

MINISTRY OF EDUCATION AND TRAINING
THAI NGUYEN UNIVERSITY

HOANG THI THUY

**STUDYING THE AGRO-BIOLOGICAL CHARACTERISTICS
AND SOME CULTIVATION TECHNIQUES FOR SEEDLESS
LINES AND CULTIVARS OF CITRUS FRUIT**

**SUMMARY OF PHILOSOPHY DOCTORAL
DISSERTATION IN AGRICULTURE**

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Supervisor: Asso. Prof. Ngo Xuan Binh, Ph.D

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INTRODUCTION

1. Rationale of the study

The main fruit trees of Vietnam are orange, mandarin, lemon and pomelo trees that have a long history of development and have been planted throughout the ecoregions nationwide. In the past many decades, citrus fruit remains one of the key export products and demand of domestic consumption is very large (Hoang Ngoc Thuan, 2004). The study of development of citrus fruit trees in our country had been officially developed since the 30s of the previous century. Increasingly, domestic and foreign authors are interested in research and development of orange trees and mandarin trees in Vietnam. Many new technical advances have been studied and applied in production. They are studies on growth and development, diversity of genetic resources of orange and mandarin trees; clonal rootstocks and multiplication techniques for orange, mandarin, lemon, and pomelo trees that are free of diseases by multiplication in vitro and micrograft; technical measures for an integrated intensification, prevention and elimination of pests and diseases, etc. However, so far, productivity of citrus fruits in our country generally is much lower than that of some countries in the region and around the world. In terms of quality, there are also many limitations: beautifulless appearance of fruits, high sugar content, but low acid content, although taste quality of some cultivars can be comparable with the world famous cultivars (oranges from Vinh Long, Tien Giang and Ha Giang, mandarins from Bac Son in Lang Son, Da Xanh pomelos). Our country has joined the World Trade Organization (WTO) and is standing on the threshold of free trade agreements with Southeast Asian countries, USA market, Europe market, and markets of alliances of Eurasian countries, the issue of agricultural product quality is a major challenge. Therefore, the study of technical measures to improve productivity, quality of oranges, mandarins, pomelos is an urgent requirement in the current period (Tran The Tuc et al, 1996), etc.

It has been showed a rapid increase in growing area and productivity of fruit trees and also dramatically decrease in area destroyed annually. (Le Thi Thu Hong, 2000). Therefore, it is urgent to sole issues of selection of multiplication citrus fruit trees without disease infected, high quality, suitable for different ecological zones, focusing on seedless cultivars, few seed fruits, etc. Production of citrus

fruit trees in our country have increase rapidly, but it has still faced many difficulties due to the disease and seed quality. Most popular cultivars planted in our country are cultivars that do not meet the needs for using as fresh fruits and fruit processing due to their fruits with low quality and many seeds (Do Nang Vinh, 2005).

The phenomenon creating seedless fruits is based on study of mechanism creating seedless fruits such as male and female sterility features, the self-disharmony feature, embryonic atrophy phenomenon, etc. There is a significant influence of different pollen grains sources on fructification rate, the number of seeds and the productivity and quality of fruits ultimately (Ngo Xuan Binh, 2009).

Although many studies on fruit citrus trees in Viet Nam have carried out, there are not many studies on creating seedless fruits for citrus trees. Thus, it is necessary to study on creating seedless fruits for citrus trees, we implement: *"Studying the agro-biological characteristics and some cultivation techniques for seedless lines and cultivars of Citrus fruit"*

2. Overall objective and specific objective of the study

2.1. Overall research objective

It is to determine agrobiological features as a scientific basis for the study of the mechanism creating seedless fruits, and to intervene with technical measures in order to improve productivity and quality of fruits of citrus trees

2.2. Specific objective

- It is to study agronomic features of studied subjects as a premise for the study of the mechanism creating seedless fruits.

- It is to study the mechanism creating seedless fruit in some experimental lines/cultivars.

- It is to study applications of some technical measures to improve productivity and quality of fruits in some citrus tree cultivars.

3. Scientific and practical significance of the study

3.1. Scientific significance

- Research results of the study will add the scientific literature on the biological characteristics related to a number of lines/cultivars of

citrus genus that is a prospect to plant in Thai Nguyen Province. This contributes to enrich archives about citrus fruits in general in Vietnam.

- The materials from this study have significantly in contributing to the creation of high quality fruits for citrus trees.

- Research results of the study will be the significant reference material in teaching, studying and learning about citrus plants in general.

3.2. Practical significance

- Research results of the study is a scientific basis to solve biological issues of some lines/ cultivars of citrus plants available with seedless feature and propose technical solutions to serve the task of goods in the long run.

- The results of the study are significant in helping gardeners in pure plantation or intercropping plantation to improve productivity and quality of fruits of citrus trees.

- Research results of technical measures are significant in intervene properly the plant period to help improvement of productivity and quality of fruits of citrus plants.

4. New scientific findings of the dissertation

- It determines agro-biological characteristics related to ability to produce seedless fruits of the experimental lines/cultivars.

- It is to identify some technical measures concerning producing seedless fruits of experiment lines/ cultivars.

Overall structure of the dissertation

There are 134 pages, excluding appendices, the dissertation is divided into 5 sections (forewords, page 4, Chapter 1: Literature review, 50 pages, Chapter 2: Contents of research methods, 14 pages, Chapter 3: results and discussion, 69 pages, Section: the conclusions and recommendations, 3 pages. The dissertation includes 39 tables, 6 image and 34 illustrations. There are 95 reference documents, of which, 48 documents are in Vietnamese, and 47 documents are in English.

Chapter 1 LITERATURE REVIEW

1.1. Scientific basis of the research

A phenomenon creating seedless fruit of Citrus trees is explained as following cases, namely, sterility male gametes, sterility female gametes, self-incompatible feature, 3n (triploid), the phenomenon of embryonic atrophy (Ngo Xuan Binh, 2009), etc. In that, the phenomenon of self-incompatible feature often occurs. This phenomenon is not to have fertilization although there is pollination due to the pollen tubes without long existence in pistils (Wakana A., Uemoto S., 1988).

