



PHYSICO-CHEMICAL STUDIES ON DEFORMATION OF POKKANTANGERINE FRUITS

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Abstract

Mandarin fruit trees undergo cycles of heavy fruit load (ON-Year) in one year, followed by low fruit load (OFF-Year) the following year, a phenomenon known as alternate bearing. Roughing disorder is an important quality barrier in citrus fruit, prevalent on easy-peeling mandarins; it appears on trees that carry a light load of fruits. That phenomenon in fruits can be distinguished morphology by large fruit size with excessively thick and rough peel. This study focuses on the knowledge of this phenomenon in the mandarin fruits (smooth and curly) which focused on the fruit external shape and its relation to physical and chemical properties of fruit and nutritional status of trees (leaves, fruits and seeds) as well as hormonal situation of those fruits to find out the causes of this phenomenon. This study was performed during 2016 and 2017 seasons on eight years old mandarin Ponkan trees grafted on Volkamer lemon rootstock, grown in sandy soil conditions under drip irrigation system, of a private orchard located at El-Beheira Governorate Egypt. The results indicated that the concentrations of potassium, magnesium, sodium and copper were higher in the leaves and fruits (peel and pulp) in affected fruits more than in healthy fruits. Also, results showed that there increases of all growth regulators in leaves of smooth fruits except the Zeatin was the highest in curly fruits. It can be concluded that, the morphological shape change of citrus fruits can be attributed to yield production fruit/tree; there was problems of feeding trees or hormonal status of trees.

Key Words: Citrus Ponkan tangerine fruits, physical and chemical properties, nutritional and hormonal status.

Introduction

Citrus fruits and juices are an important source of bioactive compounds including antioxidants such as ascorbic acid, flavonoids, phenolic compounds and pectin that are important to human nutrition Ghasemi *et al.*, (2009), Stuetz *et al.*, (2010), Abad-Garcia *et al.*, (2012), Zhang *et al.*, (2014). Mandarin (*Citrus reticulata* Blanco) is a group name for a class of oranges with thin, loose peel; include a diverse group of citrus fruits that are characterized by bright colored peel and pulp, excellent flavor, easy-to-peel rind and segments that separate easily. It is considered as one of the most important and popular citrus fruit in the world and in Egypt. It is cultivated widely in the tropical/subtropical regions 35°N to 35°S of equator as well as temperate regions. In recent times, with changing environmental conditions (climate - soil – irrigation – fertilization- rootstock) has led to a noticeable change in the familiar shape of mandarin

fruits which was focused in wrinkle in the outer rind in addition to thickened rinds, which threat of production, consumption and export Valiente and Albrigo (2004), because it leads to instability of fruit quality, fruit supply and price Knauer *et al.*, (2011), Goldschmidt (2013). Curly peel (CP) is a serious barrier in citrus fruit quality, spread in easy-peeling mandarins, characterized by excessively thick, rough peel and large fruit size Monselise and Goldschmidt (1982), Liu *et al.*, (1988), Kubo and Hiratsuka (1998, 1999). This characteristic appears on trees that carry a light load of fruit where curly peel fruits are predominant, it could be commonly observed during Satsuma mandarin fruit development, occurs through a complicated process and is influenced by hormones, soil, air humidity and rootstock Erner *et al.*, (1975, 1976).

Shalom *et al.*, (2014) who said two main factors often lead to roughing disorder (RD) in Satsuma mandarin plants. The first one is low fruit load. Hormones are another important factor behind Roughing disorder in

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Satsuma mandarin. Fruit on mandarin trees tending to have RD had higher GA content than sideward fruit with smooth peel. Thus, increased gibberellin signal transduction likely promotes fruit let development and drives starch hydrolysis in RD peel. RD occurrence on the peel was influenced significantly by GA, especially abundant GA before July. These changes may mean a significant alteration in excessive thick of RD peel. Liu (2012), Lu *et al.*, (2017) reported that the primary metabolites of sugars, organic acids and amino acids are dramatically changed in RD peel. The RD peel was always characterized by higher magnesium content during development. Gene set enrichment analysis suggested that RD significantly altered starch and GA metabolism in peel. Higher starch content and hydrolysed chain status were found in RD peel at cell division stage. This is probably associated with gibberellin signal activity in RD peel, which is an important accelerator for starch hydrolysis.

