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Impact of Anatomical, Molecular and Physiological Aspects on Some Navel Orange cvs. Potentialities under New Reclaimed land

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Abstract: This experimental was conducted for two successive seasons 2016 and 2017 at a private citrus orchard in South El Tahrir, El Beheira governorate, Egypt on three navel orange cultivars (*Citrus sinensis* (L.) Osb.) namely 'Washington', 'New hall' and 'Navelate' all were budded on Volkamer lemon (*Citrus volkameriana*) grown in sandy soil under drip irrigation system to investigate their aptitudes according to their anatomical, molecular and physiological features under our local ecological conditions. Random amplified polymorphic DNA (RAPD) in this study was conducted to estimate genetic relationship among studied cultivars. Tree canopy, fruit set, yield, fruit quality and leaf chemical compositions were determined. Generally, considering overall the studied cultivars; leaf anatomical structure revealed that, 'Washington' cultivar had the highest values in most of anatomical characters (thickness of lamina, palisade, spongy and mid vein) which reviled in its tree canopy, fruit set and fruit number as it achieved the uppermost figures in these characters. likewise, 'New hall' came in the second rank concerning these features and recorded high values in length and width of main vascular bundle which reflected in enlargement of its fruit size and weight. Genetic similarity showed that, a high value of genetic similarity (88.9) was observed between 'Washington' and 'New hall' cultivars while the least value was with 'Navelate' (69.3). An appreciable association among different studied parameters and the relative similarity between cultivars under study could be suggested from preceding data. Anatomical, molecular and physiological features could be viable relevant leading indicators in relation to will establish cultivar i. e., 'Washington' navel to prospect newly introduced cultivar behavior. Yet, more studies need to be done in this issue to inspect such associations. Elucidated data signifying to that, 'Washington' navel orange as an economical yielding cultivar with advantageous fruit characteristics could be recommended mainly to the abroad markets and 'New hall' may be more appropriate for the domestic ones.

Key words: Washington · New hall · Navelate · Anatomical studies · RAPD · Genetic similarity

INTRODUCTION

Navel oranges are one of the most recognized and favorable citrus fruits worldwide. They are a mainstay of the fresh fruit business because of a distinctive flavor and their tendency to produce fruit larger than other sweet oranges. Navel orange trees are less reliable in their cropping than other sweet orange cultivars because of genetic and climatic factors; also, they are less tolerant to environmental stresses and are more subject to various physiological disorders [1].

Egypt is the seventh largest orange producer and the third biggest exporter [2]. A single mutation in a Selecta orange tree planted on the grounds of a monastery in Bahia, Brazil, probably yielded the first navel orange between 1810 and 1820. The parent Washington navel orange tree is a tree grown by Eliza Tibbets in Riverside, California, in 1873 [3]. Possibly all 'Washington' navel orange trees throughout the world are derived from this one parent tree [4]. Many other navel orange cultivars like 'New hall' and 'Navelate' were newly imported and now are important to the citrus industry [5]. 'Navelate' sweet orange was occurred from bud mutation on a 'Washington' navel tree in Vinaros (Castellón), Spain [6]. Moreover, Hodgson [7] stated that, the bud line 'Newhall' originated as a limb sport in the Duarte area that was propagated by Paul Hackney of the Newhall Land and Water Company of Piru.

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In the past, systems of citrus genetic classification were based mainly upon morphological characteristics [8]. A number of molecular marker techniques have been used to overcome the limitations of morphological and biochemical markers in citrus genetic classification [9]. They include restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPD) and have been employed to elucidate genetic diversity, determine parentage and reveal phylogenetic relationships among various citrus species [10, 11].

Evaluation of genetic diversity and genetic relationships among various accessions is of fundamental importance such information which can provide predictive estimates of genetic variation within a species, thus facilitating breeding material selection [12].

Significant variations were observed between all the navel orange cultivars. It is not expected that the introduced cultivars will perform similarly in various regions of Egypt where different ecological conditions exist. The potential of newly introduced cultivar via examining their vigor in the orchard under local conditions may determine by long term field trials their yielding. A more efficient screening method would be helpful to shorten the testing period. Techniques that can help predict vigor have been used in other crops. Anatomical studies have shown that proportion of bark and vessels in roots can be used to classify mango rootstocks into various vigor classes, the number of stomata per unit leaf area, phloem transport area and total phenolic content and mineral nutrition can also be used as a criterion for prediction vigor [13-16].

Thus, the objective of this study is to investigate the relationship between the anatomical, molecular and physiological characteristics of the studied cultivars and their attitude under our local ecological conditions in new reclaimed land aiming to find out leading indicators that could predict the newly introduced cultivars behavior.

MATERIALS AND METHODS

The present study was carried out during two successive experimental seasons 2016 and 2017 in a private citrus orchard virtually at latitude of 30°53'59.7"N and longitude of 30°08'58.9"E in El Beheira governorate, Egypt. Three navel orange cultivars (*Citrus sinensis* (L.) Osb.) namely 'Washington', 'Navelate' and 'New hall' navel orange all budded on Volkamer lemon (*Citrus volkameriana*), were adopted. Trees were eleven years old grown in sandy soil at 4 × 6 m under drip irrigation system were used for all parameters determination except, for the molecular studies, the parent was used as well as the three studied cultivars (The parent was a tree imported to the farm of the studied cultivars from the United States of America representing the origin of parent Washington Navel orange). The total number of trees used for this experiment was forty five trees (3 cultivars x 5 replicate x 3 trees in each replicate).

