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EVALUATION OF HUANGLONGBING TOLERANCE IN CITRUS BREEDING POPULATIONS

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ABSTRACT

Huanglongbing (HLB) or greening disease is one of the most destructive disease of citrus industry all over the world. This disease had become widespread in Florida, USA since 2005. It is caused by a phloem limited bacterium called Candidatus Liberibacter asiaticus (CLas). Hybrids and populations produced by citrus breeding programs have likewise been ravaged by the disease. Thousands of unique genotypes have been exposed to natural CLas infection in the field for more than 10 years now, citrus researchers have had an opportunity to observe very substantial differences in the speed with which different types become affected by HLB, as well as the severity of symptoms. In the winter of 2015-16, we examined HLB responses in over 5000 citrus trees from hundreds of crosses made by the UF-CREC breeding program since 1986. The parentage of the crosses included pomelo, mandarin, sweet orange, grapefruit, trifoliate orange, and other miscellaneous citrus species. Ploidy levels in the populations included diploid, triploid and tetraploid. Depending on our field evaluation and symptoms of HLB disease on the trees, we divided all the trees to 5 categories according to the following ranking system (1=tolerant, 2=good looking, 3=medium, 4=bad, 5=dead). In total, only 5.3% trees among all other categories showed a very healthy appearance and no symptoms for HLB that we characterized them as HLB tolerant trees. Crosses {(Clementine x Temple) x C. ichangensis} followed by {(Clementine x Temple) x Swingle} had most tolerant trees among all of them trees. By using qPCR to determine CLas titer in visual tolerant trees, we conducted that 48.7% were CLas-positive, which have CT value less than 32, while 47.3% were CLas-negative with CT value equals or more than 32, it means that *some trees have the bacteria but still don't show any symptoms for the disease. The crosses [DPI 4-6 (Red Java) x H.B, (Clementine X Minneola) X Chinotto, VB Temple X (Nova + Ortanique), VB Temple X (Nova + Succari), (Clementine X Temple) X c. ichangensis] present the best crosses because they have CT value =40, that means no CLas in those trees. On the other hand, some characters of this category of tolerant trees had been studied such as, production of fruits, seediness and taste of fruits. A positive correlation between CT values and production of mature fruits among the tolerant trees. Concerning to seediness of fruits, most of seedless trees have been infected by Clas, while seedy trees tend to produce more non-infected trees with HLB. Taste of fruits from non-infected trees was better than those from infected trees.*

***Conclusively**, from these results, it could be concluded that, within individual species and among some citrus hybrid, number of progenies can be found that display substantial tolerance, and an ability to overcome and sometimes outgrow symptoms, this disease tolerance is not well correlated with estimated CLas populations. Future research utilizing these results for genomic selection in citrus populations will be performed.*

Keywords: Huanglongbing; Citrus; Evaluation; Crosses; Tolerance.

INTRODUCTION

Huanglongbing (HLB) or citrus greening is considered one of the most dangerous and destructive diseases of citrus trees all over the world. This disease is caused by a phloem-limited bacterium called *Candidatus librebacter* which has not been cultured yet. Three major strains of this bacterium, Asiaticus, Africanus and Americanus have been differentiated on the basis of environmental conditions and insect vector (Coletta-Filho *et al*. 2004; Garnier *et al*. 2000). HLB is transmitted through different means; infected propagation sources, the parasitic plant dodder (*Cuscuta* sp.), and insect vectors in nature. Citrus psyllid have been identified as the most potent insect vector for the transmission of the disease. Two species, *Diaphorina citri* and *Triozaerytreae,* are known as vectors of specific strains such as Asiaticus, Americanus and Africanus of bacterial inoculum, respectively (do Carmo Teixeira *et al*. 2005; Halbert and Manjunath 2004). Transmission of the pathogen has been described and reviewed in detail by Manjunath *et al*. (2008). Pathogen populations inside the host tree induce the release of a specific volatile chemical, methyl salicylate, which attracts the vector population to feed on the infected tree and the pathogen is then ingested by the vector (Mann *et al*. 2012).

CLas' infection causes a weakness and death of infected trees, loss of fruit because of early abortion, also affects the fruit, and consequently, the juice. Symptomatic fruit are smaller, misshapen, and often contain aborted seeds compared to fruit from noninfected trees. Fruit from HLB infected tree tend to fall prematurely and those that remain on the tree fail to mature correctly and retain their green color, hence the name greening for this disease. Early studies on HLB symptomatic fruit in South Africa have reported that the juice was of poor quality and tasted bitter (McClean and Oberholzer 1965; McClean and Schwarz 1970). The fruits do not color properly, remaining green on the shaded side (hence the name "greening disease") (Bové 2006; Gottwald *et al*. 2007; Halbert and Manjunath 2004).

The symptoms on leaves diverge from full yellowing, asymmetric blotchy-mottling, or other chlorotic patterns which sometimes looks like mineral deficiency to intensive vein corking. Massive accumulation of starch and disruption of chloroplasts were found to be associated with vein phloem collapse in *C*Las -infected leaves and could account for the appearance of symptoms.

Nearly all cultivated and wild citrus species are likewise sensitive to HLB in varying degrees. However, within individual species and among some citrus hybrid families, number of accessions can be found that display substantial tolerance manifest as minimal symptom expression. Recently, some citrus cultivars released by the UF-CREC breeding program have exhibited tolerance to HLB, specifically 'LB8-9' (Sugar Belle) and '7-6-27' mandarin. To understand the mechanism of HLB tolerance, previous studies have either used sweet oranges or mandarins as susceptible types in comparisons with rough lemon (*C. jambhiri*) and HLB tolerant hybrids between *Poncirus* and *Citrus* (Albrecht and Bowman 2012). This approach complicates the understanding of plant-microbe interactions because the effect of candidate genes in tolerant trees may differ in the genetic backgrounds of susceptible trees due to epistasis.

Therefore, the objective of this study was to assess field tolerance to HLB among citrus breeding populations, which may lead to the development of efficient breeding methods utilizing HLB tolerant germplasm in a citrus cultivar improvement program.

MATERIALS AND METHODS

Field Assessments

In the winter of 2015-2016, we examined HLB responses of 6609 trees from 1248 crosses made or mutations induced by the UF-CREC breeding program during years 1986-2008 (Table 1S). The parents used for these families included 89 different accessions of pomelo, mandarin, sweet orange, grapefruit, trifoliate orange, and other miscellaneous citrus species. Ploidy levels of the breeding populations included diploid, triploid and tetraploid. Eight field trials at the CREC, or in Haines City and Vero Beach, have been maintained using typical Florida citrus production practices.

