

Regression and correlation studies of some morphological traits in Tunisian orange (*Citrus sinensis L*) cv. Maltese Ballerin

Naziha El Bey*, Mohamed Karim Aounallah, Ali Sahli

Laboratory of Horticulture Sciences, National Agronomic Institute of Tunisia, Tunis, Tunisia

Received: 03 Nov 2020; Received in revised form: 03 Dec 2020; Accepted: 15 Dec 2020; Available online: 26 Dec 2020

©2020 The Author(s). Published by AI Publications. This is an open access article under the CC BY license

<https://creativecommons.org/licenses/by/4.0/>

Abstract— *Citrus sinensis* (L.osbeck) or sweet orange is an important fruit crop originated from South East Asia which is cultivated widely in tropical, subtropical and Mediterranean regions. It is consumed all over the world as an excellent antioxidant. The main objective of the present work is to identify various relationships in shoots and leaves of Tunisian sweet orange (*Citrus sinensis L.*) cv Maltese Ballerin. Significant relationships were obtained between primary and secondary growth (length and diameter of spring shoots), between the elongation and organogenesis (length, leaf number, node number) and between elongation and leaf area. Several non-destructive methods are proposed to estimate the leaf area, which is closely related firstly to the rectangle constructed from the length and width of the leaf ($R^2=0.9$) and secondly to the leaf area obtained by the method of supervised classification using Envi software ($R^2=0.96$). A significant linear relationship between the dry weight of the leaf and its area is found ($R^2=0.74$).

Keywords— *Citrus sinensis L.*, leaf area, shoot, growth, regression, correlation.

I. INTRODUCTION

The genus *Citrus* belongs to the family Rutaceae, it is the most important fruit tree crop in the world, with an annual production of approximately 75 million tons and with an annual production of 144.5 thousands thons in Tunisia (Faostat, 2018). The orange, *Citrus sinensis L.* Osbeck, is the most representative and recognizable species of this group. Oranges probably originated from south East Asia, and were cultivated in China by 2500 BC (Nicolosi et al., 2000). Blood oranges were typically cultivated in the Mediterranean area, but recently, their cultivation has spread to other citrus growing areas, such as China, Australia and United States. It is very likely that the ancestors of blood oranges originated in southeast Asia and were later introduced into Europe (Chapot, 1963; Hodgson, 1967). The distinctive red flesh color of the blood orange is due to the presence of anthocyanins. It is a variety most closely associated with Tunisia, which is the sole producer and exporter. It is of exceptional quality when the variety is cultivated in the region of the Cap-Bon in Tunisia (Swingle,

1943). Weiner and Thomas (1992) found strong correlations between stem height, stem diameter and plant biomass of 3 species of annual plants. Different dimensions of a plant are assumed to be related to each other (Corner, 1949). Various attempts have been to study the correlations between dimensions of organs at both the vegetative (eg leaf and stem size, Barcellos et al., 1986; Bond and Midgley, 1988; Brouat et al., 1998; Baret et al., 2003) and vegetative-reproductive (eg. Stem and inflorescence size; Lauri et al., 1996; Lauri and Trottier, 2004) level. The recent use of scanners, image edition and analysis, supported by specific software, allows for fast estimation of those measurements in both, fruits and leaves, with high precision (Caldas et al., 1992). The area of individual leaves can be estimated from non-destructive measurements of leaf width (W) and leaf length (L) (Wargo 1978, Ramkhelawan and Brathwaite 1990), and their ratios give some indication about the constancy of leaf shape with size. Humphries and French (1964) estimated leaf size by comparing leaves with simple geometric shapes, such as circles and ellipses. Considering the insufficiency of studies about regression and

correlations on *Citrus sinensis* L. in Tunisia, this research was conducted to find the relationships between some of morpho-physiological characteristics of *Citrus sinensis* L.