So, the target of this study was investigating the physical and chemical characteristics as well as nutritional status and hormonal situation of Citrus Ponkan Tangerine in both fruits (smooth and curly) as a comparison.

Materials and Methods

Citrus Orchard

Mandarin oranges (*Citrus reticulata* Blanco) trees on eight years old grafted on Volkamer lemon (*Citrus volkameriana*) rootstock, which spaced at 4×3 m, distance (830 trees ha⁻¹), grown in sandy soil conditions under drip irrigation system, of a private orchard, located at El-Beheira Governorate Egypt.

The Trees Received the Horticultural Practices as The Following:

Fertilization

Fertilizer applications were used as following: Farmyard manure 42 m³ ha⁻¹ during December – January. NPK rates were 240 Kg N/ha⁻¹ as ammonium nitrate (33.5% N) and calcium nitrate (15.5% N), 70 Kg P₂O₅/

ha⁻¹, as a super phosphate (15% P₂O₅) and phosphoric acid (60% P₂O₅) and 240 Kg K₂O ha⁻¹ as potassium sulfate (48-52%) were using as fertigation and divided along the growing season, combinations of magnesium, iron, manganese, zinc sulphate and boron were sprayed at three times, February, June and September.

Irrigation

The irrigation of orchard was depending on season growth and environmental conditions. Drip irrigation system was divided to three regimes, during flowering and set fruiting (start from mid-March to mid-June) at 1.5–2.0 hours/day (three time/week), during fruit growth (start from mid-June to end-October) at 3 hours/day (three times/week) and during fruit ripening (start from November to January) at 0.5–1.0 hour, once/week. In addition to, gives two times heavy irrigations for the orchard, the first when entering December month and the second one when out of February month. Volume of water spray used was (1400 L ha⁻¹).

Water Samples: Were taken up after 0.50 h from beginning the pump.

Soil Analysis: Soil samples were tested for texture, pH and electric conductivity (EC) using water extract (1:2.5) method, total calcium carbonate (CaCO₃%) determined with calcimeter method and for organic matter (O.M%) was determined using potassium dichromate Chapman and Pratt (1978). Phosphorus was extracted using sodium bicarbonate, Olsen *et al.* (1954). Potassium (K), calcium (Ca) magnesium (Mg) and sodium (Na) were extracted using ammonium acetate Jackson (1973). Iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were extracted using DTPA, Lindsay and Norvell (1978).

Soil Samples: Were collected before experiment initiated from the root zone tips of the tree at the end of the canopy (60 cm depth) table 2.

The results of soil analysis was indicated that physical properties such as soil texture is a sandy soil, as well as high pH and EC and very low calcium carbonate and organic matter in both sites. Chemical properties were

Table 1: Water physico-chemical properties of the experimental site.

pH	EC(mS/m)	SAR	CO ₃ +HCO ₃	Cl	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
8.44	3.72	9.14	1115	290	14.4	126.5	41.0	462.5	0.026		Traces	

Table 2: Soil physico-chemical properties of the experimental site.

Sample Type	Mechanical analysis %			Tex. Sandy	Physical properties				Macronutrients (mg/ 100 g)					Micronutrients (mg/ Kg)			
	Sand	Silt	Clay		pH	EC (mS/m)	CaCO ₃ (%)	OM (%)	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu
Smooth	87.2	6.0	6.8		8.12	1.05	0.44	0.22	1.50	7.2	250	16.1	96.1	4.9	5.2	2.4	6
Curly	89.2	6.0	4.8		8.15	2.25	0.88	0.15	1.75	8.0	250	17.2	154.0	4.8	4.6	3.5	4

explained the available phosphorus and calcium is moderate level and the very low of potassium, magnesium, iron and manganese in both sites according with Ankerman and Large (1974).

The location of curly fruit tree showed that pH, electrical conductivity, calcium carbonate and most elements except manganese and copper were increased for than the sites smooth fruit tree.

Nutrition Status: Nutrient element compositions in the leaves, fruits (rind and flesh) and seeds of healthy and affected fruit were analyzed. All samples washed, dried at 70° C till constant weight, then grind and digested to determine the macro nutrients (N, P, K and Mg) and micronutrient (Fe, Zn and Mn) according to Rebbeca (2004).