Many scientific studies on the fruit trees (except for fruit seedless cultivars) have shown that there is a significant influence of different pollen grains sources on fructification rate, the number of seeds and the productivity and quality of fruits ultimately (Walter Reuther et al., 1978). In some fruit trees namely persimmon (D. Kaki) that has 2 main breed groups, cross pollination and self-pollination, of which, fruits of the cross pollination group, when self- pollinating, may often fall and may fall off 100% (Chapot H. D., 1975). It implements passive self-pollination for grape, orange and mandarin trees to produce seedless fruits with productivity of high quality (Inoue H., 1990). When pollination experiments with different pollen sources are conducted, hybridization combinations and pollen grainy sources for high productivity and high quality fruits can be determined (Soost R. K. and Burnett R. H., 1961).

To determine the characteristics of sexual reproduction in plants in general and Citrus trees in particular, the study should be focused on following issues such as germination of pollen grains (pollination process); ability to complete the female flowers for reception of fertilization (fertilization process); ability to combine between male gametes and female gametes to form a zygote (fertilization process); the process of fructification and seed creation from sexual embryos (seeding process). Pollination is essential for production of seeds and even in stimulating growth of the ovary in almost seedless cultivars (Ngo Xuan Binh, 2009).

In issues related to sexual reproductive features of citrus trees, we need to concern issues related to seed embryos, process of fertilized pollination and pollen grain features (Ngo Xuan Binh, 2010 and Tran Thi Dieu Linh, 2012).

Survey results indicated that about 95% Nam Roi pomelo garden intercropped with other citrus crops appear seed fruits (Pham Thi Chu, 1996 and Nguyen Huu Dong and et al, 2003). In terms of the appearance of fruits, it is very difficult for us to distinguish the seeded fruits from seedless fruits. This phenomenon appearing more seeds also happened to Da Xanh pomelo and this is caused by cross-pollination (Chapot H. D.,

1975). On some pomelo cultivars of seedless fruits, when there is cross-pollination seed, these cultivars have numerous seeded fruits. It is usually around 100 seeds per fruit (Nguyen Thi Minh Phuong, 2007).

In practical production, technical measures were applied in production farms to intervene biological phenomenon of Citrus genus to improve productivity, quality and economic efficiency. They take advantage of the phenomenon of multiple embryos to produce rootstock source, seeds evenly; self-pollination to create seedless fruits with high yield and good quality in Nam Roi pummelo and Da Xanh pomelo (Do Dinh Ca and Le Cong Thanh, 2006)...; intercropped plantation with other crops cultivars to provide additional pollen, to stimulate cross-pollination for increasing fructification rate and improving productivity and quality, against degradation in Doan Hung pomelo, Phuc Trach pomelo and Dien pomelo (Pham Thi Chu, 1996 and Vu Viet Hung, 2011), etc.

Therefore, we need to study and explain the causes of lines/cultivars of Citrus genus with seeded fruits or seedless fruits. It is also to find out applications of measures to improve productivity and quality of fruits by intervening processes on pollination, fertilization, seeding production from different pollen grain sources.

Chapter 2

MATERIALS, CONTENTS AND METHODS

2.1. Location, materials and scope of the study

2.1.1. Studied location

Tuc Tranh Commune, Phu Luong District, Thai Nguyen Province.

2.1.2. Research Materials

It is to study on 19 lines/cultivars of Citrus trees (pomelos, oranges, mandarins) from material source selected to create cultivars. It including special cultivars of Vietnam's Phuc Trach, Da Xanh, Nam Roi, Red pummelos, Xa Doai and Tuyen Quang oranges, Duong Canh and Bac Kan mandarins and 11 lines (pomelos and oranges) created by the Faculty of Biotechnology and Food Technology, University of Thai Nguyen. These trees have planted since 5-6 years and stabilizing fruits.

2.1.3. Research scope

The study has implemented from 2011 to 2012

2.2. Contents of the study

2.3. Methods of the study

2.3.1. Studied method on agronomic features

2.3.1.1. Method of experimental arrangement

2.3.1.2. Monitoring indexes

2.3.1.3. Leaf monitoring indexes

- Measuring length of leaves, width of leaves, petiole (cm):

2.3.1.4. *Flower monitoring indexes*

Quantity of stamens and petals:

Indexes monitoring yield, fruit and fruit characteristics:

- The number of fructification per tree:
- The volume of edible parts (weight of edible fruit elements):
- Shape, size, diameter and height of fruits, number of pieces of citrus fruits, number of seeds, etc.

- The sensorial evaluation: Total score = 100 (Hoang Ngoc Thuan, 2004).

- It is to analyze ingredients (performed at laboratory of Faculty of Agronomy - Thai Nguyen University of Agriculture and Forestry)

It is to measure Brix: Total sugar content (%): total acid content (%): Vitamin C.

2.3.2. *Research methods on biological characteristics related to creating seedless fruits in some experimental lines/ cultivars.*

2.3.2.1. *Research methods on features of multiple embryo phenomenon of some experimental lines/cultivars.*

- *Research methods*

2.3.2.2. *Research methods on biological characteristics related to create seedless fruits in some experimental lines/cultivars.*

Research methods is based on Japanese research method of crossbreeding of Citrus cultivars.

a, Research methods on biological characteristics related to male sterility feature of Citrus trees include as follows:

- The method for determinating germination of pollen grains:
- The method for determinating flowers with malformed anthers:

At the time of blooming flowers, it is to harvest flowers, to observe each filament of each flower and deformed anthers, to determine the percentage of flowers available malformed anthers.