II. MATERIALS AND METHODS

The trials were carried out in a field located in Northeast Tunisia (36 °N, 10°E). It is a citrus orchard of the ‘Maltese Ballerin’ (*Citrus sinensis* (L) Osbeck) variety grafted on ‘Sour orange’ (*Citrus aurantium* (L.)) rootstock. Trees were planted at 6 x 4 m spacing on a sandy soil. The climate is Mediterranean, with hot, dry summers and mild winters. A set of 90 spring shoots were taken at random from the test plot. The length, the basal, middle and apical diameter, the number of leaves and nodes and the total leaf area of each shoot were measured. A set of 439 leaves were taken at random from the sampled spring shoots. The length and width of each leaf were measured with a caliper. The surface of each leaf was measured using a planimeter. To examine the possibility of using image processing as a means of estimating the leaf area, a subsample of 85 leaves were selected and each leaf was photographed. The treatment was performed using the software Envi. Once these measurements and photos taken, each leaf is dried in an oven at a temperature of 70 ° C for 48 hours. All measures of dry mass are performed using an analytical balance with an accuracy of 0.0001 g (Model: AB 204, Mettler Toledo, Switzerland).

III. RESULTS AND DISCUSSION

The diameter, the number of leaves, the number of nodes and the leaf area were analyzed by branch in more detail based on the length of the branches (Table 1). Strong correlations ($R^2 \geq 0.8$) show a relationship between primary and secondary growth, a relationship between the elongation and organogenesis activity and a relationship between elongation and leaf area. These relationships were also confirmed by the observations of Kozłowski (1971) on different species, Costes et al. (2000) on apricot, Mezghani Aichi (2009) on the olive tree. However, the correlation between shoot length and median diameter is moderate ($R^2 = 0.65$) and this correlation is weak with apical diameter ($R^2 = 0.15$) (Table 1).

Correlations ($R^2 = 0.81$) were found between the square root of leaf area and the square root of shoots length (Figure 1) and between the square root of leaf area and log of (number of leaves) ($R^2 = 0.77$) (Figure 2). These are in agreement with studies by Blom and Tarara (2007) which showed significant linear relationships between these parameters in *Vitis labruscana*. To estimate the total leaf area/branch, Mabrouk and Carbonneau. (1996) proposed a simple model based on correlations between total leaf area and length of main and lateral branches of the vine. In addition, the length of shoots is not always correlated closely with the leaf surface especially with the main branches (Lopes and Pinto 2000; Tregoa et al., 2001.).

Table 1. Regression Matrix among the studied variables (n = 90)

Parameters							
	L (cm)	D _b (mm)	D _m (mm)	D _a (mm)	N. L	N. N	LA (cm ²)
L (cm)		R²=0.80 (Y=7.6576 x-11.122)	R²=0.66 (Y=7.1685 x-7.8256)	R²=0.15 (Y=4.2018 x+3.1842)	R²=0.81 (Y=1.7658 x-1.0117)	R²=0.84 (Y=1.7466 x-3.5731)	R²=0.80 (Y=0.0473 x+1.183)
D_b (mm)	R²=0.8 (Y=0.1048 x+1.7488)		R²=0.79 (Y=0.9243 x+0.4623)	R²=0.37 (Y=0.7378 x+1.4353)	R²=0.76 (Y=0.2006 x+1.5327)	R²=0.73 (Y=0.1899 x+1.3152)	R²=0.62 (Y=0.0049 x+1.8911)
D_m (mm)	R²=0.66 (Y=0.0916 x+1.6416)	R²=0.8 (Y=0.8633 x+0.1451)		R²=0.5 (Y=0.8489 x+1.0166)	R²=0.62 (Y=0.1745 x+1.4587)	R²=0.56 (Y=0.1603 x+1.3118)	R²=0.57 (Y=0.0045 x+1.708)
D_a (mm)	R²=0.15 (Y=0.0365 x+1.5569)	R²=0.37 (Y=0.4871 x+0.5377)	R²=0.5 (Y=0.5766 x+0.4225)		R²=0.2 (Y=0.0788 x+1.4185)	R²=0.17 (Y=0.0732 x+1.3445)	R²=0.11 (Y=0.0016 x+1.6169)