The Following Parameters Were Investigated

Anatomical Studies: Leaf and floral buds samples used for the anatomical studies were taken at the third week of March throughout the 2nd growing season of 2017. Leaf lamina, palisade, spongy and mid vein thickness and length and width of main vascular bundle were detected, in addition different tissues in the floral bud in the previous studied cultivars. Measurements (μ) of the different tissues were taken and averages of ten readings from five slides were calculated using a micrometer eye piece and micrometer stage. The anatomical procedures were carried out according to Nassar and El-Sahhar [17]. Photomicrographs were taken at Botany Department Laboratory, Faculty of Agriculture, Cairo University.

Molecular Studies

Plant Material: Fresh leaves from the three studied cultivars as well as the parent of these cultivars were collected at the first week of April throughout the 2nd growing season of 2017 and transferred to laboratory in an icebox and were stored at -20°C until use.

Extraction and Purification of Genomic DNA: Total genomic DNA were extracted from young and fresh leaves of four orange genotypes i.e., 'Washington', 'New hall', 'Navelate' and parent using modified CTAB protocol [18]. RAPD fragments were amplified according to Machado *et al*. [19]. Amplification reaction for RAPD consisted of 1.5mM 10X buffer, 1.5mM $MgCl_2$, 100μM dNTPs (25μM each), 0.2 μM primer, 1U Taq polymerase, 50 ng of DNA and sterile water up to 25 μl. RAPD amplification was conducted as follow: an initial step at 94°C for 5 min. 40 cycles for each cycle 1 min. at 94°C, 1 min. at 37°C and 2 min. at 72°C and final extension step at 72°C for 5 min.

Tree Canopy: Tree canopy volume was determined at the end of February during two experimental seasons; tree canopy volume was estimated according to the formula of Obreza [20].

Tree canopy volume (m) = H × D × 0.5238

where, as H = tree height (m) and D = diameter of tree periphery (m).

Fruit Set: Total number of flowers was counted on four branches in four main directions at the third week of March and number of fruitlets was counted at the third week of June and then fruit set percentage (%) was calculated according to the equation:

Fruit set% = (number of fruitlets/ number of flowers) ×100

Yield: At harvest time (at the first week of December under these experimental conditions) fruits of each tree were harvested and the yield was estimated as number of fruits and weight in Kg.

Fruit Quality: At harvest stage, representative sample of 10 fruits was randomly taken from each tree and the following characters were determined:

Fruit Physical Properties: Average fruit weight (g), average fruit size (cm^3), fruit height and diameter (cm) were measured and fruit shape index (length/diameter) was calculated, peel thickness (m) and fruit firmness (l.b/ $inches^2$) were measured.

Also, fruit rind colour was quantified after McGuire [21] using Minolta colorimeter (CR-200, Ramsey, NJ) (Minolta Corporation Instrument Systems) through determining the three color components (L) lightness, (C) chroma and (H) hue angle.

Fruit Chemical Properties: Total soluble solids (TSS %), acidity % (as mg citric acid/100 cm^3 juice), TSS/ acid ratio and vitamin C (ascorbic acid as mg/100 ml juice) were determined according to A.O.A.C. [22].

Leaf Chemical Composition: Both total indoles and total phenols were determined in fresh leaves three times (March, May and July) at the two experimental seasons. Total indoles were determined according to Larsen [23], total phenols were determined according to Swain and Hillis [24]. Total carbohydrates in dried shoots of spring cycle were determined at September by using 3,5-dinitrosalicylic acid method according to Miller [25]. N, P, K, Fe, Mn and Zn concentrations in dried leaves were determined at September of the two experimental seasons. Total N% was determined by semi-micro Kjeldahl method described by Plummer [26]. Phosphorus was estimated colorimeterically by using the chlorostannous reduced molybdophosphoric blue colour method as described by King [27]. Potassium concentration was determined by using the flame photometer. Fe, Mn and Zn concentrations were determined by using atomic absorption spectrophotometer.

Statistical Analysis: One way completely randomized design was used for the experiment. The data statistical analysis carried out according to Snedecor and Cochran [28]. The multiple comparisons of means were performed according to Duncan's multiple test range [29] using COSTAT computer program.

RESULTS AND DISCUSSION

Leaf Anatomical Studies: Results in Table (1) and Fig. (1) indicate that lamina thickness recorded the highest values 970 µm in 'Washington' followed by 955µmin 'New hall', while 'Navelate' recorded the lowest one 787 µm. The increase in lamina thickness was due to an increase in thickness of mesophyll tissue. Meanwhile, palisade tissue recoded 710, 700 and 660 µm, for 'Washington', 'New hall' and 'Navelate', respectively. Also, spongy tissue was the highest in 'Washington' (847 µm) while in 'New hall' was 835 µm and in 'Navelate' they recorded 720 µm. On the other hand, mid vein thickness recorded the highest values in 'Washington' and 'New hall' amounting to 1453 and 1410 µm, respectively, while 'Navelate' recorded the lowest value (1290 µm). In addition, length and width of main vascular bundle recorded 1000 and 1110 µm for 'Washington' respectively. Whereas, 'New hall' recorded 1085 and 1205 µm, respectively, for this trait. 'Navelate' showed the lowest values for length (980 µm) and width (1090 µm) of the main vascular bundle. These results are in harmony with João *et al*. [30] on citrus.