- Method for determining malformed pollen grains:

Flowers at 1-2 days before blooming are harvested and removed petals and anthers are put in petri dishes in temperature laboratory (25°C), while anthers bloom, it is to observe pollen grain morphologies on microscope and to determine the rate of deformed pollen grains.

b, Method for collecting pollen grains

Pollen grains are collected from flowers about to bloom, before the 1-2 days of blooming flowers, it is to collect flowers, to open artificial petals and then place flower in a petri dish covered to ensure ventilation. The petri dish with flower is placed in laboratory conditions (25 °C) or a cool place to prevent from infection of strange pollen grains. It is until the anther blooming to implement direct pollination or or stored in cold temperatures to pollinate later

c, Methods of pollination

- Methods of self-pollination and natural pollination:

According to the method of pollen grains (Ngo Xuan Binh, 2001), it is to select flowers 1-2 days before blooming flowers, then to open the petals on a Petri dish for anthers blooming in room temperature conditions. Pollination is conducted immediately after anthers blooming; cross-pollinating: choose flowers of mother plant about to bloom or 1-2 days before blooming, then remove the petals, pollination is carried out by slightly sweeping on anthers bloomed of father trees on heads of pistils of flowers of the mother trees removed anthers. After pollination, pollinated flowers are isolated by wrapping in dedicated breeding bags. With formula of natural pollination, mark flowers to follow up and pollinate naturally. With formula of self-pollination, use pollen grains (flowers of father trees) and female flowers (the mother trees) on the same trees.

- Methods of elimination of male and pollination:

According to the method of pollination with flowers on trees needed to pollinate (Ngo Xuan Binh, 2001), it is to select flowers about to bloom or preferably 1-2 days before blooming, then remove the petals, eliminate male by removing the anthers. After that it is to implement artificial pollination by slightly sweeping on anthers bloomed of father trees on heads of pistils of flowers needed to pollinate, then pollinated flowers are isolated immediately by wrapping in dedicated breeding bags. After 12-15 days, these isolated bags are removing and bred flowers are marked for monitoring. Flowers, after emasculation of perianth and without conditions for pollinating immediately, are isolated by dedicated bags above. By this method, flowers can pollinate well in 2-3 days after that.

After flowers of fructification and ripening fruits, it is to evaluate fructification rate, the number of seeds of each combination of pollination.

d, Method for determining pollen grain germination rate.

Pollen grains of father trees is collected as described above, then cultured and identified the germination rate by the method of quick count (Ngo Xuan Binh, 2001).

Medium was prepared in sterile conditions (one liter of medium is mixed with 20 mg boric acid, 20 g sugar and 7 g agar; the medium mixed, boiled, poured in thin petri dishes approximately 1 - 2 mm).

Pollen grains are sowed by slightly sweeping anthers bloomed on culture medium (in Petri dishes), and then Petri dishes are sealed to prevent dehydration. after the 8-24 hours, cultured dishes are observed by a microscope: randomly mark on the pollen parts that can be counted on the medium, count 3 times per point for total pollen grains and pollen grains of germination, calculate the medium value on average. the total pollen grains counted must is greater than 1000 seeds.

- Methods of observation of mechanism of fertilization pollination through observation of pollen tube growth in the female flowers (pistil and ovary): it is conducted by the method of (Ngo Xuan Binh, 2001). Flowers pollinated after 8 days shall be collected samples. Growth of pollen tubes is observed by fluorescence microscopy at wavelength between 260 nm and 360 nm. It is to count numbers of pollen tubes in different parts of the pistils.

- Method cutting flowers to observe the growth of pollen tubes: pollinated flowers are collected and treated in acetic acid with ethanol (ratio of 1 to 3). After 24-48 hours, they are washed and stored in ethanol 70% at 4°C until use. When observing, each pistil is cut into five sections (as the following table): the stigma, style cut into 3 sections (upper, middle and bottom segments of style) and ovary. Samples were softened by immersion in sodiumhydroxide 0.6 to 0.8 N for about 24 hours at room temperature, rinsed with distilled water and stained with 0.2% aniline blue in 0.1M potassium phosphate about 24 hours at room temperature. Then these samples are observed by fluorescence microscopy at wavelengths between 260 and 500nm.

Pistil (including ovary) is cut to 5 separate sections from top (the stigma) to bottom (ovary) to observe the growth of pollen tubes in each section.

+ Self-pollination: tracking of the number of fructification/number of monitored flowers, fruit weight (g), number of seeds/fruit (big seeds, small seeds and floaters, the total number of seeds).

+ Cross pollination: tracking of the number of fructification/number of monitored flowers, fruit weight (g), number of seeds/fruit (big seeds, small seeds and floaters, the total number of seeds).

+ Comparing fruit weight of difference of 3 methods of self-pollination, emasculation of perianth and flower envelope, natural pollination, the other pollen grain sources.

Control is the formula for natural pollination for contents.

- Indexes of study of the processes of pollination and fertilization through the observations of growth of pollen tubes in pistils.

- The number of pollen tubes in the positions: top of the pistil (stigma); the upper one-third of the style; the middle two-third of the style; the lower one-third of the style and ovary.

- Experimental indexes of capability of fructification and seeds of experiments of perianth (to prevent from the cross-pollination with fruit formation). Percentage of fructification, number of seeds/fruits, fruit volume above.

e, Research method of preservation of pollen grains

Flowers of experimental plants are harvested when starting or prepare for blooming (1-2 days before flowering). Flowers are preserved

in cold temperatures 5°C. It is to rate capability of germination of pollen grains after the intervals of 10, 20, 30, 40 and 50 days.

f, Method of determination of the coefficient reflecting ability to produce seedless fruits (P)

The coefficient reflecting ability to produce seedless fruit (P) is determined by the method of Ngo Xuan Binh and Wakana Akira.

The formula $P = A/B \times C/D \times 100$.

- Coefficient in the formula of perianth: A is percentage (%) of fructification of the formula of perianth; B is percentage (%) of fructification of the formula of natural pollination; C is weight of fruits of the formula of perianth; D is weight of fruits of the formula of natural pollination.

- Coefficient in the formula of male elimination and perianth: A is percentage (%) of fructification of the formula of male elimination and perianth; B is percentage (%) of fructification of the formula of natural pollination; C is weight of fruits of the formula of male elimination and perianth; D is weight of fruits of the formula of natural pollination.