N. L	$R^2=0.8$ ($Y=0.4571x+1.827$)	$R^2=0.76$ ($Y=3.7945x-4.1253$)	$R^2=0.62$ ($Y=3.535x-2.4456$)	$R^2=0.2$ ($Y=2.3484x+2.4376$)	$R^2=0.82$ ($Y=0.8748x-0.4649$)	$R^2=0.8$ ($Y=0.0241x+1.8322$)
N. N	$R^2=0.84$ ($Y=0.4835x+3.0686$)	$R^2=0.73$ ($Y=3.8412x-2.7186$)	$R^2=0.56$ ($Y=3.472x-0.7314$)	$R^2=0.17$ ($Y=2.3349x+4.0087$)	$R^2=0.82$ ($Y=0.9354x+2.0014$)	$R^2=0.74$ ($Y=0.024x+3.4078$)

Parameters: L= length, D_b= Basal Diameter, D_m= Medium diameter, D_a= Apical Diameter, NL = Number of leaf, NN= Number of node and LA = Leaf Area.

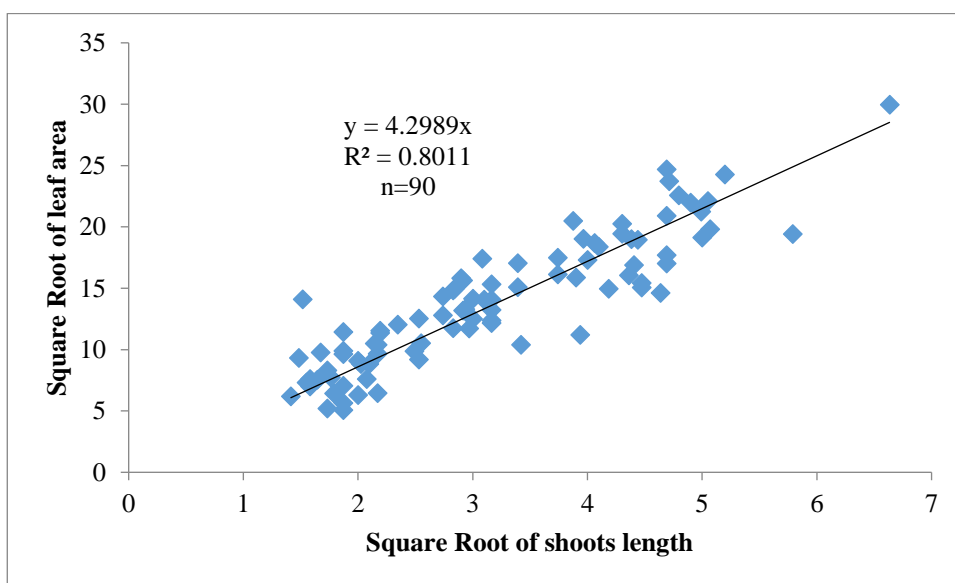


Fig.1: Relationship between the square root of the leaf area and the square root of the shoot length