Floral Bud Anatomical Studies: Longitudinal sections through the floral bud of the studied cultivars are shown in Fig. (2). It is clear that the floral bud is surrounded by calyx which consists of five united sepals, each comprised of two epidermal layers in all cultivars and about 9 layers of ground tissues in between in 'New hall', while in 'Washington' 11 layers. There are number of oil glands extending through the ground tissue in 'Washington' and 'Navelate'. The corolla consists of five separated petals.

Table 1: Anatomical characters of 'Washington', 'New hall' and 'Navelate' leaves during the second season (2017)

	Characters (µm)					
					Main vascular bundle	
Cultivars	Lamina thickness	Palisade thickness	Spongy thickness	Mid vein thickness	Length	Width
Washington	970	710	847	1453	1000	1110
New hall	955	700	835	1410	1085	1205
Navelate	787	660	720	1290	980	1090

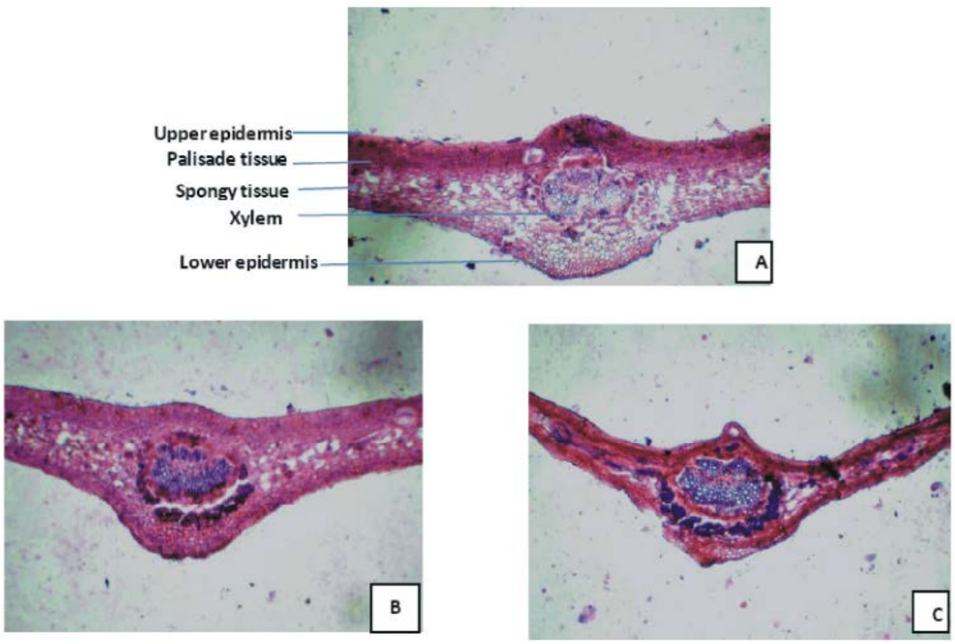

Fig. 1: Transverse sections through the middle part of leaf, (A) Washington (B) New hall (C) Navelate (X 40)

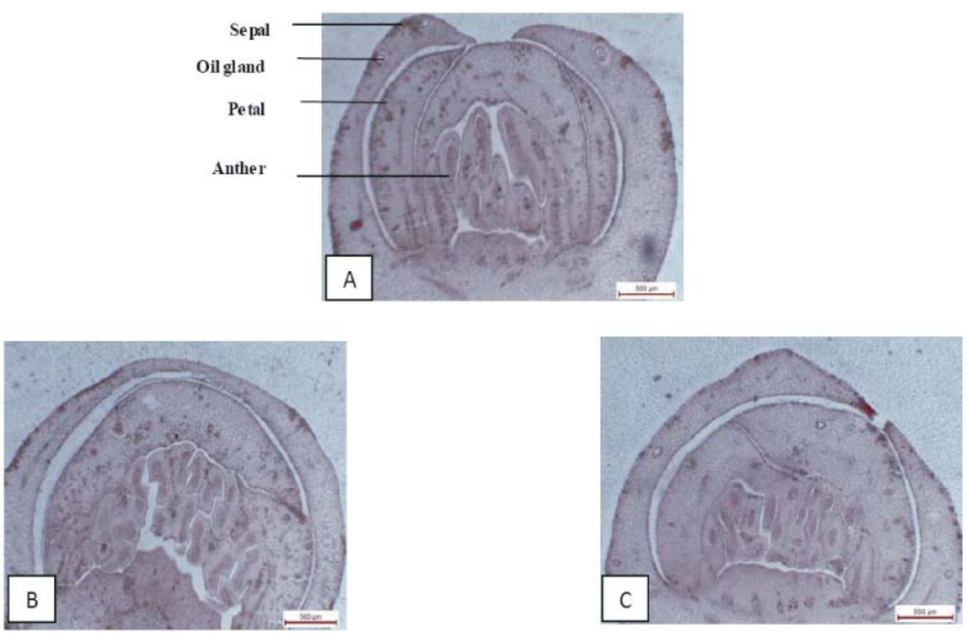

Fig. 2: Longitudinal sections through the floral bud (A) Washington (B) New hall (C) Navelate (X 40)

Each segment of corolla consists of two epidermal layers and about 15 to 20 layers of slightly rounded parenchyma cells in 'New hall' and 'Navelate', respectively forming the mesophyll of each petal. The androecium consists of numerous separate stamens. The filaments united in different number of bundles. The gynoecium is comprised of unlimited united carpels. The ovary consists of numerous locules. Placentation is central. These finding are in accordance with João *et al*. [31] on citrus.