Fig. 1. Field evaluation of HLB infection in different crosses. Pictures illustrates the difference between tolerant (0% symptoms), good (0 to 25% symptoms), medium (26 to 50% symptoms), bad (51 to 99% symptoms), respectively in the field.

We observed very substantial differences in the speed which different individuals became affected by HLB, as well as in the severity of symptoms in the breeding populations. Examining of trees for disease symptoms was carried out using HLB field identification guides (Spann *et al*. 2010). Generally, the trees were divided into 5 grades according to the following ranking system (1=tolerant, 2=good looking, 3=medium, 4=bad, 5=dead) trees (Fig. 1). These rankings were made based on the severity of the disease symptoms, tolerant trees (%0 symptoms), good trees (less than %25 symptoms), medium (%25 to %50 symptoms), bad trees (more than %50 symptoms), dead trees were completely died (Albrecht and Bowman 2011), those symptoms like asymmetrical blotchy leaf mottle which was observed on most of the trees; bright yellow corky leaf veins, foliar yellowing and defoliation, leading to a thin canopy, twig and branch dieback and tree decline, which was substantial in large number of trees.

The fruit produced by infected trees is small, green, underdeveloped, and misshapen, with aborted seeds and bitter in taste. Fruits also have higher acid and lower sugar content. The fruits do not color properly, remaining green on the shaded side (hence the name "greening disease") (Bové 2006; Gottwald *et al*. 2007; Halbert and Manjunath 2004). These symptoms were substantially noted in the diseased trees, but they could also be observed in some healthy trees, as well.

After HLB visual evaluation for every tree in the field, we selected the tolerant trees according to our visual characterization, to assess the amount of production especially mature fruits by counting the number of fruits per tree and we gave this character four categories depending on number of fruits; (0= no fruits, 1= few (less than 10 fruits per tree), 2= medium (between 10 to 20 fruits on the tree) and 3= high (more than 20 fruits per tree). Stover *et al*. (2016) all scion displayed increased cropping, greater than 20 fruit/tree.

Also, fruit taste had been assessed that is varied between (0= bad, 1=medium and 2= good taste). Also, seediness was assessed by checking and counting the number of seeds in the fruits from each tree (0=abortion seeds (brown and aborted seeds), 1=seedless (no seeds), 2=few seeds (less than 4 seeds) and 3=many seeds (more than 4 seeds)).

Pathogen Detection with PCR Analysis

Twelve fully expanded and old leaves were collected randomly from each tolerant tree with or without symptoms of HLB. The leaves were kept in plastic bags and refrigerated, then sent out to a commercial HLB diagnostic lab for DNA extraction and qPCR detection of Ct values. DNA was extracted from leaf midribs and petioles by using the plant DNeasy Mini Kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Real- time PCR assays were performed according to Li *et al.* (2006) **.** Amplifications were performed using a real-time PCR system Agilent Mx3005P System (Agilent Technology) using a Brilliant III Ultra-Fast QPCR Master Mix (Agilent Technology). The industry standard for HLB diagnoses were applied; specifically, cycle threshold (Ct) values <32 were considered *C*Las-positive. The statistical analysis of the results was done by using JMP software.

RESULTS

Field Assessment

Tolerant trees

The present results illustrate that the tolerant trees represent very low percentage (5.3%) comparing to the other categories in the field evaluation for symptoms of HLB disease (Fig. 2). Based on the number of trees in every cross, we can notice that more than 30 % of cross {(Clementine x Temple) x C. ichangensis} were tolerant trees, followed by {(Clementine x Temple) x Swingle} with ratio of 22.72% tolerant trees, (Fig.3A).

Good trees

From our studying we found 23.7% good trees between all evaluated trees. (Fig. 2). This cultivar (C528/Cleo) had 96.5% good trees between all its trees, followed by those crosses {(Ellen x (page+Ortanique) x (Rhode Red+Dancy)), (Riley NR1 Pomelo 2 x McRed) and (Nules x (Hamlin+Dancy))} with more than 70% good trees between all of them trees (Fig. 3B).

Medium trees

Medium trees with HLB symptoms between (25%- 50%) represent the highest category in this study (32.8%) between all the other categories (Fig.2). Cross (DPI 4-6 (red Java) x H.B.) has 70% from its trees that were medium trees, following by (H.B. x DPI 4-6 (Red Java)), (King Mandarin x Seedless Kishu). (Lee x Murcott) and finally (Clementine x Temple) x ortanique, with (60.56%, 58.06%, 52.17% and 50%), respectively (Fig. 3C).

Bad trees

The trees older than 7 years showed 15.4 % bad trees between them (Fig. 2). Those trees were really infected, and the symptoms covered more than 50% from the tree branches. Fallglo x (Nova + Osceola) had most of bad trees while Citrus. ichangensis had the least percentage of bad trees between all its trees, (44% and 32.3%, respectively) (Fig. 3D). On the other hand, there were some crosses didn’t have any bad trees for example {(Nules x W.murcott), (Nules x 921), (Red Java x Page), (Lee x Murcott), (Nules x (Succari + Page)), (Shan Tian You)} as shown in Table 1.

Table 1. The cultivars which have no bad trees**.**

Nules X W.Murcott
Riley NR1 Pummelo 2 X McRed
13-51 X W. Murcott
Blk 3 14-45 X (Page + (Clementine X Satsuma))
14-45 X (Page + Ortanique)
14-45 X (Murcott + Ortanique)
Nules X 921
Red Java X Page
Temple X (Page + (Clementine + Satsuma))
13-51 X (Page +(Clementine+Satsuma))
C528
13-51 X (Page + Ortanique)
DPI Fortune X Minneola
Mandalate
Orie Lee's Temple X Hamlin 4x
Ellendale X (Page + Ortanique) X (Rhode Red + Dancy)
Lee X (Page + Ortanique)
(Clementine X Minneola) X (Page + Ortanique)
Lee X Murcott
Nules X (Succari + Page)
Shan Tian You

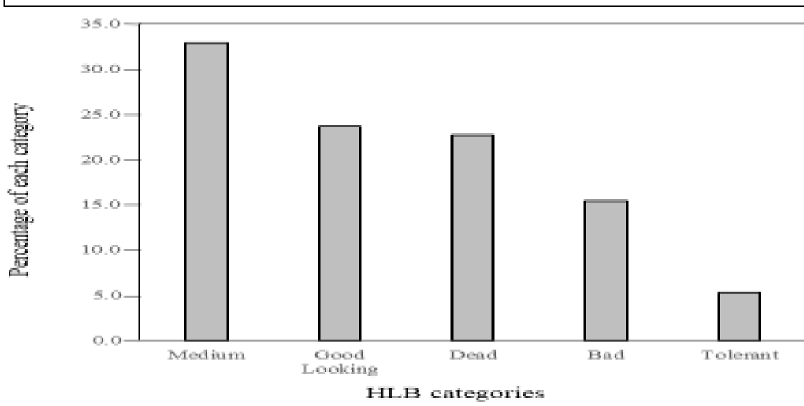

Fig. 2. Different categories of HLB in Field evaluation and percentage of each category.