- Coefficient (P): 0-10: very low; 10 - <25: low; 25- <40: medium; 40 - <60: High; > 60 very high.

2.3.3. Research on some technical measures to improve productivity and quality in a number of experimental lines /cultivars

2.3.3.1. Research on influence of technique of perianth and without perianth on the yield and quality of some experimental lines /cultivars.

Experiments with two formulas: Formula 1: perianth; Formula 2: without perianth (natural pollination).

Research method:

Monitoring indexes:

- Ratio of fructification = Number of fructification to number tracking flowers.

- The volume of fruits (g): Weigh number of fructification and calculate the mean value.

- The number of seeds/fruit: Count the seeds of fruits of fructification and calculate the mean value.

2.3.3.2. Study of the effect of GA3 on yield and quality of fruits in the experimental lines/cultivars when perianth.

Choosing trees for the experiment:

Spraying formula: there are four formulas:

Methods of GA3 treatment

Number of times and pointimes of drug treatment: drug is treated 3 times, the first time 10 days before followers blooming, the 2nd time when flowers blooming and the 3rd time after 10 days of flower remnants.

Spray wetly the entire young fruit bud clusters.

Spray in the afternoon coolly.

Indexes and monitoring methods: Fructification rate (%), average fruit weight (g/fruit), Number of seeds per fruit (seed):

2.3.3.3. *Study of the effect of GA3 on fruit productivity of some experimental lines/cultivars when natural pollination*

Choosing trees for the experiment:

Spraying formula: there are four formulas in the experiment:

Methods of GA3 treatment

Fructification rate (%), average fruit weight (g/fruit), Number of seeds per fruit (seed): Calculating number of seeds in mean value

2.3.3.4. *Research on influence of some foliar fertilizers on yield, quality of fruit in some experimental lines and cultivars when natural pollination.*

Choosing trees for the experiment (2.3.3.3).

Spraying formula: there are four formulas in the experiment:

Treatment 1: white mouse foliar fertilizer 209:

Treatment 2: fertilizer Thanh Ha, KH:

Treatment 3: Foliar fertilizers Gibb-1 (GA3):

Treatment 4: without spraying (control)

Tracking indexes

- The fructification rate (%), fruit weight in average (g/fruit), number of seeds/fruit (seeds)

2.4. Methods of treatment of the data

The data is treated by: IRRISTAT, SAS, Excel and the mathematical statistical methods.

Chapter 3 RESULTS AND DISCUSSION

3.1. Agronomic characteristics of some experimental lines/cultivars

- Evaluation of polyploidy of some lines/cultivars experiment.
 - Morphological characteristics of stems, branches of the experimental lines/cultivars (Table 3.2). Morphological characteristics of leaf set (Table 3.3). Features of flowers (Table 3.4). Characteristics of fruits of the experimental lines/cultivars (Table 3.5). Growth characteristics: The reproductive cycle in years of some experimental lines/cultivars (Table 3.6: flowering characteristics of of some lines/cultivars (Table 3.7): Yield of fruits of a number of experimental lines/cultivars (Table 3.8) and (Table 3.9)/2012. Several indicators of fruits of the experimental lines/cultivars of Citrus genus (Table 3.10)/2011 and (Table 3.12)/2012. Assessing the fruit quality of the

experimental lines/cultivars: (Table 3.12) Results of biochemical analysis results of a number of the experimental lines/cultivars in 2012 showed that these lines /cultivars are rich in nutrients and vitamins.

From Table 3.1 to table 3.12, it is showed that growth, development, fructification and productivity of the experimental lines /cultivars are normal and stable.

3.2. Assessment of some biological characteristics relevant to the ability creating seedless fruits of the experimental lines / cultivars

3.2.1. Research results of multi embryonic phenomenon of some experimental lines/cultivars

3.2.1.1. Rates of single embryo seeds and multiple embryo seeds of some experimental lines /cultivars.

Research results of rate of multiple embryo seeds in the experimental lines/cultivars are presented at the table 3.13 and the table 3.14: through study on multi-embryo ratio in two-year duration, it is showed that 100% seeds of the experimental lines/cultivars of pomelos is the single embryo seeds. Contrary to Mandarin (in 2011) that has a very small proportion of single embryo seeds (1%). However, in the year (2012), this variety has 100% of multiple embryo seeds. the lines /cultivars of oranges (TN1, TN6, TN12, TN13, XB-6, Xa Doai and Tuyen Quang oranges) has a ratio of single embryo seeds, the rest are multiple embryo seeds. Bac Kan mandarin is 100% of the multiple embryo seeds for all 2-years of research.

In multiple embryos seeds, cloned embryos germinated for better trees and tends to more similar to the mother trees, therefore, when crossbreeding, their seedlings are often weak and died causing difficult to choose creation and take care hybrid seedlings. These cultivars should apply only as rootstocks in seedling production. However, the single embryo cultivars (embryos created from process of fertilized pollination) are capable of sexual reproduction. Therefore, they can be used as mother trees in breeding plants.

3.2.1.2. The ability to bring multiple embryos seeds of some experimetal lines/cultivars

Table 3.15. The number of embryos/seed of the experimental lines/cultivars in 2011. Table 3.16. The number of embryos/seed of the experimental lines/cultivars in 2012. The number of embryos/seed ranged from 1.07 to 2.8 embryos/seed (2011) and 1.06 to 2.40 embryos/seed (2012). Number of embryos/seeds of different lines/cultivars of oranges and mandarins is ranged from 2-3 embryos/seed. Therefore, study of the multiple embryo phenomenon of the experimental lines/cultivars and some documents show that the higher ratio of embryos it is, the weaker capability of sexual reproduction it is.

3.2.2. Research results of biological characteristics related to male sterility ability of the experimental lines/cultivars

3.2.2.1. The germination of pollen grains of the experimental lines/cultivars at flowering period.

Experimental results presented in Table 3.17 shows that pollen grains of the lines/cultivars germinate at different levels. Pollen grain germination rates of four lines (XB-130, TN1, TN6 and TN13) are very low rates. This means that that lines have male sterility feature because the most pollen grains can not afford to germinate.