DNA Fingerprint as Detected by RAPD Marker

Polymorphism Detected by RAPD Markers: In the present study twelve primers were screened with the DNA of the four orange genotypes ('Washington', 'New hall', 'Navelate' and parent). These primers generated reproducible and easily scorable RAPD profiles (Fig. 3). The total number of amplicon results from the twelve primers was 83 with an average of 10.4 amplicon/ primer. The amplified amplicons ranged from 7 to 15 (Table 2). Polymorphic amplicons ranges from 2 to 6. Primer OPO-07 produced the highest number of amplicons. While, the lowest number of amplicons was produced by OPA-07 and OPA-14. The highest number of polymorphic amplicons (6) was produced by OPD-07. While, the highest (50%) percentage of polymorphism was produced by OPD-04. The average number of polymorphic fragments was 3.8.

Similar results were obtained by Machado *et al.* [19] and Coletta *et al.* [32] who reported that, in mandarins the average number of polymorphic bands was 2.2 and 1.95, respectively as well as Golein *et al*. [33] who noticed that, the isolation and characterization of seven polymorphic microsatellite loci in citrus. These markers produced between 4 and 9 alleles per locus (with an average of 6.14) in 32 evaluated cultivars of *C. limon*. A highest and lowest gene frequency of 0.5953 and 0.2418 was observed in OPA-06-04 and OPQ-18-07 loci, respectively [34].

Genetic Similarity: The genetic similarity ranged from (69.3) among 'Washington' and 'Navelate' to (88.9) between two genotypes of 'Washington' and 'New hall', while, it was (80.1) between 'Navelate' and 'New hall'. The highest value of genetic similarity was observed between 'Washington' and 'New hall' cultivars (Table 3). Such genetic relationships were supported via dendrogram expressed in (Fig. 3) which represented clusters analysis of genetic distances between the studied genotypes.

On the other hand, it was clear that, parent genotype was far from the studied cultivars with in genetic similarity except for 'Navelate'; e.g. it recorded genetic similarity of 66.7 with 'New hall', 70.1 with 'Washington' and 77.8 with 'Navelate'. Moreover, 'Washington' cultivar showed a lower genetic similarity with 'Navelate' (69.3). The range of the genetic similarity in citrus cultivars was estimated by Federici *et al*. [35] as well as Nicolosi *et al*. [36] who noticed that, there are high genetic similarity found amongst lemons and limes.

In this respect, sweet oranges usually show low level of genetic diversity [37]. ISSR markers revealed a high level of genetic similarity ranging from 0.83 to 0.98 among the twenty orange accessions [38]. This narrow genetic base among the sweet orange accessions has been previously reported in many publications [39].

Table 2: Total number of amplicons, monomrphic amplicons, polymorphic amplicons and percentage of polymorphism revealed by RAPD markers.

Primer		Sequence	Total number of amplicons	Monomorphic amplicons	Polymorphic amplicons	Percentage polymorphism
1	OPA-05	ACGGGTCTTG	8	6	2	25.00
2	OPD-04	TCTGGTGAGG	8	4	4	50.00
3	OPA-07	GAAACGGGTG	7	4	3	42.90
4	OPD-05	TGAGCGGACA	11	7	4	36.40
5	OPA-14	TCTGTGCTGG	7	4	3	42.90
6	OPD-07	TTGGCACGGG	13	7	6	46.20
7	OPA-16	AGCCAGCGAA	14	11	3	21.40
8	OPO-07	CAGCACTGAC	15	10	5	33.30
Total		-	83	53	30	298.10
Mean		-	10.4	6.6	3.8	37.26

Table 3: Genetic similarity matrixes computed according to dice coefficient from RAPD marker

Nh	Nh	Nv	W
Nv	80.1		
W	88.9	69.3	
P	66.7	77.8	70.1

where: Nh: 'New hall' genotype, *Nv* : 'Navelate' genotype, W: 'Washington' genotype and *P:* parent genotype

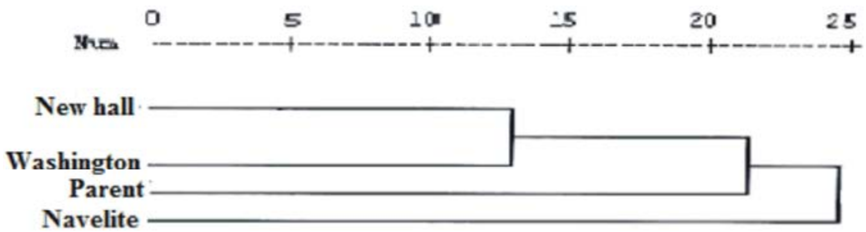

Fig. 3: Dendrogram using average linkage (between groups)

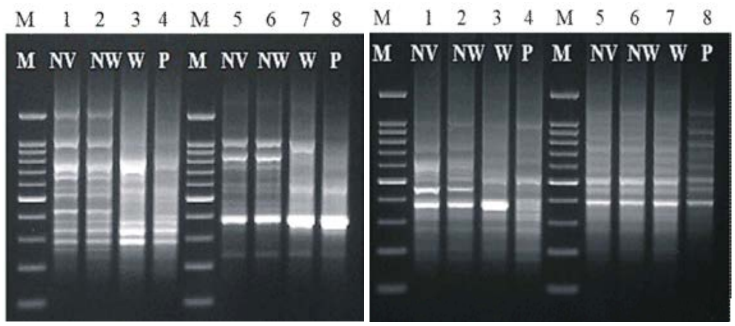

Fig. 4: Agarose-gel electrophoresis of RAPD products generated with primers for four orange genotypes. 'Navelate' (NV), 'New hall' (Nw), 'Washington' (W), parent (P) and M: molecular weight marker (1Kpb ladder marker)

Cluster Analysis: Dendrogram obtained from UPGMA cluster analysis of genetic distances (Fig. 3) reveal that, all of 'Navelate' and parent genotypes were separated in two individual clusters. Meantime, the third cluster contains 'Washington' and 'New hall' genotypes.