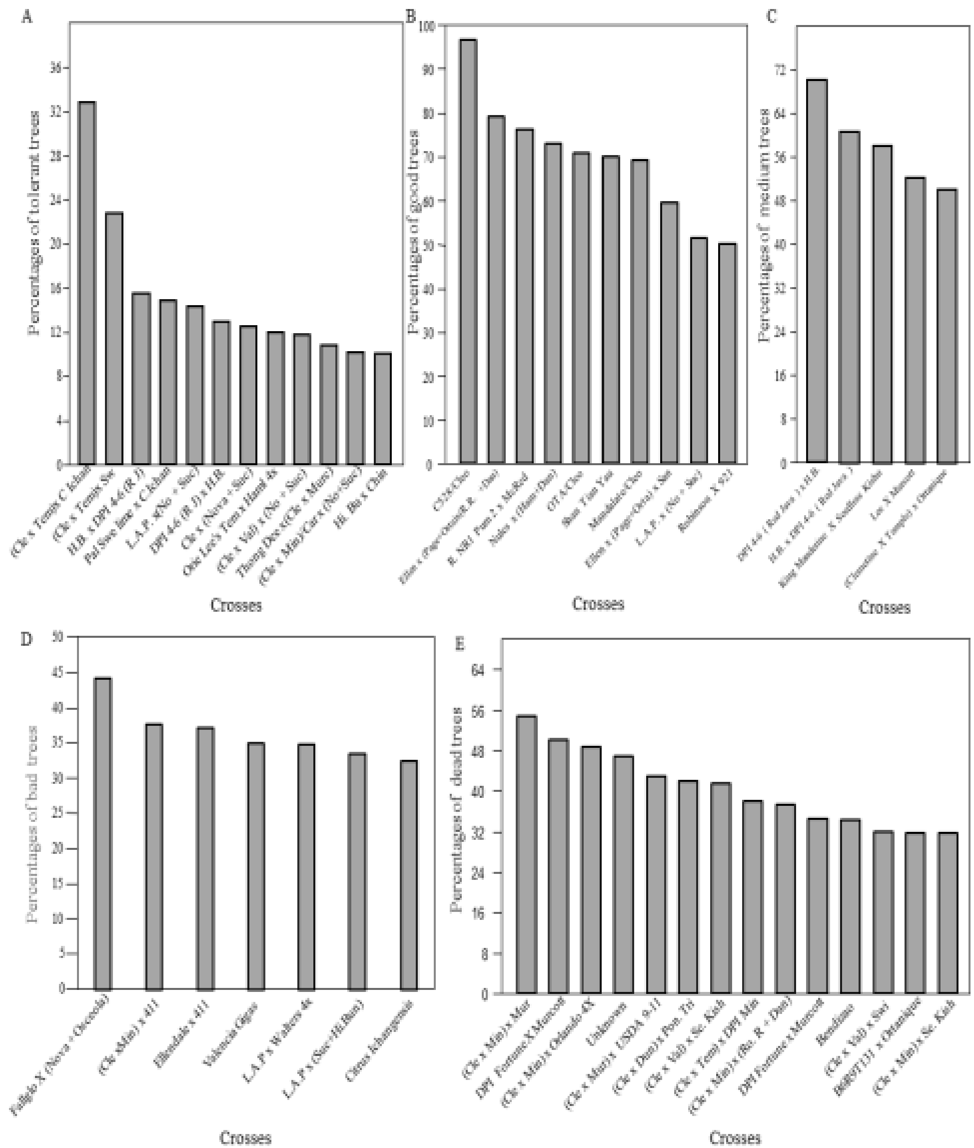

Fig. 3. Different categories of HLB visual evaluation in different crosses. And percentages of: A) Most tolerant crosses; B) Most good-looking crosses; C) Most medium crosses; D) Most of bad crosses and E) Most dead crosses. Crosses full names and abbreviations listed in (Table 2S).

Dead trees

From our field surveying, we found 22.7% dead trees in different crosses and mutations (Fig.2). Most of those dead trees were in those two crosses

Clementine X Minneola) X Murcott) with 54.70% dead trees, followed by (DPI Fortune X Murcott) with 50% dead trees, while both (Clementine x Minneola) x Seedless kishu and (Daisy Mandarin x Seedless Kishu) had the least percentage of dead trees with 31.60 % and 30.80%, respectively (Fig. 3E).

Characterization of tolerant trees

After visual characterization of all trees in all the blocks, the tolerant trees were selected for some studies such as checking CT value by qPCR analysis, cropping, taste of fruits and seediness. The correlation between CT value and cropping, seediness and taste of fruits had been assessed.

1.PCR Analysis

Among all visual tolerant trees 48.7% were *C*Las-positive, which have CT value less than 32, while 47.3% were *C*Las-negative with CT value equals or more than 32 (Fig. 4A). The best crosses for this study that have most tolerant trees with CT value =40, [DPI 4-6 (Red Java) x H.B, (Clementine X Minneola) X Chinotto, VB Temple X (Nova + Ortanique), VB Temple X (Nova + Succari), (Clementine X Temple) (Cleo) X c. ichangensis, Fallglo X (Nova + Osceola), H.B. x DPI 4-6 (Red Java), Palestine Sweet Lime x C. ichangensis, (Clementine X Minneola) X (Nova + Succari) (Fig.4E).

2. Number of fruits (cropping)

Number of mature fruits for every tolerant tree had been determined. Most of trees didn't have a big number, the greatest hybrids in number of fruits [(Clementine X Temple) X Swingle, Clementine x (Nova+Succari) and Kansu sweet orange with 40% high production compared with the other categories of production in the same cross. The infected fruits had many symptoms such as misshapen, smaller and green at the stylar end of the fruit. Generally, we noticed a positive correlation between CT values and production of mature fruits among the tolerant trees, by increasing CT value, the number of fruits had been increased too. On the other hand, the production was really infected and low all over the field not just in infected trees but the tolerant trees too. (Fig.4B).