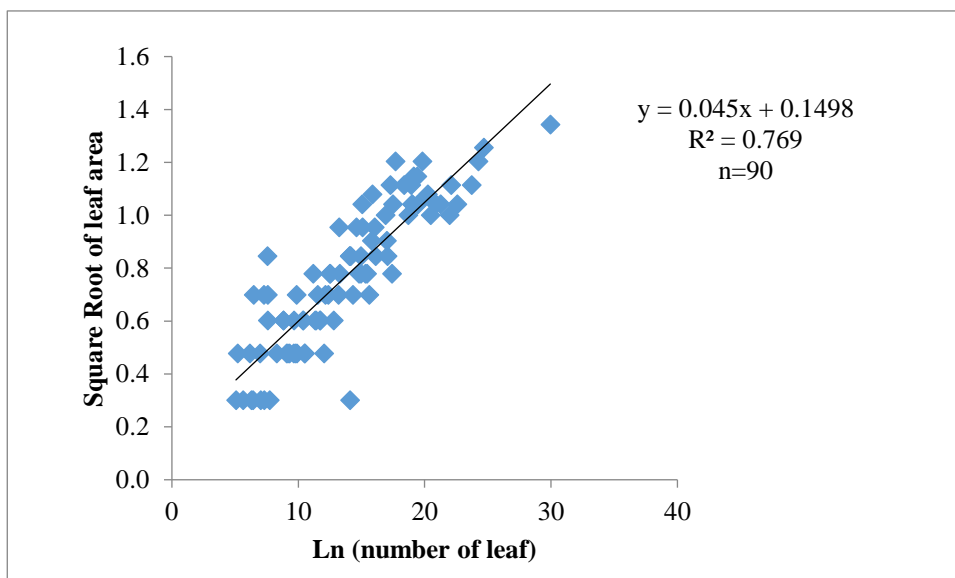


Fig.2: Relationship between the square root of the leaf area and Ln (number of leaves)

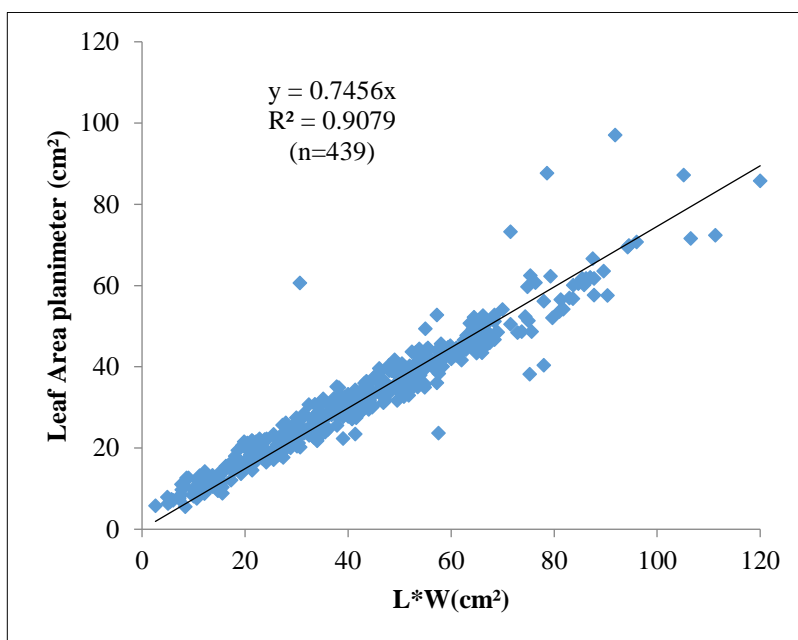
The leaf area of a crop is a determinant factor in mechanisms such as radiation, interception, water and energy exchange, growth and yield potential. The

implementation of tools for measuring and estimating crop leaf area (LA) has long been a concern for researchers. There are currently several approaches for

leaf area determination, which include direct and indirect methods. Leaf area (LA) has been estimated by many different methods. Marshall. (1968) classified these methods into two broad classes: 1) destructive and non-destructive, and 2) direct and indirect. Several non-destructive models are proposed for estimating the leaf area of several genotypes. Among the methods to estimate leaf area, mathematical models based on measures of biometric variables (leaf width and length) are widely used for various species of plants (Serdar and Demirsoy, 2006). Montgomery. (1911) first suggested that leaf area of a plant can be calculated from linear measurement of leaves using a general relationship $A=b \times L \times W$ where b is a coefficient. The leaf area of the Maltese Ballerin variety is closely related to (length and width) of leaf ($R^2 = 0.90$) (Figure 3a). Our result is in agreement with the data reported by Mazzini et al. (2010). The comparison of the LA (leaf area) results obtained by the destructive method (Planimeter) and the newly developed non-destructive image processing method by Envi software program (Figure 3b) showed a strong correlation ($R^2 = 0.96$; $n=85$) with the slope very close to one (0.967), which was strong evidence for the reliability of the image processing method. Manivel and

Weaver. (1974) found a high correlation between the length of vine leaves and their area ($R^2 = 0.91$). These simple correlations and measurement techniques can be used for estimating the leaf area of a branch by measuring only the length and width of the leaf without destroying them. Indeed, this approach has been used by Spann and Heerema. (2010) who estimated leaf area branches of 14 types of fruit trees such as almonds, citrus, nuts, olives and pistachios using equations regression similar to ours.