In this respect, the cophenetic correlation between ultrametric similarities of the tree and the similarity matrix was high (r = 0.79), suggesting that the cluster analysis strongly represents the similarity matrix. In the cluster analysis navel group sweet oranges were indicated as a separate group from Valencia group sweet oranges. 'Antalya (40)' was the most distinct accessions from the others. The same results are seen as a result of PCO and neighbor joining. Polymorphism was found to be quite low in Valencia group sweet orange. Located within the same cluster 'Navelina 7-5', 'Navelate 2-7', 'Navel 38-M' and 'Navel 39-M' were constituted in a separate the most distant group of Valencia and navel oranges [40].

Tree Canopy: It is obvious from data in Table (4) that, in the two experimental seasons, 'Washington' navel orange trees attained the highest values of tree canopy when compared with the other studied cultivars, which may be due to highest values of most leaf anatomical characters; as thickness of lamina, palisade, spongy and mid vein followed by 'New hall', which recorded high values in length and width of main vascular bundle (Table 1). Where, a high vein length per unit area can enable higher leaf hydraulic conductance, greater stomatal density and stomatal conductance and higher rates of gas exchange per leaf area [41]. In addition, thicker veins may have greater water and sugar transport capacity when they contain greater sizes and numbers of xylem and phloem cells [42]. Moreover, among the relationships with leaf characteristics, photosynthetic rate at light saturation had the strongest correlation with leaf mesophyll parameters, such as palisade cell layer thickness. Also, thicker palisade parenchyma allows full photosynthetic exploitation of high irradiance in the tropical rain forest [43].

Fruit Set: It is clear from the data presented in Table (4) that, in the two experimental seasons 'Washington' navel orange trees attained the highest values of fruit set percentage followed by both 'New hall' and Navelate trees, this may be due to the highest values of total carbohydrates concentrations of 'Washington' shoots (Table 8) comparing to the other two cvs.

There is a relationship between the nutritional status of the tree and fruit set. Fruit set has been positively correlated with carbohydrate in leaves of the previous season [44]. Moreover, there was a direct effect of the auxin on abscission, which causes a delay of abscission and may result eventually in an increase in set [45]. As, 'Washington' leaves had nearly the highest significant of leaf total indoles concentrations values followed by 'New hall' leaves at the three dates of sampling (Fig. 5).

Table 4: Tree canopy, fruit set, fruit number and fruit yield of studied cultivars

	Tree canopy (m)		Fruit set (%)		Fruit number		Yield (Kg/tree)	
Cultivars	1st	2 nd	1st	2 nd	1st	2 nd	1st	2 nd
Washington	16.30 a	16.20 a	19.22 a	15.93 a	221.67 a	218.67 a	51.77 a	56.66 a
New hall	14.60 b	15.20 b	9.26 b	7.70 b	173.33 b	202.71 a	54.56 a	59.19 a
Navelate	13.73 b	14.67 b	6.83 b	6.98 b	91.67 c	94.67 b	28.87 b	23.13 b

Means in each column fallowed by the same letter did not differ at p<0.05 according to Duncans multiple range tests

Table 5: Fruit physical properties of studied cultivars

	Fruit weight (g)		Fruit size (cm^3)		Fruit shape index		Fruit firmness (l.b/ $inches^2$)		Peel thickness (m)	
Cultivars	1st	2 nd	1st	2 nd	1st	2 nd	1st	2 nd	1st	2 nd
Washington	233.55b	259.11b	253.17b	262.67b	1.13 a	1.14 b	13.82a	14.41b	1.05a	1.19a
New hall	314.75a	292.00a	349.50a	336.33a	1.13 a	1.20 a	10.36b	11.02 b	1.15a	1.29a
Navelate	313.67a	306.33a	360.33a	373.67a	1.10 a	1.14 b	14.18a	18.89 a	1.03a	0.90b

Means in each column fallowed by the same letter did not differ at p<0.05 according to Duncans multiple range tests

Fruit Yield: The data in Table (4) showed that, the highest values of fruit yield were obtained by 'New hall' followed by 'Washington' navel orange with lack of significance at the two experimental seasons. While, in the two experimental seasons 'Washington' navel orange trees had the highest fruit number when compared with other studied cultivars as a result of its superiority in fruit set.

This results are in agreement with those obtained by Sayed and Abdel- Aziz [46] and Nasser *et al*. [5] who found that, the highest significant yielding values were obtained by 'New hall' followed by 'Navelina' and 'Lane Late' cultivars, as well as Jia *et al*. [47] who noticed that, 'New hall' is very productive.

Concerning fruit number, the data in Table (4) demonstrated that, 'Washington' navel orange cultivar trees bore the highest number of fruit followed by 'New hall' orange trees at the two experimental seasons.