3. Seeds

The obtained data showed that 35.7% of tolerant trees had fruits with many seeds, on the other hand, 5.9% from the tolerant trees have few seeds in

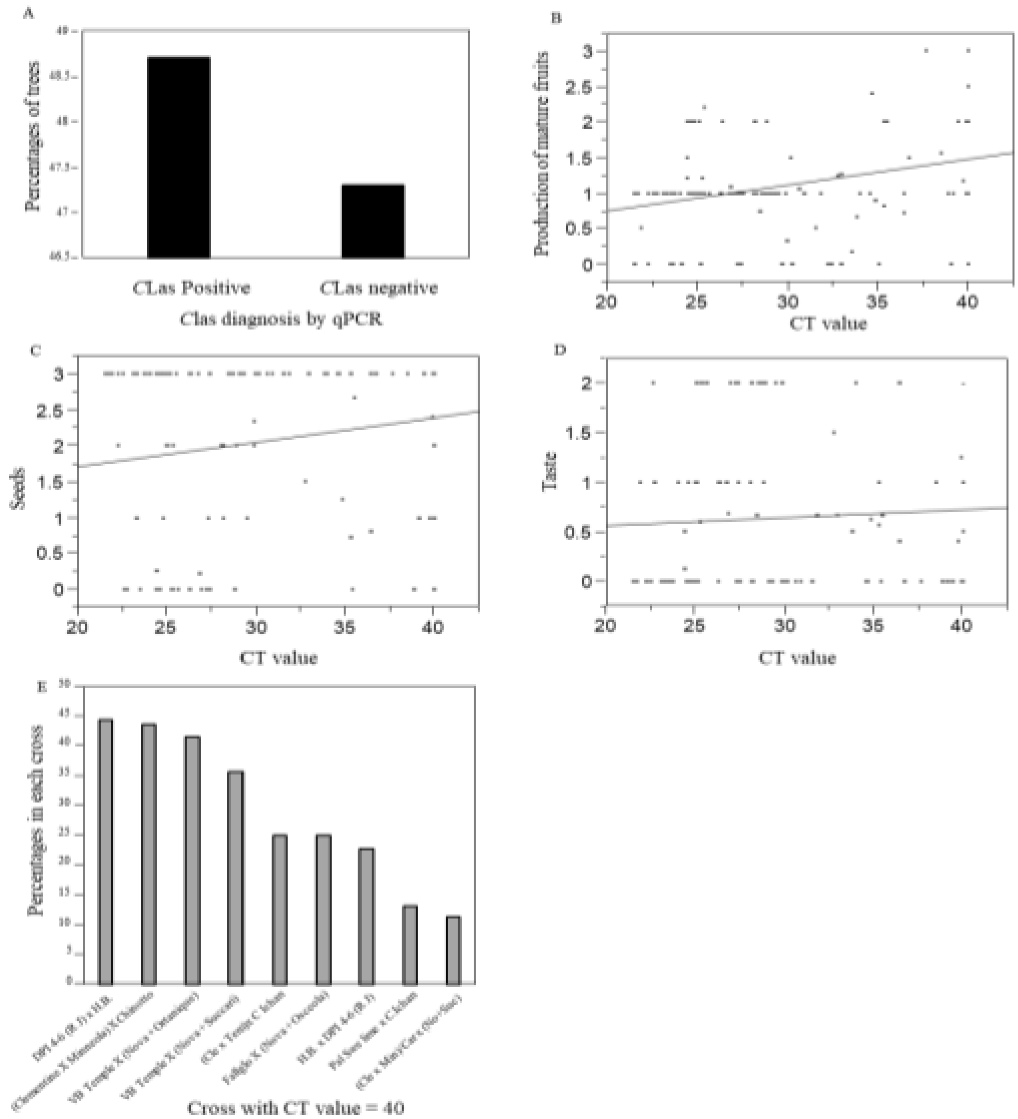

Fig. 4. *C*las diagnosis for visual tolerant trees by qPCR and correlation between CT value and some studied characters. A) The ratio between *C*Las positive and *C*Las negative between all visual tolerant trees; Correlation between CT value and B) Production of mature fruits; C) Seediness; and D) Taste of fruits and E) Tolerant crosses with CT value=40 and percentage of those tolerant in each cross.

the fruits, while 18.7% from those trees had seedless fruits, most of them in this triploid hybrid ((Clementine X Minneola) X (Nova + Succari)), and 4.8% had abortion seeds, this kind of seeds is considered one of HLB symptoms, that's why we noticed that the cross with biggest number of abortion seeds was [Fallglo x (Nova + Osceola)] and it is the same cross with the biggest number of bad trees, this result can reflect the effect of HLB disease on citrus seeds quality. From the relationship between seediness and CT value we found that most of seedless trees have been infected by *C*las, while seedy trees tend to produce more non-infected trees with HLB (Fig. 4C). This result can lead to good way for fighting this serious disease by breeding program, maybe there is an effect for presence of seeds in fruits and tolerance to HLB.

4. Taste of fruits

The fruits of all tolerant trees were tasted, 18.4% of them had good taste, most of them in this cross ((Clementine X Minneola) X (Nova + Succari)), 7.4% trees had medium taste (not good or not bad), while most of the visual tolerant trees had fruits with bad taste (40.2%). (Fig. 4D) illustrates that the taste of fruits from non-infected trees was better than those from infected trees.

DISCUSSION

Huanglongbing (HLB) has become a global problem that fights the production of citrus. The disease is widely spread in Asia, Africa and not long ago in America (Jagoueix *et al*. 1994; do Carmo Teixeira *et al*. 2005)Unfortunately, most of cultivated and wild citrus species are likewise sensitive to HLB in varying degrees. Although, some citrus species and relatives were considered tolerant to this disease (McClean and Schwarz 1970; Miyakawa 1980). This disease is really hard to be assessed in the field because the symptoms resemble other disease (such as stubborn disease) and nutritional deficiencies (such as zinc), those results illustrated by(Grafton-Cardwell *et al*. 2006).

One of the major difficulties for detection of HLB disease is that, symptoms are unevenly distributed within infected trees (Folimonova *et al*. 2009). That's why we didn't depend on just visual evaluation for characterization the trees, but more studies had been conducted for the tolerant trees.

This study illustrated that very little percent from all the studied trees were tolerant compared with the other categories. That means very big loss happened as an effect to HLB disease. HLB has become the most destructive citrus disease all over the world, where it generates substantial economic losses (Bové 2006). The cross {(Clementine x Temple) x C. ichangensis} includes most of HLB tolerant trees in visual evaluation. These results mean that this cross considered the best cross in this study. May be this related to Temple parent, (Stover *et al*. 2015) assessed HLB in diverse cultivars in commercial groves with high HLB incidence and conducted that 'Temple' had the least symptoms for HLB comparing to 'Murcott' had the most symptoms.