3.2.2.2. Anther features of some experimental lines/cultivars at flowering point

Results at the following table 3.18 and 3.19 show that in 2011, 3 lines of TN1, TN6 and TN13 bearing malformed anthers account for 100%, 98% and 100%. Followers observed of other lines/cultivars have not malformed anthers. It is not observe flowers bearing malformed anthers of three lines of TN1, TN6 and TN13 in 2011 and continued to have them in 2012 account for 100%, 94.7% and 100%, respectively. Other lines/cultivars have the same results as in 2011. This means that it is not detect flowers bearing malformed anthers. This suggests that flowers bearing malformed anthers have genetic factors (table 3.18).

3.2.2.3. Research on opening characteristics of anthers in the experimental lines/cultivars

Research results on proportion of malformed anthers in 2011 are shown in Table 3.19. Malformed anthers have only 03 lines, including the TN1, TN6 and TN13, with a relative high rate. Through the investigation in 150 flower of each line, it indicates that rates of malformed anthers of TN1, TN13 and TN6 lines are 64.6, %, 67.5% and 72.8%, respectively. Other lines/cultivars observed have not malformed anther.

Data in the tables 3.20 on research results of rates of malformed anthers in 2012 showed that rates of malformed anthers are only to repeat at TN1, TN6 and TN13 lines observed availability of malformed anthers in 2011 and account for 73.2%, 62.1% and 87.5%, respectively. So, rates of germination of pollen grains in the lines/cultivars (XB-130, TN1, TN6 and TN13) are very low, there are 3 lines (TN1, TN6 and TN13) that have very high rate of malformed anthers. Usually, morphology of finishing anthers in Citrus trees is oval mass with two proportionate heads. Morphology of malformed anthers is abnormal deformation, two disproportionate heads and indefinite shape. Also, observe results also showed that there is duplication of lines (TN1, TN6 and TN13) that have very high rates

of malformed anthers. When flowering, anthers do not completely open, with a certain ratio of unopened anthers. This also means that the ability to extricate pollen grains during pollination of these lines is very low. Rates of malformed anthers on the possibility of repeated opening of anthers in three lines also are observed continuously in two years of study. That shows that foregoing properties are affected significantly in elements of genetics.

3.2.3. Results of evaluation of the morphological characteristics of the pollen grains of experimented lines and cultivars

The results are shown in table 3.21, the experimental line/cultivars have certain percentages of malformed pollen grains. In 2011, the rates of malformed pollen grains are ranged from 1.6% to 81.5%, of which, 3 lines of TN1, TN6 and TN13 have the highest rates of malformed pollen grains and account for 78.3%, 81.5% and 62.3%, respectively. It is similar in 2012, 3 foregoing lines still have the highest rates of malformed pollen grains and account for 87.4%, 71.8% and 69.1%, respectively. Foregoing lines having the highest rates of malformed pollen grains are lines that have low rates of pollen grain germination and very high rates of flower available malformed anthers and malformed anthers (tables of 3.17, 3.18, 3.19 and 3.20).

The study results above showed that in the experimental line/cultivars of Citrus trees, rates of pollen grain germination of 3 lines of TN1, TN6 and TN13 are very low, namely: TN1 (2.71% in 2011 and 1.15 % in 2012), TN6 (1.48% in 2011 and 1.13% in 2012), TN13 (0.79% in 2011 and 0.66% in 2012). Rates of these lines available malformed pollen grains and flowers bearing malformed pollen grains are very high; these lines are identified as incomplete male sterility lines. Other lines and cultivars have not male sterility features. The rate of low pollen germination of XB-130 is low, because this is triploid line, process of division of chromosomes to form gametes (pollen grains) is disordered leading to loss of pollen grain germination capacity. Research results show that 3 male sterility lines above can be used as crossbreeding materials or continues to select creation of new cultivars.

3.2.2.4. Research on preservation of pollen grains of the experimental lines/cultivars

It is observation of rates of pollen grain germination of 19 lines/cultivars of pomelo, oranges and mandarins involving in the experiment after certain period of preservation at 5⁰C. Results obtained at table 3.22 shows that rates of pollen grain germination of the lines/cultivars reached the highest rate in a 10-day period and decrease rapidly over storage duration of 20 and 30 days. Specifically, rates of pollen grain

germination of Phuc Trach pomelo at flowering time, after 10 days, 20 days, 30 days, and 40 days of preservation are 24.95%, 18.3%, 9.8%, 1.2% and 1.2%, respectively. Pollen grain germination of different cultivars of Da Xanh, Nam Roi, Red pummelos and other lines of 2X-B, TN3, TN4, TN5, TN15, TN12 are similar rates. However, rate of pollen grain germination of XB130 at the flowering time is 0.02%, its capacity of the pollen grains germination completely loses after 10 days. That of oranges and mandarins is similar, germination capacity decreases with preserved time. However, pollen grains of three lines (TN1, TN6 and TN13) have germinated at the flowering time. After 10 days of preservation, there is no longer capacity of germination.

Therefore, the study showed that capacity of germination of the lines/ cultivars is good at flowering time and decreases gradually after 10 days, 20 days and 30 days of preservation. After 40 days of preservation, the pollen grains of the lines/cultivars ceases to germinate. Capacity of germination of XB130, TN1, TN6, TN13 lines/cultivars is low; they are not to germinate after 10 days of preservation. In crossbreeding, we should use the pollen grain source stored at 5⁰C in a duration as short as possible.

3.2.4. The research results on seedless fruit creating characteristics related to self-incompatibility of the experimental lines/ cultivars

3.2.3.1. Assessment of capability creating seed of the experimental line/cultivars s in natural conditions (natural pollination)

Table 3.23 and table 3.24 show that the lines/cultivars when pollinating naturally (natural conditions) are also to have seeded fruits. Number of seeds of lines/cultivars are different. The lines/ cultivars are often to have many seeds in fruits. It is only two lines of pummelos TN3 and triploid line (XB130) available less seeds. Orange lines (TN1, TN13) have less seed. Other lines/cultivars of oranges and mandarins have numerous seeds.