In the present study a good correlation found between leaf dry weight and leaf area (Figure 4). Linear function without intercept described the best relationship between the two factors ($DW=0,0117 \times LA$; $R^2 = 0.74$; $n=439$). This relationship is used to define the specific weight of the leaves of sweet orange Maltese Ballerin cv, which is in the order of 0.012 g/cm^2 . Studies of *Vitis vinifera L.* prove the existence of a correlation between the dry weight of the leaf and its area (Costanza et al., 2004). It was concluded that leaf area, fresh weight and dry weight of leaves of *Citrus sinensis* plants can be estimated or simulated as a linear function of $L \times W$ with reasonable accuracy.



(a)

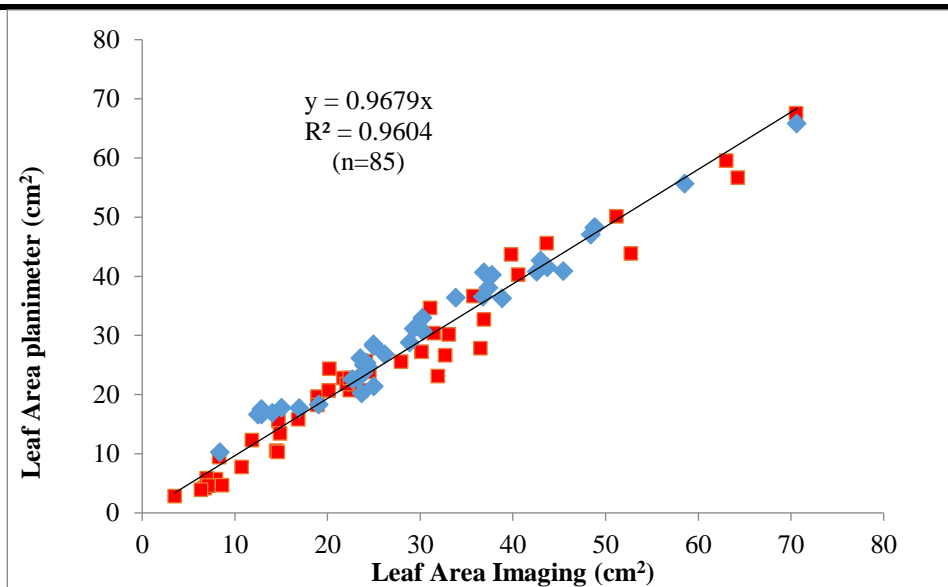


Fig.3: Regression through the origin relating

(a) leaf area (LA) obtained by the destructive method (Planimeter) and L*W

(b) leaf area (LA) obtained by the destructive method (Planimeter) and the non-destructive method (processed digital images Envi software)