Concerning to tolerant trees, just 8.8 % from all tolerant trees had big production, these results refer to very big loss in the production of trees and this had been conducted before by Bové (2006). The fruits in most of trees had many symptoms such as misshapen, smaller and green at the stylar end of the fruit, those results had been conducted before by Grafton-Cardwell *et al*. (2006). PCR results for visual tolerant illustrated that almost 1:1 ratio, *C*Las-positive to *C*Las-negative. Data illustrated that, 80%from those *C*Las-negative have CT value =40.The cross (Clementine X Temple) X C. ichangensis has most of those tolerant trees with CT value =40. (Stover *et al*. 2015) resulted that 'Temple' showed the lowest (Las) titer comparing to 'Murcott' had the highest titers. Taste of fruits differed between all tolerant trees, 18.4% of them had good taste, most of them been included in this somatic hybrid ((Clementine X Minneola) X (Nova + Succari)), that was one of the best tolerant progenies in this study, also had most trees of seedless fruits.

In recent years, HLB has caused substantial economic losses to the citrus industry by shortening the tree life and reducing productivity with poor quality colorless fruit left on the trees (Bové 2006). The greatest hybrids in number of fruits [(Clementine X Temple) X Swingle], this can be related to Temple that showed significantly greater cropping than 'Hamlin' (Stover *et al*. 2016).

A significant correlation had been noticed between CT values and the taste of fruits. Most of bad taste fruits were included in infected trees. The infected fruits were bitter and sour. And this had been summarized before by Grafton-Cardwell *et al.* (2006), HLB infected fruits are small, green, under developed, misshapen and bitter in taste. Thus, fruit cannot even be marketed as juice.

Most of abortion seeds were included in the infected trees with CT value less than 32 and reduced in non-infected trees with CT value more than 32, those results agree with (Capoor *et al.* 1974) and (McClean and Schwarz 1970). They noticed that seed abortion is common symptom for HLB infected fruits despite the absence of apparent disease symptoms on fruit and leaves.*et al*

From our study, we illustrated that most of seedless trees have been infected by *C*las, while seedy trees tend to produce more non-infected trees, but these results don't agree with (Albrecht and Bowman 2011), Fruit average seed did not differ significantly ($P > 0.05$) between symptomatic and symptomless trees.

In summary, HLB tolerance is complex quantitative trait, and controlled by many genes. To provide HLB tolerant cultivars as well as good flavor, genomic assisted breeding could increase the efficiency and precision of citrus improvement.

CONCLUSION

Use of resistant or tolerant citrus plants could be the most economically way of managing HLB disease. The objective of this study was to look at the impacts of genetically modified individuals of citrus on HLB disease. The cross (Clementine x Temple) x C. ichangensis, was the best cross in the field evaluation, showed less severe symptoms than all the other trees. Also, it has most of tolerant trees with CT value=40**.** The severity of HLB disease is often measured by visual assessment. Visual assessment is rapid and useful when hundreds of trees should be evaluated. Although, it is not enough for evaluation.

As expected, some differences were observed in the performance of the HLB infected trees on the genetically diverse individuals included in the study. However, it should be noted that the study was done over a short period of time (less than a year). Longer studies and other additional field trials along with greenhouse studies will be done to validate any conclusions.

Table 1S. All cultivars in this study.

The crosses	Number of trees	The crosses	Number of trees
(Clementine X Minneola) X (Nova + Succari)	243	Shadette	33
(Clementine X Minneola) X Seedless Kishu	212	White Mandarin (Ponkan)	33
Unknown	177	(Clementine X Duncan) X Seedless Kishu	32
Daisy Mandarin X Seedless Kishu	172	(Clementine X Minneola) X Walters 4x	32
(Clementine X Minneola) X Orlando 4x	168	(Clementine X Temple) X (Hamlin + Dancy 4x)	32
VB Temple X (Nova + Ortanique)	163	(Clementine X Temple) X Ortanique	32
Nules X (Hamlin + Dancy)	159	Orie Lee's Temple X Valencia 4x	32
(Clementine X Minneola) X Chinotto	136	Citrus ichangensis	31
Clementine X (Clementine X Minneola)	130	King Manderin X Seedless Kishu	31
Palestine Sweet Lime X C. ichangensis	128	13-51 X (Page + Clementine X Satsuma)	30
Fallglo X (Nova + Osceola)	125	(Clementine X Minneola) X Ruby	29
B6R9T131 X Ortanique	123	BLK 3 14-45 X (Page + Clementine X Satsuma) X Orlando Tangelo 4x	29
VB Temple X (Nova + Succari)	103	C5282	29
Low Acid Pummelo X Walters 4x	98	Ellendale X 411	27
(Clementine X Valencia) X (Nova + Succari)	94	LBER5T36 X Imperial 4x	27
Nules X W. Murcott	90	3 13-51 X (Page + Ortanique)	26
(Clementine X Minneola) X (Nova + Succari)	89	DPI Fortune X Murcott	26
(Clementine X Duncan) X *Poncirus trifoliata*	88	DPI Fortune X Minneola	26
Riley NR1 Pummelo X McRed	88	Mandalate	26
Low Acid Pummelo X (Succari + Hirado Buntan)	87	Flying Dragon 2	25
Marisol X 411	80	Orie Lee's Temple X Hamlin 4x	25
(Clementine X Minneola) X 9-11 USDA	78	(Clementine X Valencia) X Swingle	24
Robinson X (Nova + Succari)	73	Ellendale X (Page+Ortanique) X (Rhode Red+Dancy)	24
(Clementine X Temple) X DPI Minneola	71	Lee x X (Page + Ortanique)	24
Fortune X (Nova + Succari)	71	OTA	24
H.B. X DPI 4-6 (Red Java)	71	(Clementine X Minneola) X (Page + Ortanique)	23
DPI 4-6 (Red Java) X H.B.	70	Lee X Murcott	23
Low Acid Pummelo X (Nova + Succari)	70	Nules X (Succari + Page)	23
Low Acid Pummelo X Valencia 4x	70	Valencia Gigas	23
(Clementine X Minneola) X (Hamlin + (Clementine X Minneola))	69	(Clementine X Minneola) (B7R14T33, 34) X Vermilian	22
(Clementine X Temple) X C. ichangensis	67	(Clementine X Temple) X Swingle	22
(Clementine X Duncan) X Duncan	65	(Clementine X Murcott) X USDA 9-11	21
(Clementine X Minneola) X Hamlin 4x	65	Hirado Buntan X Chinotto	20
(Clementine X Minneola) X Murcott	64	Nules X (Murcott + Ortanique) X (Nova + Osceola)	20
Clementine X Orlando 4x	61	Sha Tian You	20
(Clementine X Minneola) X (Rohde Red + Dancy)	59	(Clementine X Valencia) X Walters 4x	19
13-51 X W. Murcott	59	Fremont	19
DPI Fallglo X Swingle	59	Hirado Buntan X (Succari + Hirado Buntan#5)	19
(Clementine X Minneola) X Fortune	57	Temple X (Nova + Osceola)	19
(Clementine X Minneola) X Carrizo	56	Thong Dee X Minneola Tangelo	19
(Clementine X Minneola) X Orlando Tangelo	55	(Clementine X Duncan) X Paperrind 4x	18
DPI Fortune X Murcott	55	(Clementine X Minneola) X Big Tangelo	18
(Clementine X Valencia) X Seedless Kishu	53	13-51 X (Murcott + (Clementine X Minneola))	18
14-45 X (Page + Clementine X Satsuma)	53	Monreal X Orlando 4x	18
Lee X Seedless Kishu	52	Sour Orange X Ridge Pineapple	18
B6R9T133 X Hamlin 4x	51	(Clementine X Minneola) X Paperrind 4x	17
14-45 X (Page + Ortanique)	51	Hirado Buntan X Hudson	17
Fortune X Valencia 4x	49	Hudson 4N	17
(Clementine X Minneola) X 411	48	Lee X Fairchild	17
(Clementine X Valencia) X Swingle	47	Mariol X (Page + Clementine X Satsuma) X (Nova + Osceola)	17
Ellendale X (Page + Ortanique) X Sanguinelli	47	Temple X Seedless Kishu	17
Nakon X (Clementine X Minneola)	47	(Clementine X Minneola) X Murcott X (Valencia + Page)	16
(Clementine X Minneola) X (Thong Dee X Pomeroy trifoliate orange)	46	B6R9T132 X Ruby 4x	16
Clementine X Orlando 4x	42	Clementine seedlings	16
14-45 X (Murcott + Ortanique)	41	Ellendale seedlings	16
Clementine X (Nova + Succari)	40	Nakon X Minneola Tangelo	16
Nules X 921	40	Shiranui	16
Red Java X Page	40	(Clementine X Minneola) X US 119	15
(Clementine X Minneola) X Orlando	38	B6R9T131 X Dancy 4x	15
Temple X (Page + Clementine X Satsuma)	37	Ellendale X Orlando 4x	15
Thong Dee X (Clementine X Murcott)	37	Hirado Buntan X Hudson	15
Robinson X 921	36	Nakon X Page	15
Bendizao	35	Southern Farms citranges	15
(Clementine X Minneola) X Orlanlo Tangelo X Valencia 4x	34	(Clementine X Hamlin) X Swingle	14