Leaf Samples: Were collected around the tree from the fully mature leaves at October month (4 to 5 from the shoot top) to determine the nutrient contents.

Fruit Samples: Were taken at harvest time “mid-November” in the two seasons; three fruits from four sides of each tree “three replicates, tree/replicate” were picked up randomly and used for determination fruit quality assessments in both types of fruits.

Fruit Quality Estimation

Physical Characteristics

Fruit fresh and dry weights (g), fruit length “L” (cm), fruit diameter “D” (cm), index (L/D) ratio, crust thickness (cm), fruit weight (g), pulp weight (g), crust weight (g), ratio of both crust and pulp to fruit, ratio of crust to pulp, number of seeds and lobes and average weight of 100 seeds (g).

Chemical Characteristics

Chemical characteristics of fruit juice, *i.e.*, total soluble solids percentage (TSS%) by using hand refractometer, total acidity (TA%) was estimated as percentages of Citric acid and vitamin C “Ascorbic acid” were analyzed as described by AOAC (2000).

Growth Regulators

Extraction, Separation and Estimation of Plant Hormones

The frozen plant material (leaves, peels and fruits) were extraction according to the method of Shindy and Smith (1975) and described by Hashem (2006). To estimate the amounts of acidic hormones (IAA, IBA, ABA and GA), the plant hormone fractions and standard ones were methylated according to Vogel (1975) to be ready for GC analysis. On the other hand JA was determined according to Kramell (1996). Peak

identification was performed by comparing the relative retention time of each peak with those of IAA, GA, ABA and JA standards.

Cytokinins fractions (Zeatin and Benzyl Adenine) were extracted as previously mentioned for the acidic hormones and were detected by HPLC analyzer, standards of Cytokinin, Zeatin and Benzyl Adenine were used and the peak area of the standard was also used in identification and detection of Zeatin and Benzyl Adenine in each sample Muller and Hilgenberg (1986).

Statistical Analysis

Experimental design Randomized Complete Block Design (RCBD) with three replicates was adopted. The results were submitted to analysis of variance Snedecor and Cochran (1980), Differences among treatment means were determined by using the LSD test at a significance level at 0.05 of probability Waller and Duncan (1969).

Results and Discussion

Nutrition Status

Nutrient concentrations in leaves, fruit parts and seeds in the two shapes of citrus Ponkan Tangerine fruits: The nutritional status of the tree “macronutrient concentrations and micronutrients” plays a very important role in the growth, productivity of trees and fruits nutritive quality. Data in Fig. 1 refer to there is a difference in nutrient concentrations for different parts of trees, such as leaves, fruit rind, fruit flesh and seeds and also influenced by trees type, curled or healthy. From the first glance of the Figure nutrient concentrations were highest in healthy trees parts than affected trees parts. It worth mentioned, the results indicated that the highest significant nutrient contents of nitrogen, phosphorus, calcium, iron, manganese and zinc were obtained by leaves, fruits (rind & flesh) and seeds of smooth fruits. Other wise, the curly fruits had the highest statistical concentrations of potassium, magnesium, sodium and copper in leaves and fruits parts more than in healthy fruits. It may be due to high nutrient contents of P, K, Mg and Na in the soil which grow of curled fruits tree except P element in leaves and fruit parts. Also, the high phosphorus concentrations in smooth fruit tree parts can be the result of increased root propagation, increased pH, or increased salt concentration in the soil. Meanwhile, the high nitrogen concentration may be the result of increased concentration of organic matter in the soil for smooth trees. Despite the high concentration of calcium carbonate in the soil of the crumpled fruit trees, which reached twice as much as the calcium concentration in the soil of the two type’s fruit trees, the concentration of calcium in the parts of the tree (leaves, fruits and seeds) was lower than smooth