3.2.3.2. The ability of fertilization through observing the pollen tube growth in the pistils of experimental lines/cultivars with different pollen grain sources

Table 3.25 shows that at the stigma (SM), a huge amount of pollen grain germinates and creates pollen tubes grown in the pistil. Pollen tubes continue growth towards ovary (towards the ovule). In complex of pollination, pollen tubes at the upper style (US) are numerous, while pollen tubes in self-pollination complex is the fewest number (Phuc Trach pomelo available 195 pollen tubes). The number of pollen tubes in the complex of natural pollination gains 351 (US) for Phuc Trach pomelo. The number of pollen tubes at the middle style (MS) is 10.3 for

self-pollination of Phuc Trach pomelo and 302 for natural pollination of Phuc Trach pomelo. There is not any pollen tube at low style (LS) for self-pollination of Phuc Trach pomelo. In formula of natural pollination of Phuc Trach pomelo, there are 278 pollen tubes. This also means that there is not process of fertilization occurring to pollinate in perianth, pollen tubes have to reach from 206 to over 500 in number with formula of natural pollination and cross pollination with other pollen grain sources. It is similar to Phuc Trach pomelo, other lines/cultivars observed also have the same results.

The table 3.26 shows that after one day of pollination, it is not to see the growth of pollen tubes in the pistil of combination of self-pollination. In combination of self-pollination, there is a majority of pollen tubes stopping growth at the top pistil and stamen after from 1 to 6 days of pollination and 100% of the pollen tubes does not grow to the ovary. In which, the pollen tubes of combination of self-pollination of pomelos of Phuc Trach, Red, Nam Roi, Da Xanh, 2XB, XB13 and TN13 stop to grow altogether at the segment from the stigma to the middle one-third of the style (MS) after 6 days of pollination. Pollen tubes of combination of self-pollination of Da Xanh pomelo stop to grow more quickly (after 4 days of pollination) and also stop growth at the upper one-third of the style (US).

Experimental results on pollen tube growth in the pistil show that formulas of self-pollination of pomelos (including Phuc Trach x Phuc Trach; Red Pummelo x Red Pummelo; Da Xanh x Da Xanh; Nam Roi x Nam Roi; 2X-B x 2X-B; TN13 x TN13) have numerous pollen tubes observed at the stigma (SM; > 1000 pollen tubes) and not any pollen tube at the low one-third of the style (LS) and ovary (OV). This shows that the protein S gene can concentrate more in the stigma and the style parts. It acts to inhibit, prevent the pollen tubes to grow (carrying male gametes) towards ovule in the ovary. In the the formula of self-pollination, there is not the process of self-fertilization occurring.

When self-pollinating, pollination process takes place in the pistil of experimental lines/cultivars and pollen tubes germinate and grow. However, after 4-6 days of pollination, the pollen tubes (bearing male gametes) are inhibited and stop growth at the segment from the stigma to the middle one-third of the style (MS). Thus, the line/cultivars of self-pollination pomelos create seedless fruits or have low fructification rates because self-incompatible feature controls of fertilization process (Table 3.27. The number of pollen tubes in the pistil of combinations of cross pollination in 2012)

Results of observations of the growth of pollen tubes of some lines/cultivars (pomelos, oranges and mandarins), when conducting cross-pollination, are follows: The combinations of cross-pollination studied are also to have many pollen tubes growing to the ovary. After 8 days of pollination, number of pollen tubes in the style and ovule of combinations of cross-pollination often reaches > 200. In which, the pollen tubes of combinations of pomelos of Phuc Trach x Da Xanh; Red Pummelo x Phuc Trach, Da Xanh x Nam Roi, Nam Roi x Phuc Trach, 2X-B x Red Pummelo, XB130 x Da Xanh, TN13 x Duong Canh mandarin show that pollen tubes are growth to the ovary after 6 days of pollination. Growth of pollen tubes of some combinations is more quickly. It is only 4 days after pollination, pollen tubes seen appearing in the ovary (OV) are combinations of pomelos of Phuc Trach x Da Xanh, Nam Roi x Phuc Trach.

Thus, through observation, pollen tubes of combinations of cross-pollination of experiments are rapid growth and prolonged into perianth after 4-6 days of pollination to make the process of fertilization.

3.2.3.3. Research on the influence of pollination on fructification rates, fruit weight and seed number/fruit.

Table 3.28 and 3.29 table have a significant difference in fructification rate between lines/cultivars:

One of the characteristics of Citrus trees is self-incompatible feature that can be demonstrated feature against inbreeding when there are good pollen grains, good ovule, good pollination but without fertilization process. While pomelos have 100% of self- incompatible feature. But characteristic of some types of Citrus trees has no fertilization process, fruits are still big because of availability of their endogenous auxin. In some lines/cultivars, endogenous auxin is only formed after fertilization. Therefore, the lines/cultivars having this feature required fertilization occurring, endogenous auxin created help growth of fruits. It is to study the lines/cultivars of pomelos and oranges of self-pollination. They create seedless fruits or have low fructification due to self-incompatible feature to control the fertilization process. Research shows that 12 in 19 experiemental lines/cultivars having self-incompatible feature are pomelos of Phuc Trach, Da Xanh, Nam Roi, Red pomelo, 2X-B , TN3, TN4, TN5, TN15, XB130, TN1 and TN13. When self-pollinating, they create seedless fruits.

In remaining lines/cultivars of oranges and mandarins. 7 in 19 lines/cultivars have compatible feature. They are TN6, TN12, XB-6, Xa Doai orange, Tuyen Quang orange, Duong Canh mandarin, Bac Kan mandarin when self-pollination, they create high fructification rate, and seeded fruits.