(Clementine X Minneola) X Valencia	14	*Citrus junos*Carrizo	9
(Clementine X Minneola) X Valencia 4x	14	*Citrus sunki**Citrus junos*	9
(Clementine X Minneola)/Carrizo	14	Fiwicke orangeorange X Argentine TF*Citrus sunki*	9
(Clementine X Hamlin) X *Poncirus trifoliata*	14	*Fortunella polyandra*Fiwicke orangeorange X Argentine TF	9
Liang Ping Yau	14	Hirado Buntan X Chinotto*Fortunella polyandra*	9
Rice Manderin	14	LB4-19 X SwingleHirado Buntan X Chinotto	9
Robinson X Fairchild	14	Robinson X Paperrind 4X4xLB4-19 X Swingle	9
Robinson X US 119	14	SF-12-2-33Robinson X Paperrind 4x	9
VB Clementine 4x X 411	14	Tahiti orange X Argentine TFSF-12-2-33	9
(Clementine X Minneola) X (Valencia + Page)	13	(Clementine X Minneola) Tahiti orange X Argentine TF	9
104/US 897	13	(Clementine X Temple) X (Valencia+ Robinson X Temple) (Clementine X Minneola)	8
Clementine X (Pink Marsh + Murcott)	13	(Clementine X Valencia) X Orlando Tangelo 4x (Clementine X Temple) X (Valencia+ Robinson X Temple)	8
Ichangensis	13	(Clementine X Temple) X (K+V) (Clementine X Valencia) X Orlando Tangelo 4x	8
Marisol X Succari 4x	13	Cao Ju (Clementine X Temple) X (K+V)	8
Palestine Sweet Lime X Gou Tou	13	DPI King H4 X MarshCao Ju	8
Pimpled Manderin	13	Fallglo X MurcottDPI King H4 X Marsh	8
Chironja (Clementine X Minneola) X Orlanlo Tangelo X Hamlin 4x	12	Golden PeaFallglo X Murcott	8
Dominican Wild Grape fruit Chironja	12	Mercer PageGolden Pea	8
Fallglo X Fair child Dominican Wild Grapefruit	12	Robinson X RubyMercer Page	8
M132Fallglo X Fairchild	12	SR3RU01-MB2/CZORobinson X Ruby	8
Sue Linda Temple X (Nova + Succari) M132	12	St. VincentSR3RU01-MB2/CZO	8
3931Sue Linda Temple X (Nova + Succari)	12	(Clementine X Minneola) X 80-9St. Vincent	8
LB 4-8 X Ortanique3931	11	(Clementine X Valencia) X Seedy Marsh (Clementine X Minneola) X 80-9	7
Lisbon X ChinottoLB 4-8 XOrtanique	11	(Clementine X Valencia) (B7R14T36) X Vermilian Hong (Clementine X Valencia) X Seedy Marsh	7
NB-7-34/US897Lisbon X Chinotto	11	Clementine X (Succari + Dancy) (Clementine X Valencia) (B7R14T36) X Vermilian Hong	7
Nules X W. MurcottNB-7-34/US897	11	Ellendale X (Page + Ortanique) Clementine X (Succari + Dancy)	7
(Clementine X Minneola) X (Page + Clementine X Satsuma) Nules X W. Murcott	11	Fiwicke Orange X Flying Dragon TF Ellendale X (Page+Ortanique)	7
(Clementine X Minneola) X Ortanique (Clementine X Minneola) X (Page + Clementine X Satsuma)	10	Hong Nin Mon Fiwicke Orange X Flying Dragon TF	7
(Clementine X Murcott) X Paperrind 4x (Clementine X Minneola) X Ortanique	10	Lee X (Murcott + (Clementine X Minneola)) Hong Nin Mon	7
(Clementine X Valencia) (B7R14T35, 36) X Bendizao (Clementine X Murcott) X Paperrind 4x	10	R3NA02-RALee X(Murcott + (Clementine X Minneola))	7
(Clementine X Temple) X Sunburst (Clementine X Valencia) (B7R14T35, 36) X Bendizao	10	Ruby X Flying Dragon TFR3NA02-RA	7
Clementine X (Hamlin X (Clementine X Minneola)) (Clementine X Temple) X Sunburst	10	Snack/CICCRuby X Flying Dragon TF	7
Fiwicke orangeorange X CarrizoClementine X (Hamlin X (Clementine X Minneola))	10	Vangasay Lemon X (Dpi 6- 13) X 1575-21Snack/CICC	7
Orie Lee's Temple X Orlando 4x Fiwicke Orange X Carrizo	10	(Clementine X Valencia) X HamlinVangasay Lemon X (Dpi 6- 13) X 1575-21	7
Sanford Sweet Orange X Argentine TF Orie Lee's Temple X Orlando 4x	10	(Clementine X Valencia) X Ruby (Clementine X Valencia) X Hamlin	6
Sanford Sweet Orange X Flying Dragon TF Sanford Sweet Orange X Argentine TF	10	(Clemetine X Temple) (Cleo) X Swingle (Clementine X Valencia) X Ruby	6
Tahiti orange X Flying Dragon TF Sanford Sweet Orange X Flying Dragon TF	10	2014 (Clemetine X Temple) (Cleo) X Swingle	6
Wilking seedlings Tahiti orange X Flying Dragon TF	10	22242014	6
(Clementine X Duncan) (B7R14T23,24,25) X Valencia 4x Wilking seedlings	10	Eureka X Chinotto2224	6
(Clementine X Duncan) X (Nova + Succari) (Clementine X Duncan) (B7R14T23,24,25) X Valencia 4x	9	Fortune X ValenciaEureka X Chinotto	6
(Clementine X Minneola) X Murcott X Seedless Kishu (Clementine X Duncan) X (Nova + Succari)	9	Gft. Chimera 3Fortune X Valencia	6
(Clementine X Minneola) X Valencia 4x (Clementine X Minneola) X Murcott X Seedless Kishu	9	LB 4-3 X ChinottoGft. Chimera 3	6
(Clementine X Minneola)/Cleo (Clementine X Minneola) X Valencia 4x	9	Murcott 4N/CleoLB 4-3 X Chinotto	6
(Clementine X Murcott) X Valencia (Clementine X Minneola) /Cleo	9	Riley Fortune X (Valencia + Robinson X Temple) Murcott 4N/Cleo	6
(Clementine X Valencia) X (Nakon X Flying Dragon TF) (Clementine X Murcott) X Valencia	9	Rootsprout Riley Fortune X (Valencia + Robinson X Temple)	6
B23ER5T36 (Low Acid Pummelo) X Walters Clementine X Valencia) X (Nakon X Flying Dragon TF)	9	Ruby X Argentine TFRootsprout	6
CarrizoB23ER5T36 (Low Acid Pummelo) X Walters 4x	9	Satsuma X SunburstRuby X Argentine TF	6

