	5.43	15.18	0.0001
Genotype × year	34	1.31	3.68	<.0001
Error	451	0.35		
<u>Geometric diameter of the seed</u>				
Genotype	34	20.00	1.26	0.1563
Year	1	54.60	3.43	0.0647
Genotype × year	34	12.21	0.77	0.8266
Error	455	15.91		
<u>Sphericity index of the seed</u>				
Genotype	34	0.031	1.12	0.3
Year	1	0.017	0.62	0.4332
Genotype × year	34	0.022	0.80	0.7823
Error	455	0.028		
<u>Seed surface</u>				
Genotype	34	9.57	6.66	<.0001

Follows in the next right column

Source of variation	ddl	Mean square	F-Value	Pr>F
Year	1	89.07	61.94	<.0001
Genotype × year	34	3.83	2.67	<.0001
Error	455	1.43		
<u>Seed volume</u>				
Genotype	34	1.26	6.75	<.0001
Year	1	11.57	61.70	<.0001
Genotype × year	34	0.51	273	<.0001
Error	455	0.18		
<u>Leaf</u>				
<u>Length of the leaf</u>				
Genotype	34	98.25	5.79	<.0001
Year	1	252.43	14.88	0.0001
Genotype × year	34	120.18	7.08	<.0001
Error	617	16.96		
<u>Blade length</u>				
Genotype	34	90.19	5.71	<.0001
Year	1	296.4	18.75	<.0001
Genotype × year	34	296.4	7.07	<.0001
Error	617	15.86		
<u>Blade width</u>				
Genotype	34	15.67	6.04	<.0001
Year	1	128.4	49.48	<.0001
Genotype × year	34	14.03	5.41	<.0001
Error	617	2.59		
<u>Petiole length</u>				
Genotype	34	0.59	8.64	<.0001
Year	1	1.77	25.95	<.0001
Genotype × year	34	0.33	4.51	<.0001
Error	617	0.06		
<u>Number of veins</u>				
Genotype	34	0.071	8.84	<.0001
Year	1	0.075	8.05	0.0047
Genotype × year	34	0.076	8.30	<.0001
Error	617	0.008		
<u>Physicochemical parameters</u>				
<u>Soluble solid content (°Brix)</u>				
Genotype	36	12.45	19.74	<.0001
Year	1	201.55	319.42	<.0001
Genotype × year	31	5.76	9.14	<.0001
Error	71	0.63		
<u>Acidity</u>				
Genotype	36	13.91	5.82	<.0001
Year	1	205.51	85.89	<.0001
Genotype × year	31	4.92	2.06	0.0064
Error	71	2.39		
<u>pH</u>				
Genotype	36	0.33	14.17	<.0001
Year	1	59.88	2506.15	<.0001
Genotype × year	31	0.19	8.21	<.0001
Error	71	0.02		

weight and flesh ratio among two years. These traits showed high values in 2016 with 42.73 g and 0.83 respectively, in comparison to those obtained in 2015 with 41.02 and 0.81 respectively. The remaining traits are much more stable. Regarding the effects of

genotype, year and their interaction on the fruit traits, the results showed high significant effect of genotype on all traits ($p < 0.0001$), while the year effect was significant only on fruit weight and flesh ratio ($p < 0.05$). Whereas, the effect of genotype \times year interaction was very significant on all traits analyzed ($p < 0.0001$), except on geometric diameter of fruits.

For the seed results, in 2015 the averages of weight, surface and volume were higher (2.58 g, 7.23 mm² and 1.85 cm³ respectively) than those obtained in 2016 (2.36 g, 6.36 mm² and 1.54 cm³ respectively). This result was confirmed by the significant effects of genotype, year and their interaction on average weight, surface and volume of the seed ($p < 0.0001$). The rest of traits such as geometric diameter and sphericity index of seed seems to be not affected by this factor. As results, there is a significant combined effect between genotypes, year and genotype \times year interaction on the weight, volume and as well as seed surface.

Moreover, the average values of leaf length, blade length, blade width and number of veins were higher in 2016 (23.71 cm, 22.5 cm, 7.43 cm, and 0.43 cm respectively) compared to those obtained in 2015 (22.47 cm, 21.16 cm, 6.46 cm, and 0.43 cm respectively). While, the petiole length recorded the highest value in 2015 (1.31 cm). In addition, the results of ANOVA showed highly significant effects of genotype, year and genotype \times year interaction on all leaf traits studied ($p < 0.001$).