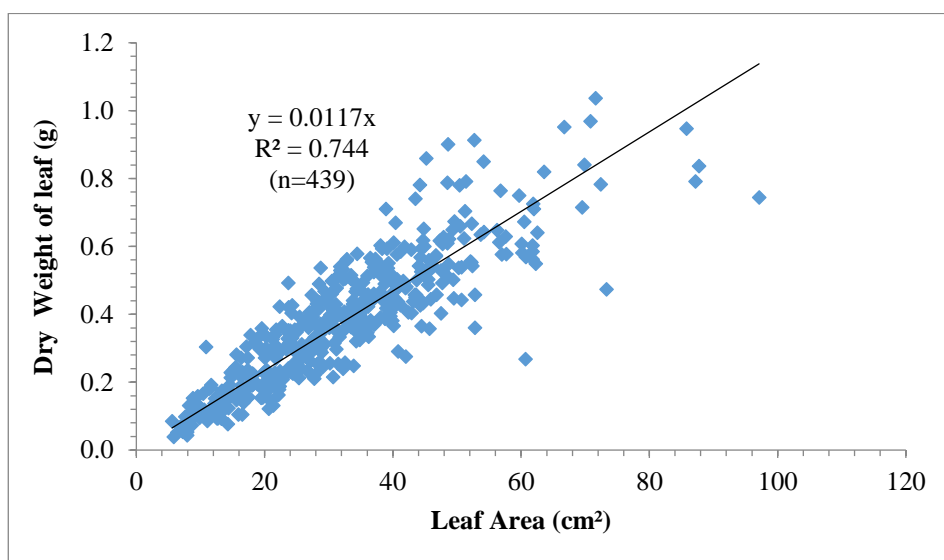


Fig.4: Relationship between leaf dry weight and leaf area

IV. CONCLUSION

In recent years, non-destructive methods have been developed for estimating plant growth and fruit parameters with the aim of replacing the destructive ones. Clear relationships and high correlations were found in the Sweet orange *Citrus sinensis* L Maltese Ballerin cv. throughout shoots and leaves in the climatic conditions of Cap Bon in Tunisia.

ACKNOWLEDGEMENT

The authors are grateful to the Laboratory of Horticulture Sciences, National Agronomic Institute of Tunisia for the academic and practical support of this study.

REFERENCES

- [1] Bange MP, GL Hammer, SP Milroy and KG Rickert, 2000. Improving estimates of individual leaf area of sunflower. *Agronomy Journal*, 92: 761- 765.