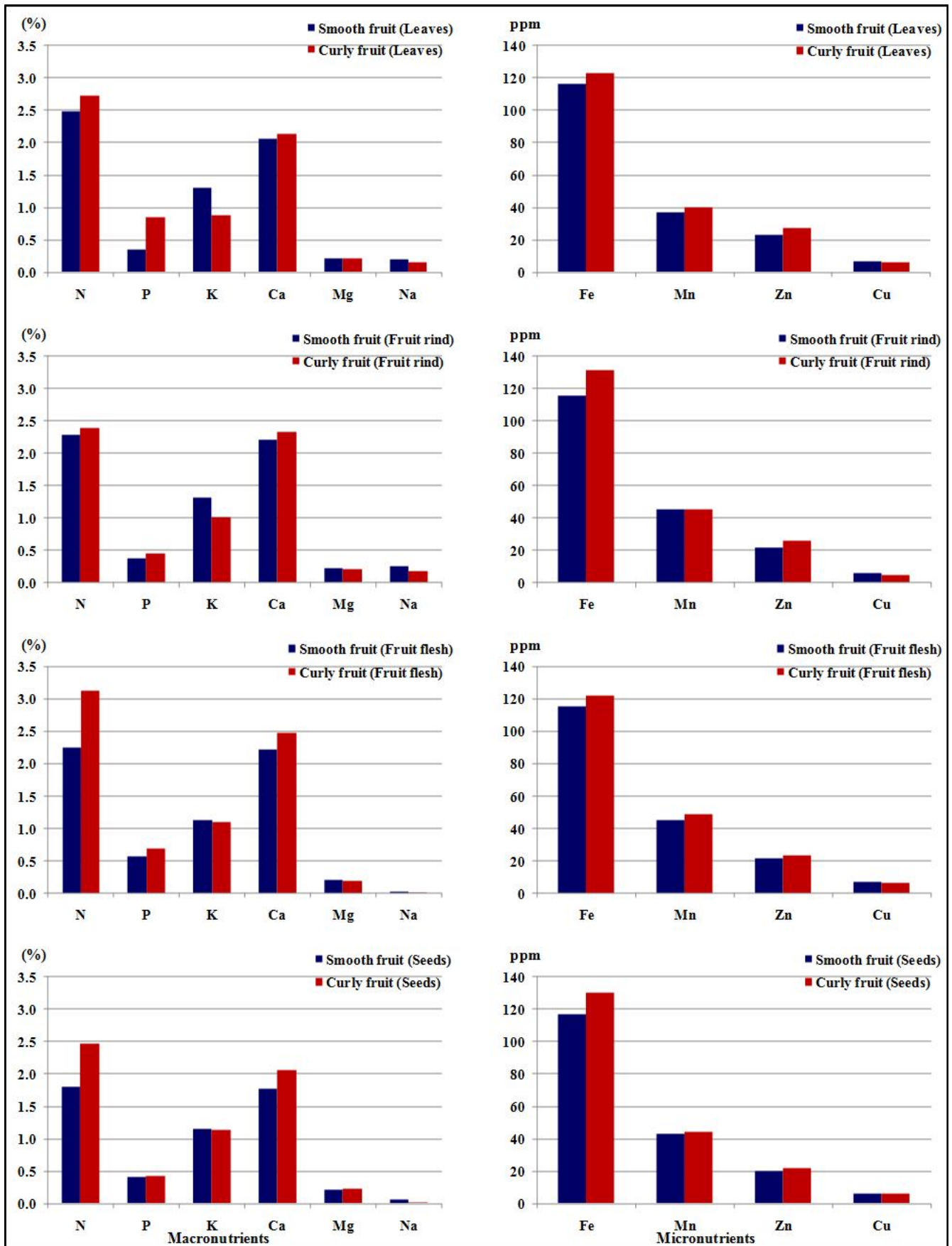


Fig. 1: The nutrient concentrations in leaves, fruit parts and seeds of Citrus Ponkan Tangerine Fruits.

trees.

These results agreed with Camara *et al.*, (2003) who stated that Volkamer lemon was clearly leaf mineral composition and, to some degree, tolerance to environmental stress. Curly fruits analyses revealed that higher Mg and Cu levels occurred in mature peel Liu *et al.*, (1988, Kubo and Hiratsuka (2000), Mahouachi *et al.*, (2009). The higher Mg content might contribute to the chlorophyll biosynthesis, carbohydrate accumulation and delayed degreening in roughing disorder peel Lu *et al.*, (2017).

Fruit Quality Estimation

Physical Characteristics

From visual observations, appear that the shape and size of the fruit varies depending on the degree of bearing. The smooth fruit trees were high in yield and smaller fruits size when compared to the crumpled fruit trees Fig. 2.