This results are in agreement with those obtained by Sayed and Abdel-Aziz [46] who found that, 'New hall' and 'Washington' navel orange achieved higher fruit number per tree followed by 'Navelina' and 'Navelate' cultivars without significant difference between each of them in the two experimental seasons.

It worth to mention that, despite that 'Washington' navel orange achieved the highest values of fruit set and consequently fruit number per tree, but 'New hall' cultivar surpass it achieving the highest fruit yield due to having the heaviness of its fruit weight (Table 5). While, 'Navelate' trees had the lowest fruit yield as it had the lowest fruit set of the three cultivars.

Fruit Quality

Physical Properties: Fruit size has become as important as yield in the determination of the profitability of citrus plantings and an economic premium is usually obtained through the increase in fruit size even at the expense of a reduction in crop yield [48]. Tabulated data (Table 5) revealed that, the highest values of fruit weight and fruit size, at the two experimental seasons were recorded by 'Navelate' trees and 'New hall' trees, with insignificant differences between them followed by 'Washington' trees. Enlargement of 'Navelate' fruits size may be related basically to their relatively limited number as this cultivar had the lowest fruit set values comparing to the other ones (Table 4) since, an increase in set reduces fruitlet growth rate [48] and the trees with faster growing fruitlets set a lower number of them but of a bigger size [49]. While, the large size of 'New hall' fruit may be correlated to the impact of vascular system cross sectional area as it had the highest main vascular bundle measurements figures (Table 1) as a direct relationship between fruit size at maturity and pedicel diameter has been reported for many crops i.e., Valencia orange [50], Fortune mandarin [51] and Marsh grapefruit [52]. Where, during the phase of cell division, long before the completion of the differentiation of the vascular system, both phloem and xylem cross sectional area in the pedicel are directly and tightly related to fruitlet size [52].

The obtained data are in harmony with those reported by Nasser *et al*. [5] who found that, 'New hall' gave the highest values of yield and fruit weight followed by 'Navelina' and 'Lane Late' but 'New hall' gave large fruit size which is not accepted in export and local market.

Table 6: The three colour components lightness (L), chroma (C) and hue angle (H) during two successive seasons of the fruit studied cultivars

	1st			2 nd		
Cultivars	H	L	C	H	L	C
Washington	76.07 b	56.89 b	66.85 a	74.12 b	56.89 a	67.58 a
Navelate	85.62 a	58.51 b	69.43 a	86.29 a	56.23 a	63.58 a
New hall	80.39 ab	62.25 a	68.99 a	77.47 b	54.53a	68.29 a

Means in each column fallowed by the same letter did not differ at p<0.05 according to Duncans multiple range tests

Table 7: Fruit chemical properties of studied cultivars

	Vit. C (mg/100ml)		T.S.S (%)		Acidity (%)		T.S.S/ acid ratio	
Cultivars	1st	2 nd	1st	2 nd	1st	2 nd	1st	2 nd
Washington	40.14 a	44.00 a	10.56 a	10.70 a	0.820 a	0.852 a	12.95 a	12.55 a
New hall	35.73 a	37.50 b	9.51b	9.65 b	0.892 a	0.901 a	10.69 b	10.73 b
Navelate	36.67 a	38.60 b	9.03 b	9.41 b	0.892 a	0.880 a	10.41 b	10.71 b

Means in each column fallowed by the same letter did not differ at p<0.05 according to Duncans multiple range tests

Concerning fruit firmness, the highest values were obtained by 'Navelate'. Whereas, peel thickness highest value was recorded by 'New hall' cultivar trees followed by 'Washington' navel orange with lack of significance.

In this respect, fruit with thick peel are usually low in juice, while those with thin peel are prone to splitting and are sensitive to postharvest problems that can occur during shipping and storage [53]. Rind thickness has a significant importance to overall citrus quality both at pre-harvest and postharvest stages. Fruits with a thick rind would be more resistant to fruit splitting problem at farm stage and handling damage at postharvest stage. However, such fruits tend to have lower juice content [54].

The data in Table (5) revealed that, in the second season the highest values of fruit shape index were obtained by 'New hall'.

The results are in agreement with Nasser *et al*. [5] who noticed that, the significant highest values of fruit shape were obtained by 'New hall' and 'Fukumoto' cultivars.

As for rind colour, the data presented in Table (6) depends mainly on hue angle (H) values, regardless of lightness (L) & Chroma (C) values. Our data of rind colour at harvest situated generally between the hue angle values (60-80), whereas 60 refers to the deeps orange, while 80 to greenish yellow.

It is clear from the data that, of the two experimental seasons that 'Washington' navel orange had the lowest value of hue angle (H) values and this results means that 'Washington' navel orange attained deep orange colour fruits with good appearance when compared with the other studied cultivars.

Chemical Properties: It is clear from the data in Table (7) that, the highest values of vitamin C in the second season, T.S.S% as well as T. S.S/ acid ratio in the two experimental seasons were recorded by 'Washington' navel orange fruits when compared with the other studied cultivars.

'Washington' navel orange fruits had the highest values of T.S.S% as well as T. S.S/ acid ratio may be due to high potassium concentration of 'Washington' navel leaves (Table 8), which play vital role in citrus fruit quality. Whereas, potassium acts as an osmotic agent in the opening and closing of stomata. It plays an important role in controlling the acidity of the citrus fruit juice. It functions in charge balancing and membrane transport [55].