- [2] Barcellos De Souza, I., P. E. Lauri, and P. Blanc., 1986. Evolution de parametres morphologiques au cours du cycle de croissance de *Callisia fragrans* (Commelinaceae). *Canadian Journal of Botany* 64: 1664–1670.
- [3] Baret, S., E. Nicolini, T. Le Bourgeois, and D. Strasberg., 2003. Developmental patterns of the invasive bramble (*Rubus alceifolius* Poiret, Rosaceae) in Reunion Island: an architectural and morphometric analysis. *Annals of Botany* 91: 1–10.
- [4] Blom, P. E. et Tarara, J.M., 2007. Rapid and Nondestructive Estimation of Leaf area on Field grown Concord (*Vitis labruscana*) grapevines. *Am. J. Enol. Vitic.* 58:3p.
- [5] Bond, W., and J. Midgley., 1988. Allometry and sexual differences in leaf size. *American Naturalist* 131: 901–910.
- [6] Brouat, C., M. Gibernau, L. Amsellem, and D. Mckey., 1998. Corner's Rules Revisited: Ontogenetic And Interspecific Patterns In Leaf-Stem Allometry. *New Phytologist* 139: 459–470.
- [7] Byrne, D. H., Nikolic, A. N., & Burns, E. E., 1991. Variability in sugars, acids, firmness, and color characteristics of 12 peach genotypes. *Journal of the American Society for Horticultural Science*, 116(6), 1004-1006.
- [8] Chapot, H., 1963. Quelques oranges sanguines. *Cah. Rech. Agron. Rabat* 18, 61–87.
- [9] Clayton, M.; Amos, N.D.; Banks, N.H.; Morton, R.H. Estimation of apple fruit surface area. *New Zealand Journal of Crop and Horticultural Science*, v.23, p.345-349, 1995.
- [10] Corner. E.J.H., 1949. The durian theory of the origin of the modern tree. *Annals of Botany* 13:368-414.
- [11] Costanza, P., Tisseyre, B., Hunter, J.J. and Deloivre, A., 2004. Shoot Development and NonDestructive determination of Grapevine (*Vitis vinifera* L.) Leaf Area. *S. Afr. J. Enol. Vitic*, Vol.25, No.2.
- [12] Costes, E., D. Fournier, et J.C. Salles., 2000. Changes in primary and secondary growth as influence by crop load in Fantasma apricot trees. *J. Hort. Sci & Biotech.* 75(5): 510-519.
- [13] Food and Agriculture Organization of the United Nations., 2020. <http://www.fao.org/faostat/en/#home>
- [14] Hodgson, R.W., 1967. History, world distribution, botany and varieties. In: Reuther, W., Webber, H.J., Batchelor, L.D. (Eds.), *The Citrus Industry*, vol. I. University of California, Berkeley, CA, pp. 431–591.
- [15] Humphries, E.G. and S.A.W. French. 1964. Determination of leaf area by rating in comparison with geometric shapes. *Ann. Appl. Biol.* 54:281–284.
- [16] Kozłowski T.T. 1971. Growth and development of trees, Vol. 2. New York: Academic Press.
- [17] Lauri, P. E., T Rouanne, and J. M. Lespinasse. 1996. Quantitative Analysis Of Relationships Between Inflorescence Size, Bearing-Axis Size and fruit-set: an apple tree case study. *Annals of Botany* 77: 277–286.
- [18] Lauri, P. E., and C. Trottier. 2004. Patterns of size and fate relationships of contiguous organs in the apple (*Malus domestica* Borkh.) crown. *New Phytologist* 163: 533–546.
- [19] Lopes, C.M.; Pinto, P.A., 2000. Estimation de la surface foliaire principale et secondaire d'un sarment de vigne. *Prog. Agric. Vitic.* 117, 160-166.
- [20] Mabrouk, H.; Carbonneau, A., 1996. Une méthode simple de détermination de la surface foliaire de la vigne (*Vitis. Vinifera* L.). *Prog. Agric. Vitic.* 113, 192-398.
- [21] Manivel, L. and R.J. Weaver. 1974. Biometrics correlations between leaf area and length measurements of 'Grenache' grape leaves. *HortScience* 9:27–28.
- [22] Marshall, J.K., 1968. Methods for leaf area measurement of large and small leaf samples. *Photosynthetica* 2:41–47.
- [23] Mazzini, R.B., Vasconcelos R.R., Mary.P.R., 2010. A simple and non-destructive model for individual leaf area estimation in citrus. *Cambridge University Press.* Vol 65. pp 269-275.
- [24] Mezghani, M., 2009. Thèse de Doctorat. Déterminisme de la diversité caulinaire et radicaire chez l'olivier (*Olea europea* L.) et modélisation de la croissance. Approche morphogénétique. Institut National Agronomique de Tunisie. 324p.
- [25] Montgomery, E.G., 1911 correlation studies in corn. *Agricultural Experiment station of Nebraska* 108-159.
- [26] Nicolosi, E., Deng, Z. N., Gentile, A., La Malfa, S., Continella, G., Tribulato, E., 2000. Citrus phylogeny and genetic origin of important species as investigated by molecular markers. *Theoretical and Applied Genetics*, 100(8), 1155-1166.
- [27] Ortega S., Fuentes S., Retamales J.B., 1998. Models for predicting fruit diameter of Packham's Triumph Pears. *ACTA HORT.* 475: 295-302.
- [28] Ramkhelawan, E., R.A.I. Brathwaite., 1990. Leaf area estimation by non-destructive methods in sour orange (*Citrus aurantium* L.). *Trop. Agric.* 67:203–206.
- [29] Schultz, H.R. 1995. Grape canopy structure, light microclimate and photosynthesis. 1. A two-dimensional model of the spatial distribution of surface area densities and leaf ages in two canopy systems. *Vitis*, v.34, p.211-215.
- [30] Serdar Ü, Demirsoy H., 2006. Non-destructive leaf area estimation in chestnut. *Sci. Hortic.* 108(2): 227-230.
- [31] Spann, T.M., Heerema, R.J. 2010. A simple method for nondestructive estimation of total shoot leaf area in tree fruit crops. *Scient Horticult.* 125: 528-533.
- [32] Swingle, W. T., 1943. The botany of Citrus and its wild relatives in the orange subfamily. *The citrus industry*, 1, 128-474.
- [33] Tregoat, O., Ollat, N., Grenier, G., Van Lauuwmen, C., 2001. Survey of the accuracy and rapidity of several methods for vine leaf area assessment. *J. Int. Sci. Vigne Vin*, 35. 31-39 pp.
- [34] Wargo, P. 1978. Correlations of leaf area with length and width measurements of leaves of black oak, white oak and sugar maple. *USDA For. Serv. Res. Note* NE-256, 3 p.
- [35] Weiner, J., Thomas, S. C., 1992. Competition and allometry in three species of annual plants. *Ecology*, 73(2), 648-656.