Xie ShanSatsuma X Sunburst	6	B6R7T131 X Hamlin 4x3875	5
(Clementine X Minneola) X (Succari + Dancy) Xie Shan	6	B6R7T131 X Valencia 4xB6R7T131 X Hamlin 4x	5
(Clementine X Minneola) X (Valencia + Minneola) (Clementine X Minneola) X (Succari + Dancy)	5	Kara (SRA)B6R7T131 x Valencia 4X	5
(Clementine X Minneola) X Orlando Tangelo (Clementine X Minneola) X (Valencia + Minneola)	5	(Clementine x X Mineola) x X Murcott X (Nova + Osceola) Kara (SRA)	5
(Clementine X Minneola) X Orlando Tangelo X Rubyblood 4x (Clementine Minneola) X Orlando Tangelo	5	Monreal X Orlando (Clementine X Mineola) X Murcott X (Nova + Osceola)	5
(Clementine X Minneola)/Swingle (Clementine X Minneola) X Orlando Tangelo X Rubyblood 4x	5	Palestine Sweet Lime X SwingleMonreal X Orlando	5
(Clementine X Murcott) X Paperrind (Clementine X Minneola)/Swingle	5	S4HA01-MLB? Palestine Sweet Lime X Swingle	5
(Clementine X Valencia)/Cleo (Clementine X Murcott) X Paperrind	5	Sue Linda Temple X (Hamlin + (Clementine X Minneola))S4HA01-MLB?	5
(Clementine X Valencia) X Walters 4x (Clementine X Valencia)/Cleo	5	Temple X OrlandoSue Linda Temple (Hamlin + (Clementine XMinneola))	5
3637(Clementine X Valencia) X Walters 4x	5	Tu Nin MonTemple X Orlando	5
38753637	5	Tu Nin Mon	5

Table 2S. The full names and the abbreviations for some crosses

Full names	Abbreviations
(Clementine x Temple) (Cleo) x C Ichangensis	(Cle x Tem)x C Ichan
(Clementine x Temple) (Cleo) x Swingle	(Cle x Tem)x Sw
H.B. x DPI 4-6 (Red Java)	H.B. x DPI 4-6 (R J)
Palestine Sweet Lime x C. Ichangensis	Pal Swe lime xC Ichan
Low Acid Pummelo x (Nova + Succari)	L.A.P. x(No + Suc)
DPI 4-6 (Red Java) x H.B.	DPI 4-6 (R J) x H.B.
Clementine x (Nova+Succari)	Cle x (Nova + Suc)
Orie Lee's Temple x Hamlin 4X	Orie Lee's Tem x Haml 4x
(Clementine x Valencia) x (Nova+Succari)	(Cle x Val) x (No + Suc)
Thong Dee x (Clementine x Murcott)	Thong Dee x (Cle x Murc)
(Clementine x Minneola)/Car x (Nova + Succari)	(Cle x Min)/Car x (No+Suc)
Hirado Buntan x Chinotto	Hi. Bu x Chin
C528/Cleo	C528/Cleo
Ellendale x (Page+Ortanique) x (Rhode Red+Dancy)	Ellen x (Page+Orta)x(R.R. +Dan)
Riley NR1 Pummelo 2 x McRed	R. NR1 Pum 2 x McRed
Nules x (Hamlin+Dancy)	Nules x (Ham+Dan)
OTA/Cleo	OTA/Cleo
Shan Tian Yau	Shan Tian Yau
Mandalate/Cleo	Mandalate/Cleo
Ellendale x (Page+Ortanique) x Sanguelli	Ellen x (Page+Orta) x San
Low Acid Pummelo x (Nova + Succari)	L.A.P. x(No + Suc)
Robinson x 921	Robinson x 921
Flying Dragon 2	Flying Dragon 2
Fallglo x (Nova + Osceola)	Fallglo x (Nova + Osceola)
(Clementine x Minneola)x 411	(Cle xMin) x 411
Ellendale x 411	Ellendale x 411
Valencia Gigas	Valencia Gigas
Low Acid Pummelo x Walters 4X	L.A.P x Walters 4x
Low Acid Pummelo x (Succari+Hirado Buntan)#5	L.A.P x (Suc+Hi.Bun)
Citrus Ichangensis	Citrus Ichangensis
(Clementine x Minneola) x Murcott	(Cle x Min) x Mur
DPI Fortune x Murcott	DPI Fortune X Murcott
(Clementine x Minneola) x Orlando 4X	(Cle x Min) x Orlando 4X
Unknown	Unknown
(Clementine x Murcott) x USDA 9-11	(Cle x Mur) x USDA 9-11
(Clementine x Duncan) x Poncirus Trifoliata	(Cle x Dun) x Pon. Tri
(Clementine x Valencia) x Seedless Kishu	(Cle x Val) x Se. Kish
(Clemetine x Temple) x DPI Minneola	(Cle x Tem) x DPI Min
(Clementine x Minneola) x (Rohde Red + Dancy)	(Cle x Min) x (Ro. R + Dan)
DPI Fortune x Murcott	DPI Fortune x Murcott
Bendizao	Bendizao
(Clementine x Valencia) x Swingle	(Cle x Val) x Swi
B6R9T131 x Ortanique	B6R9T131 x Ortanique
(Clementine x Minneola) x Seedless Kishu	(Cle x Min) x Se. Kish