Nevertheless, total acidity of citrus juices is an important factor in overall juice quality and in determining time of harvest in several citrus producing countries [53]; there was no significant variation between all studied cultivars at the two experimental seasons in total acidity.

Chemical Composition

Total Indoles: Data in Fig. (5) demonstrated that, in the two experimental seasons the highest values of leaf total indoles concentrations were obtained by the three studied cultivar in July samples when compared with other sampling dates. In addition, 'Washington' leaves had the significant highest values followed by 'New hall' leaves at the three dates of sampling, with slight exceptions. While, 'Navelate' cultivar had the lowest value in this concern.

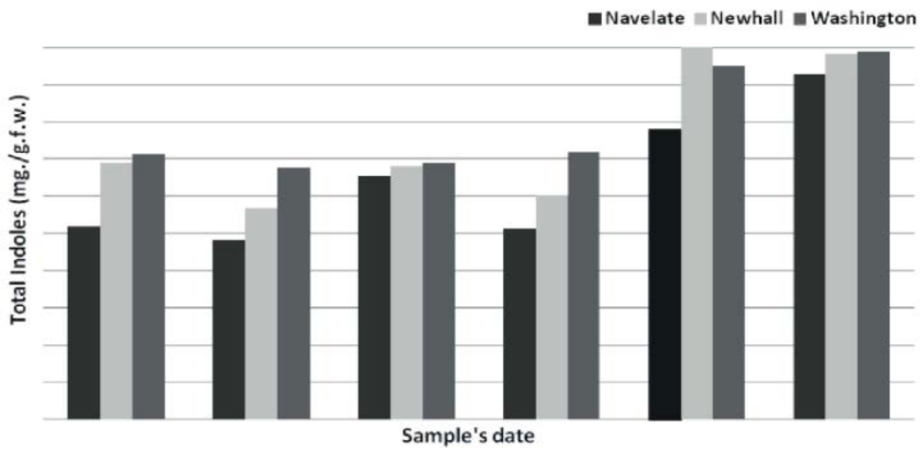

Fig. 5: Total indoles concentration (mg/g.fw) for studied cultivars at three date samples through two successive seasons

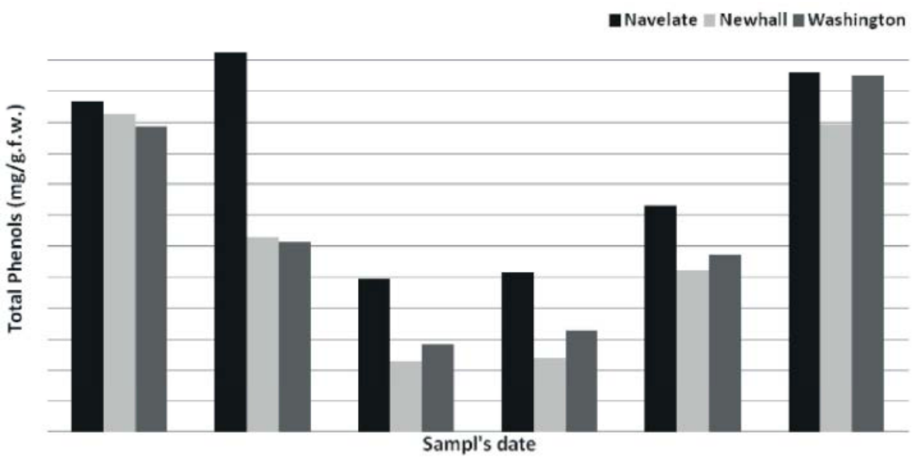

Fig. 6: Total phenols concentration (mg/g.fw) for studied cultivars at three date samples through two successive seasons

In this respect, there is a positive relationship between IAA and GA_3 and the growth of plant organs as well as the development of natural growth regulators in plants; especially in citrus fruits [56]. Correspondingly, the endogenous hormones (IAA or Zeatin) enhanced fruit growth during fruit growth and development [57].

Total Phenols: Data presented in Fig. (6) revealed that, in the two experimental seasons the highest values of total phenols concentrations was recorded by 'Navelate' navel orange leaves followed by 'New hall' orange leaves, with some exceptions in March samples. The highest values of total phenols in 'Navelate' leaves may lead to the decreasing of tree canopy of this cultivar in the two experimental seasons.

As, phenolic compounds have been shown to have both stimulatory and inhibitory effects on plant development. They play a significant role in cell wall development act in lignin biosynthesis [58]. Likewise, phenolic compounds are thought to be related to the growth vigor of trees, however, there were not a causal link between phenolic compound content and dwarfism [59]. The total phenolic content and some mineral nutrition were found to be different according to vigor capacity and moreover, there was a direct relationship between vigor and total phenol content [16].

Total Carbohydrates: The data in Table (8) showed that, the highest values of total carbohydrates in shoots were recorded by 'Washington' followed by 'New hall' shoots at the two experimental seasons, with insignificant differences from the Navelate cv.

In this respect, carbohydrates are used in the formation and development of the flowers and fruit of citrus trees [60]. Carbohydrate availability and fruit sink strength have been suggested as the most valuable factors responsible for final fruit size [61].