The data presented in table 3 showed that all physical properties of Citrus Tangerine fruits were dependent on the type of fruit. Moreover, significant progress has been made in curly fruits in most physiological measurements than soft fruits. These measurements such as the average of fruit diameter, length, crust thickness, fruit weight, pulp weight, crust weight and weight of 100 seeds superior increased than smooth fruits by about 0.12, 0.16, 0.5, 0.58, 0.57, 0.60 and 0.03% respectively. In contrast the delicate fruits were progressed in juice volume around 0.01% than creased fruits. Meanwhile, no significant differ among them in the other parameters such as fruit index (L/D ratio), crust ratio to fruit, pulp ratio to fruit, ratio of crust to pulp, number of seeds and lobes.

Table 3: Physical properties of Citrus Ponkan Tangerine Fruits.

Physical characteristics	Curly fruit	Smooth fruit	LSD _{0.05}
Fruit length "L" (cm)	7.89	7.05	0.41
Fruit diameter "D" (cm)	8.89	7.67	0.48
Index (L/D ratio)	0.89	0.92	NS
Crust thickness (cm)	0.72	0.48	0.09
Fruit weight (g)	253.22	160.19	54.1
Pulp weight (g)	170.91	108.70	38.0
Crust weight (g)	82.31	51.49	26.9
Crust ratio to fruit	0.32	0.32	NS
Pulp ratio to fruit	0.68	0.68	NS
Ratio of crust to pulp	0.49	0.47	NS
Number of seeds	14.78	13.33	NS
Number of lobes	13.67	9.67	NS
Average weight of 100 seeds (g)	12.20	11.80	0.16
Juice volume (cm)	68.33	68.89	0.09

NS: Non Significant.

There are several reports that indicated a Volkamer lemon rootstock effect on the quality of citrus fruit, yield and tree growth and tree size Economides (1976, 1977), Wutscher (1988), Hafez-Omaira (2006), also trees grafted on it were highly productive and with large fruits Ali-Akbar & Ali Al Irezanezhad (2005). Our results are in harmony with those findings Monselise and Goldschmidt (1982), Liu *et al.*, (1988), Kubo and Hiratsuka (1998, 1999), Goldschmidt (2013) they said that citrus trees produce in one year heavy fruit load (ON-Year), which inhibits return bloom and vegetative growth the next year, the second year produce few fruit, most of which are curly fruits and predominant. It is characterized by excessively thick, rough peel and large fruit size Erner *et al.*, (1976), Kubo and Hiratsuka (1998), Mohamed-Karima and Hafez-Omaira (2004), Liu (2012), Shalom *et al.*, (2014).

Chemical Characteristics

The results in the table 4 indicated that the chemical properties of fruits such as pH, total soluble solids% (TSS), total acidity% (TA) and ascorbic acid (VCmg/100ml juice) are affected by smooth and wrinkled Citrus Tangerine fruits.

The results also, referred that all chemical measurement characters that were the mentioned above were significant increase in fruits smooth than the other one. It may be because of curly fruits that are high in fiber. These results of chemical properties of Citrus Ponkan Tangerine Fruit are in agreement with those obtained by Kaplankiran *et al.*, (2005), El-Sayed *et al.*, (2007), Demirkaser *et al.*, (2009), El-Nagdi, *et al.*, (2010), Hifny *et al.*, (2012, 2013), Incesu *et al.*, (2013), Khalifa and Hamdy (2015), Hafez-Omaira *et al.*, (2017).

Hormonal Status

From the first look in table 5 below, the results showed differences in the hormonal status in the leaves and fruit parts in both smooth and curly fruits.

Generally, results indicated that the curly shape had increment in GA, ABA, BA and JA in flesh and peel fruits than in leaves. But, in contrast the previous growth regulators in the smooth shape in this regard.

Concerning the Zeatin, data showed higher content

Table 4: Physical properties of Citrus Ponkan Tangerine Fruit.