Assessment of capability carrying seedless fruits in experimental lines/cultivars related to self-incompatible feature (2011) and (2012)

Two tables 3.30 and 3.31 show that the ability to produce seedless fruit of the lines/cultivars is as follows:

- There are 6 lines/cultivars of pomelos (including Da Xanh, Nam Roi, 2X-B, TN4, TN5 and XB130). Through 2 years of research, results in assessment of capability creating seedless fruits are "high to very high rate".

- The lines/varieties of pomelos (including Phuc Trach, TN3 and TN15) rated is not ability of fructification and if any, fructification rate are very low and not ability of creation of seedless fruits.

Assessment of ability creating seedless fruits of the lines/cultivars of oranges and mandarins as follows:

- There are two orange lines TN1 and TN13 are two lines that have self-incompatible feature. Therefore, when flower envelope, emasculation of perianth and flower envelope have the self-pollinated process, due to self-incompatible feature there is no fertilization process. Producing seedless fruits: data shows that capacity of carrying seedless fruits of two these lines rated is "high to very high" when they are self-pollination and flower envelope.

- The lines/cultivars of oranges and mandarins include seven lines/cultivars (TN6, TN12, XB-6, Xa Doai orange, Tuyen Quang orange, Duong Canh mandarin, Bac Kan mandarin). They are the lines/cultivars having the self-compatible feature. When perianth is not emasculation of perianth, processes of pollination and fertilization still occur normally, and fructification rate and fruit weight equivalent to cross-pollination, when flower envelope, fruits still have seeds.

- Seven lines/cultivars of oranges and mandarins (including TN6, TN12, XB-6, Xa Doai orange, Tuyen Quang orange, Duong Canh mandarin, Bac Kan mandarin) have the self-compatible cultivars. Therefore, when having emasculation of perianth and flower envelope, they do not have capable of carrying seedless fruit. 100% of flowers available emasculation of perianth and flower envelope fall off, it is not capable of carrying seedless fruits.

3.3. Research on some technical measures to improve fruit yield and quality in a number of experimental lines/cultivars

3.3.1. Research on influence of technique of flower envelope and without flower envelope on the yield and quality of some experimental lines/cultivars

Table 3.32 shows that when self-pollination and cross-pollination, lines/cultivars produce different results on fructification rate, fruit weight and number of seeds/fruit:

Technique of flower envelope prevents from infection of strange pollen grains, pollen grains of that flower are self-pollination for that flower. This shows that if self-pollination, some lines/cultivars have low fructification rate and reduction of fruit weight and seedless fruits (including Phuc Trach, Red Pomelo, TN3, TN15). Other remaining lines/cultivars are Da Xanh, Nam Roi, 2X-B, TN4, TN5, XB-130, when self-pollinating for fructification rate, fruit weight equivalent to those at the experiment without flower envelope (natural pollination), creating seedless fruits. The lines/cultivars of mandarins and oranges have two lines (TN1, TN13) when flower envelope for fructification rate, fruit weight and number of seeds equivalent to technique without flower envelope and creating seedless fruit. Other lines/cultivars of oranges and mandarins (including TN6, TN12, XB-6, Xa Doai orange, Tuyen Quang orange, Duong Canh mandarin, Bac Kan mandarin) when flower envelope, produce fructification rate, fruit weight and number of seeds equivalent to results of technique without flower envelope.

From this experiment, it concluded as follows:

- The lines/cultivars (Da Xanh, Nam Roi, 2X-B, TN4, TN5, XB-130, TN1, TN13) with flower envelope produce high fruit rate and seedless fruits. It is to recommend that in production, lines/cultivars planted purely or enveloped flowers will produce seedless fruit.

- The lines/cultivars (Phuc Trach, Red pomelo, TN3, TN15) produce low fructification rate and seedless fruits. In order to reach high-yield, they should intercrop with cross-pollinating crops.

- The lines/cultivars of Citrus genus (TN6, TN12, XB-6, Xa Doai orange, Tuyen Quang orange, Duong Canh mandarin, Bac Kan mandarin) are the lines/arieties of self-incompatibility, when monoculture or intercropped plantation, they still produce high yield.

3.3.2. Research on the influence of GA3 on fruit yield of some experiment lines/cultivars when flower envelope with spraying and flower envelope without spraying

The experiment showed (table 3.33) when spraying GA3 for some experimental lines/cultivars of pomelos and oranges when flower envelope. This is significant for monoculture plantations. If spraying GA3 applied at various stages of the experiment, it increases fructification rate and fruit weight of some experimental lines/cultivars. The results are as follows: Phuc Trach pomelo when flower envelope without spraying gains 0.67% of fructification and fruit weight by 380g/fruit. When spraying GA3 with a 40ppm and 50ppm concentration, fruiting rate of Phuc Trach pummelo increases 1.3%, this is 0.63% of difference. Fruit weight

increased 430g/fruit sprayed at a concentration of 50 ppm. The difference between formula of flower envelope with spraying and flower envelope without spraying is 50g at a concentration of 50 ppm.

Fruiting rate and fruit weight of Da Xanh pummelo increased respectively 0.7% and 51g comparing with the formula without spraying. Fruiting rate and fruit weight of Nam Roi pummelo increased respectively 1% and 48g comparing with the formula without spraying. Fruiting rate and fruit weight of TN1 line increased respectively 1% and 60g comparing with the formula without spraying. Fruiting rate and fruit weight of TN13 line increased respectively 0.7% and 50g comparing with the formula without spraying.

It can say that when spraying GA3 (50 ppm), fruiting rate and fruit weight of the lines/ cultivars of pomelos and oranges increased respectively 0.7-1% and in range of 40 - 60g with seedless fruit.

This is very consistent with the results of previous studies and has concluded that "GA3 proven has a good effects in the increase of fruiting rate for the cultivars available fruiting gene producing seedless fruit self-incompatible genotype when there is no cross-pollination".