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تقييم التحمل لمرض الاخضرار فى عشائر الموالح المرباة

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يعتبر مرض اخضرار الموالح واحد من اخطر الامراض المدمرة لصناعة الموالح فى العالم. انتشر هذا المرض بولاية فلوريدا بالولايات المتحدة الامريكية منذ عام ٢٠٠٥ عن طريق البكتريا اللحائية المسماة(*Candidatus* Liberibacter asiaticus (*C*Las. ولقد تسبب المرض فى تدمير العديد من هجن وعشائر الموالح المنتجة عن طريق برامج التربية والتهجين. ولقد تعرضت الاف من الانماط الوراثية المميزة للموالح للاصابة الطبيعية بالمرض فى الحقل لمدة تزيد عن عشر اعوام حتى الوقت الحالى. ولقد قام باحثى الموالح بملاحظة الاختلافات الاساسية في سرعة اصابة الطرز الوراثية المختلفة بالمرض وكذلك درجات الاصابة المختلفة وحدة الاعراض. ولقد اجريت هذه الدراسة فى مركز بحوث الموالح التابع لجامعة فلوريدا بالولايات المتحدة الامريكية فى شتاء ٢٠١٥-٢٠١٦ لدراسة تأثير هذا المرض على طرز الموالح بالمنطقة ولقد اجريت الدراسة على اكثر من ٥٠٠٠ شجرة موالح من مئات الهجن التى انتجت ضمن برامج تربية الموالح بالمركز منذ عام ١٩٨٦ والتى انتجت من اباء مختلفة من الموالح مثل البرتقال الحلو واليوسفى والجريب فروت والشادوك والبرتقال ثلاثى الاوراق واجناس اخرى للموالح وكذلك اشتملت هذه الهجن مستويات تضاعف وراثى مختلفة اما ثنائية او ثلاثية او متعدده. وبناءا على هذا التقييم الحقلى لاعراض المرض على الأشجار تم تقسيم الاشجار الى خمس مجموعات تبعا لهذا التصنيف (١- اشجار متحملة لمرض ، ٢- أشجار جيدة المظهر ، ٣- أشجار متوسطة، ٤- أشجار سيئة، ٥- أشجار ميتة). فى المجمل لوحظ ان ٥.٣% فقط من كل الاشجار تحت الدراسة ذات مظهر صحى وخالية من اى أعراض للمرض والتى تم تشخيصها كأشجار متحملة للمرض. وكان الهجين [(Clementine x Temple) x C. ichangensis] متبوعا بالهجين [(Clementine x Temple) x Swingle] أفضل الهجن فى هذا التقييم الحقلى حيث اشتملت اكبر نسبة من الأشجار المتحملة للمرض بين أشجارها. كذلك تم اجراء تحليل معملى لعينات من هذه الاشجار المصنفة كأشجار متحملة للمرض باستخدام جهاز qPCR للتاكد من وجود البكتريا من عدمه وكذلك تقدير مستويات

البكتريا الموجودة لتحديد افضل هذه الهجن وتم استنتاج ان ٤٨.٧% من هذه الاشجار اعطت نتيجة ايجابية لوجود البكتريا حيث كانت قيم CT فى هذه العينات اقل من ٣٢، بينما ٤٧.٣% أعطت نتيجة سلبية لوجود البكتريا والتى كانت فيها قيم CT اكبر من او تساوى ٣٢. وهذا يعنى أن بعض الاشجار قد تحتوى البكتريا ولكن تظل بدون اى اعراض للمرض.

كذلك تم تقييم حقلى لهذه الاشجار المتحملة للمرض من حيث كل من (عدد الثمار على الشجرة حيث يقل مع شدة الاصابه، وطعم الثمار حيث يتحول لطعم حامضى قابض مع شدة الاصابة، واخيرا عدد البذور وصفاتها حيث ان البذور المجهضة الجنين عرض مميز جدا لهذا المرض). كذلك تعتبر الهجن [DPI 4-6 (Red Java) x H.B, (Clementine X Minneola) x Chinotto, VB Temple x (Nova + (Nova + Succari), (Clementine x Ortanique), VB Temple x Temple) X c. ichangensis هى أفضل الهجن حيث كانت قيم CT لها =٤٠ مما يعنى عدم وجود البكتريا فيها. ومن ناحية اخرى تم دراسة عدة صفات فى هذه المجموعة من الاشجار التى تحمل صفة التحمل للمرض مثل انتاجية الثمار وكذلك صفات وعدد البذور بالثمرة وايضا طعم الثمار. ولوحظ وجود ارتباط ايجابي بين قيم CT وانتاجية الثمار بين الاشجار المتحملة للمرض ، اما بخصوص صفات البذرة وجد أن معظم الاشجار ذات الثمار عديمة البذورمصابة بالبكتريا المسببة للمرض وعلى العكس من ذلك الثمار عديدة البذور غير مصابة بالبكتريا المسببة لمرض الاخضرار، وكذلك لوحظ ان طعم الثمار من الاشجار الغير مصابة بالبكتريا كان أفضل من طعم ثمار الاشجار المصابة.

التوصية: من نتائج هذه الدراسة يمكن استنتاج ان من بين الاجناس الفردية وكذلك بعض هجن الموالح يوجد عدد من السلالات التي تظهر تحمل كبير لمرض الاخضرار، وكذلك القدرة على التغلب على المرض وأحيانا التخلص من أعراض المرض, وأن هذا التحمل للمرض غير تام الارتباط بقيم عشائر البكتريا (*C*Las) المقدرة. بحث مستقبلى يستخدم نتائج هذه الدراسة فى تنفيذ الانتخاب الوراثى فى عشائر الموالح.