Fruit types	Chemical characteristics			
	Juice pH	TSS (%)	TA (%)	VC (mg/100ml juice)
Curly	4.13	10.33	1.32	7.13
Smooth	4.14	11.67	1.38	7.70
LSD _{0.05}	0.003	0.01	0.04	0.06











<p>Curly Fruits</p>	<p>Smooth Fruits</p>
	
<p>Bigger size and delayed fruit de- greening</p>	<p>Big size and red-orange bright colour peel</p>
	
<p>Rough surface</p>	<p>Smooth surface</p>
	
<p>Enlarged fruit segments – Thick and rough peel</p>	<p>Narrow fruit segments – Thin and smooth peel</p>
	
<p>Low seeds</p>	<p>More seeds</p>
	
<p>bigger juice sacs - white and more indistinct coloured pulp</p>	<p>Smaller juice sacs - Bright coloured pulp</p>

Fig. 2: External characteristics of Citrus Ponkan Tangerine Fruits in both shapes.

Table 5: Plant hormones contains of leaves and fruits (flesh & peel) of Citrus Ponkan Tangerine.

Plant hormones (ppm)	Leaves		Fruit (Flesh)		Peel	
	Smooth	Curly	Smooth	Curly	Smooth	Curly
Gibberellic Acid (GA)	5029.33	4744.06	182.241	420.278	669.474	1267.33
Zeatin	49.8254	85.7843	3.28854	3.5782	19.0721	27.334
IndoleAcetic Acid (IAA)	311.805	247.961	10.2794	7.26482	59.9818	95.2265
Abscisic Acid (ABA)	13.3478	9.02871	0.71851	2.86475	2.79332	15.2865
Benzyl Adenine (BA)	26.2445	19.4134	0.7975	4.05162	14.017	20.4555
Indole Butyric Acid (IBA)	141.064	Absent	5.87951	Absent	43.4175	62.0844
Jasmonic Acid (JA)	882.16	628.192	36.4613	193.196	109.69	597.927

in all aspects estimated (leaves and fruit parts) in curly shape in compared the smooth shape.

As for IAA and IBA, it can be noticed in the two growth hormones were increased in leaves and flesh fruits in smooth shape and also in peel curly shape. On the contrary, IAA and IBA were decreased in peel of smooth fruits as well as in leaves & flesh fruits in curly shape in case IAA only in this respect. Meanwhile, IBA was absent in leaves and flesh curly shape.

From the above results we can say trees produce a variety of hormones, like auxins, gibberellins and cytokinins that are called stimulators. Auxins promote cell enlargement, the main factor controlling fruit size during the rapid growth phase Iglesias *et al.*, (2007), like indole acetic acid (IAA) and Indole-3-butyric acid (IBA) play a key role in regulating growth and development of different plant parts Popko *et al.*, (2010). IAA regulation is by affecting the transcriptional factors Hayashi (2012). Gibberellins increase cell enlargement, thus enhancing fruit growth in certain species such as citrus Eman *et al.*, (2007). Gibberellins also, induce seeds to sprout, have a role in flowers development and reduction of physiological drop Ullah *et al.*, (2014). Cytokinins such as Zeatin and benzyl adenine are active in all stages of germination to regulate seed germination Riefler *et al.*, (2006), affect the activities of meristemic cells in shoots and roots, as well as leaf senescence Santner *et al.*, (2009), Hafez-Omaira *et al.*, (2013).

Cytokinins promote carbohydrate metabolism and increased sink strength, fruit size by increasing number of cell layers and fruit dry matter at harvest Monthes and Engelbrecht (1961), Emomogor (1995). Jasmonic acid (JA) and its derivatives have essential roles in plant development from germination to senescence, included number of physiological processes like vegetative growth, cell cycle regulation, senescence and fruit ripening Wasternack and Hause (2002), Yoshida *et al.*, (2009). Abscisic acid is known as growth inhibitors; it positively affects stomatal activity, seed dormancy, causes the

leaves to break off and fall in autumn and plant activities under stresses Popko *et al.*, (2010).

Our results were nearly in the same line with obtained by Liu (2012) said that hormones are another important factor behind roughing disorder in Satsuma mandarin. Fruit on “Okitsu wase” mandarin (*C. unshiu* Marc.) trees tending to have RD had higher GA content than sideward fruit with smooth

peel Shalom *et al.*, (2014).

CONCLUSION

From the above results it can be say the morphological shape change of citrus fruits with changing environmental conditions such as rootstock - soil - fertilization - irrigation - climate and it depended on a light load of fruit has led to a noticeable change in the familiar shape of mandarin fruits. Thus, it can be concluded that, the morphological shape change of citrus fruits can be attributed to tree productivity (number of fruits/tree); there was problems of feeding trees and also hormonal status of trees.

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