Furthermore, the comparison of the soluble solids content, acidity and pH results for two years indicated that the values of these parameters were higher in 2016 (9.77°Brix, 8.09 g/l malic acid, 4.52) than those registered in 2015 (7.17°Brix, 5.69 g/l malic acid, 3.18). Moreover, the statistical analysis showed high significant effects of genotype, year and genotype \times year interaction on these parameters ($p < 0.0001$).

4. Discussion and Conclusions

The comparison of the averages of the studied traits during two consecutive years and the evaluation of the effects of genotype, environment and their interactions allow to measure the stability of the characters can be integrated in breeding programs (Ebdon and Gauch, 2002). In this regard, the present study revealed a significant variation of morphological

and physicochemical traits of 35 loquat genotypes during 2015 and 2016 as well as the magnitude of the inter-annual variation of the studied traits depending on the genotype. Some traits are stable, while others showed a significant variation from one year to the next. Effectively, the average values of fruit weight and flesh ratio parameters were different between 2015 and 2016 which showed the highest values of these traits. Similarly, Elsabagh and Haeikl (2012) recorded a significant difference of fruit weight of four Egyptian loquats during 2011 and 2012. In loquat, it has been reported that fruit weight depends mainly on genotype (Gariglio *et al.*, 2001; Lin *et al.*, 1999) and the cultivation conditions, which present a notable effect on the characteristics of the fruit (Cuevas *et al.*, 2012). In fact, the increase of fruit weight can be attributed to the amount of rainfall and low temperatures recorded during the 2016 in comparison with 2015 in Berkane region. In addition, the tree load had a negative effect on fruit size, so that the proportion of large fruits increased as the number of fruits per tree decreased (Mahhou *et al.*, 2006). In addition, these good results were due to a program initiated during 2016. This program provided a supplementary training for farmers and purchasing technical equipment, packaging supplies and equipment for a refrigeration unit in order to develop and improve the loquat crop (Chellay, 2022). Concerning the seed results, the average weight, surface and volume of the seed, were superior in 2015 than values obtained in 2016, with a significant influence of genotype, year and genotype \times year interaction. In fact, the results revealed a significant combined effect between genotype, year and their interaction on seed weight, seed volume and seed area, but its effect was not significant on geometric diameter and seed sphericity index. This finding is in agreement with that reported by Elsabagh and Haeikl (2012), which found a significant difference among 2011 and 2012 of the seed weight of four Egyptian loquat trees. This result could be due to the amount of rainfall recorded in 2015, compared to 2016 which influenced the plants growth in the Berkane region (Zejly, 2016). Whereas, severe water stress during seed fill of soybean plants caused their inability to regulate seed number and changing the weight distribution of the seeds to a higher proportion of small seeds. As a consequence, a greater number of small seeds (Dornbos and Mullen, 1991). For the leaf traits, the year 2016 is characterized by higher values of leaf length, blade length, blade width and number of

veins compared to those obtained in 2015. In addition, the results revealed a highly significant effects of genotype, year and genotype \times year interaction on all the leaf variables studied. In cassava, the percentage of variation due to environment was higher than the percentage of variation due to genotype for leaf retention, indicating that the environment strongly influenced the expression of this trait (Jiwuba *et al.*, 2020). Also, the phenotypic plasticity for leaf size, specific leaf area, and leaf level of hybrid poplars are modulated by a variety of environmental factors, including light, nutrient availability, and water availability (Toillon *et al.*, 2013). Regarding the sugar content, acidity and pH, which were registered during the year 2016 are superior to those recorded in 2015. This result is reinforced by the strong effect recorded of genotype, year and their interactions on these parameters. In the Egyptian loquat, the same result was obtained by Elsabagh and Haeikl (2012). The authors observed a significant effect of the year on acidity and sugar content of some loquat varieties during 2011 and 2012. The tree charge influences soluble solids content, which increased as the number of fruits per tree decreased. Thus, the soluble solids content of the fruit increased with fruit size (Mahhou *et al.*, 2006).

According to the results obtained, the variation of morphological and physicochemical traits analyzed was very important with an high effect of genotype factor. Nevertheless, fruit size and shape, which are the most important economic criteria, were found to be stable over these two consecutive years. These finding should be considered in breeding programs for more effective control of fruit quality. Further research is needed to control the impact of these factors on the stability of the selected plant. Moreover, a future research should also investigate the effect of these factors on biochemical composition.

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