Table 8: Total carbohydrates and leaf minerals concentrations of studied cultivars

Cultivars	Total carbohydrates (mg/g. d.w.) 1st	2nd	N (%) 1st	2nd	P (%) 1st	2nd	K (%) 1st	2nd	Fe (ppm) 1st	2nd	Mn (ppm) 1st	2nd	Zn (ppm) 1st	2nd
Washington	1.879a	1.912 a	2.60b	2.23b	0.65a	0.57a	1.043a	1.250a	73.75c	65.75b	35.51a	28.46a	38.13b	40.09b
New hall	1.686b	1.780ab	2.70b	2.90b	0.55a	0.59a	0.873b	0.780b	79.91a	72.22a	30.79c	28.19a	40.31a	41.31a
Navelate	1.650b	1.683b	3.10a	3.03a	0.61a	0.64a	0.820b	0.757b	77.23b	65.07b	33.23b	27.62a	41.70a	41.45a

Means in each column fallowed by the same letter did not differ at p<0.05 according to Duncans multiple range tests

Also, carbohydrate levels seem to play a role in floral induction, fruit set, fruitlet abscission and fruit enlargement but they are not the sole factor. There is a general understanding that plant hormones and source-sink mechanisms must interact in the execution of plant performance [62].

Minerals: Regarding to leaves nitrogen concentration, the highest values were recorded by ‘Navelate’ at the two experimental seasons. Concerning to phosphorus concentration, no significant differences were obtained by the three cultivars at the two experimental seasons. While, the highest values of potassium concentration in the two experimental seasons and manganese concentration were obtained by ‘Washington’ navel orange leaves when compared with the other studied cultivars. Moreover, the highest values of iron leaves concentration were obtained by ‘New hall’ leaves at the two experimental seasons. While, in the two successive seasons the highest values of zinc concentration was recorded by ‘New hall’ and ‘Navelate’ leaves.

In this respect, the regular application of appropriate fertilizer materials is essential to proper growth and development of the citrus young tree and the subsequent development of large crops of good fruit quality in the mature tree [63]. The effects of mineral nutrient supply on crop yield response characteristics are often a reflection of sink limitations imposed by either a deficiency, or an excessive supply of mineral nutrients during certain critical periods of plant development [64]. Also, nutritional factors are one of the limiting factors for fruit set in citrus and other fruit species [65]. Where, inflorescences with persisting fruitlets accumulated more mineral elements than inflorescences with abscising fruitlets and for the phloem-mobile elements the excess accumulation was allocated to the fruitlets [66].

Generally, considering overall the studied cultivars performing; leaf anatomical structure revealed that, ‘Washington’ cultivar had the highest values in most of anatomical characters (thickness of lamina, palisade, spongy and mid vein) which reviled in its tree canopy, fruit set and fruit number as it achieved the uppermost figures in these characters. likewise, ‘New hall’ came in the second rank concerning these features and recorded high values in length and width of main vascular bundle which reflected in enlargement of its fruit size and weight, while ‘Navelate’ had a distant less values in the above mentioned characteristics. Also, it is clear that, the floral bud is surrounded by calyx which consists of five united sepals, each comprised of two epidermal layers in all cultivars. Meantime, Furthermore, UPGMA cluster analysis of genetic distances revealed that, all of ‘Navelate’ and parent genotypes were separated in two individual clusters. Meantime, the third cluster contains ‘Washington’ and ‘New hall’ genotypes. In addition, genetic similarity showed that, parent genotype was far from the studied cultivars genetic similarity except for ‘Navelate’ followed by ‘Washington’. A high value of genetic similarity (88.9) was observed between ‘Washington’ and ‘New hall’ cultivars while it was the least value with ‘Navelate’ (69.3). In addition, ‘Washington’ attained nearly the highest values of total indoles followed by ‘New hall’ thus enhancing their growth vigor in the same order, respectively. While, ‘Navelate’ leaves had the lowest values of indoles and gained also the highest values of total phenols concentrations which may have lead to the decreasing of tree canopy. Furthermore, the studied cultivars followed the same pattern as previous where ‘Washington’ followed by ‘New hall’ with lack of significance in the second season gained the uppermost of total carbohydrates in shoots respectably, reflecting such ranking in fruit set and fruit enlargement for both of them. Overall, ‘Washington’ had the highest values of fruit quality parameters, the highest value of fruit yield was recorded by ‘New hall’ and ‘Navelate’ got high values of fruit weight, fruit size relating to their relatively limited fruit set and number.

CONCLUSION

It could be concluded that 'New hall' figures were much more close to 'Washington' ones pertaining different studied cultivars properties as a result of most of their anatomical molecular and physiological characters. Anatomical futures could be viable indicators concerning tree vigor, fruit set and physical properties. In addition, the molecular studies was a moderate indicator in this concern as it proved the genetic similarity between 'Washington' and 'New hall' cultivars, while 'Navelate' showed a lower genetic similarity with 'Washington'. Moreover, the total indoles and total phenols in leaf content were reliable indicators for tree growth vigor. While, neither floral bud anatomical studies nor polymorphism detected by RAPD markers were significant indicators in this concern. So, a considerable association among different studied parameters and the relative similarity between cultivars under study could be suggested from preceding data. Such futures could be reliable relevant leading indicators in relation to will establish cultivar i. e., 'Washington' navel to predict newly introduced cultivar behavior. Yet, more studies need to be done in this issue to inspect such associations.

Still, illustrated data signifying to that 'Washington' navel orange as an economical yielding cultivar with advantageous fruit characteristics could be recommended mainly to the abroad markets and 'New hall' may be more appropriate for the domestic ones.

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