3.3.3. Research on the effect of GA 3 with the productivity and quality of fruits of some experimental lines/cultivars when pollinating naturally

At the experiment of spraying GA3 (Table 3.34), the open-pollinated plants sprayed GA3 at the concentration of 30ppm, 40ppm and 50ppm increased fruiting rate and fruit weight. Fruiting rate and fruit weight increase maximum at the concentration of 50ppm. Specifically, fruiting rate and fruit weight of Phuc Trach pomelo with formula with spraying increased respectively 0.3% and 58g comparing with the formula without spraying. Fruiting rate and fruit weight of Da Xanh pomelo increased 0.3% and 44g, respectively. Fruiting rate and fruit weight of Nam Roi pomelo increased 0.3% and 49g, respectively. Fruiting rate and fruit weight of TN1 Line increased 0.7% and 48g, respectively. Fruiting rate and fruit weight of TN6 Line increased 0.4% and 16g, respectively. Fruiting rate and fruit weight of TN3 Line increased 0.6% and 30g, respectively. In summary, when spraying GA3 (50 ppm), fruiting rate and fruit weight of the experimental lines/cultivars increased respectively in range 0.3-0.7% and 16-58g comparing with the formula without spraying.

In terms of number of seeds /fruit, number of seeds/ fruit of Phuc Trach pomelo at the formula of natural pollination is 105. When spraying GA3 at concentrations of 30ppm, 40ppm and 50ppm, results of seeds are 52.5 seeds/ fruit, 44.5 seeds/fruit and 32.5 seeds/fruit, respectively. Number of seeds/fruit of Da Xanh pomelo when using GA3 is from 20.5 and 48.5. Number of seeds/fruit of Nam Roi pummelo is from 28 to 49 comparing with the formula without spraying (96.2 seeds / fruit).

In terms of number of seeds/fruit of orange lines when spraying GA₃, number of seeds/fruit of TN1 line is in range of 4.0 and 4.4 comparing with natural pollination (6.8 seeds/fruit). Number of seeds/fruit of TN6 line sprayed with GA₃ is in range of 7.6 and 9.0 comparing with natural pollination (13.8 seeds/fruit). Number of seeds/fruit of TN13 line sprayed with GA₃ is in range of 3.8 and 4.6 comparing with the formula without spraying (7.0 seeds/fruit).

By studying spraying GA₃ with various concentrations, the number of seeds /fruit reduced significant. The results showed that if spraying GA₃ for lines/ cultivars of pomelos and oranges, number of seeds/fruits will reduce and increase fruit quality.

When spraying with growth regulators, it is not only to accelerate the process of plant growth and development, but also reduces the formation of the left floor, ensuring transport of nutrients for fruit, thereby rate of loss fruit reduce and productivity and quality of fruit increase.

Fruiting rate of the Citrus trees is dependent on many internal and external factors. Low auxin content and growth regulators are one of the basic causes leading to flowers and fruits falling off. Giberellin enhances the fruiting effect of Citrus trees. The effect to enhance fructification is significant and detected in two cultivars with seeded and seedless fruit. Therefore, the addition of growth regulators is necessary and this is one of measures to increase the proportion of fructification. The addition of substances such as growth regulators such as exogenous GA₃ is one of the measures to increase the proportion of fructification.

3.3.4. Research on influence of some foliar fertilizers on yield and quality of fruit in some experimental lines, cultivars when pollinating naturally

Results showed that when using three kinds of foliar fertilizers (table 3.35) with a concentration according to manufacturers and dosages mentioned on packages. If handled three consecutive times, with different pointtimes, fruiting rates, fruit weight, number of seeds/fruit at three formula are also higher than comparing with the control group with natural pollination and without spraying. Fruiting rates, fruit weight of Phuc Trach pummelo sprayed with foliar fertilizer increased respectively 0.4%, in range of 927.5g and 968g comparing with the control group and without spraying (910g). Number of seeds/fruit of the pomelo sprayed with foliar fertilizer is

in range of 31.5 and 34 comparing with the control group and without spraying (105 seeds/fruit).

Fruiting rates, fruit weight of Da Xanh pummelo sprayed with foliar fertilizer increased respectively 0.3%, in range of 1300.1g – 1323.3g comparing with the control group and without spraying (1279g). Number of seeds/fruit of the pomelo sprayed with foliar fertilizer is in range of 20.5 and 28 comparing with the control group and without spraying (96.2 seeds/fruit).

Fruiting rates, fruit weight of Nam Roi pummelo sprayed with foliar fertilizer increased respectively 0.3%, in range of 845.3g – 867.1g comparing with the control group and without spraying (816g). Number of seeds/fruit of the pomelo sprayed with foliar fertilizer is in range of 21 and 29.3 comparing with the control group and without spraying (96.2 seeds/fruit).

Fruiting rates, fruit weight of TN1 line sprayed with foliar fertilizer increased respectively 0.7%, in range of 218.2g and 263.4g comparing with the control group and without spraying (213g). Number of seeds/fruit of the line sprayed with foliar fertilizer is in range of 2.0 and 2.4 comparing with the control group and without spraying (6.8 seeds/fruit).

Fruiting rates, fruit weight of TN6 line sprayed with foliar fertilizer increased respectively 0.4%, in range of 330.1g and 340.5g comparing with the control group and without spraying (323g). Number of seeds/fruit of the line sprayed with foliar fertilizer is in range of 3.0 and 3.3 comparing with the control group and without spraying (18.3 seeds/fruit).

Fruiting rates, fruit weight of TN13 line sprayed with foliar fertilizer increased respectively 0.6%, in range of 301.3g – 324.3g comparing with the control group and without spraying (295.0g). Number of seeds/fruit of the line sprayed with foliar fertilizer is in range of 2.2 and 2.8 comparing with the control group and without spraying (7.0 seeds/fruit).

Thus, when nutrition supplements through leaves, fruit rate, productivity and quality of fruits of Citrus trees.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

1. In terms of agronomic characteristics of the experimental lines/cultivars, it has showed that

- 19 experimental lines/cultivars are capable of growth and flowering with normal results. Seed fruits are in natural conditions, particularly, lines of XB130, TN1, TN6, TN13 has less seed fruits. The lines/cultivars are rich in nutrients and vitamins.

- Annual growth cycle of citrus lines has underwent four bud sprout phases (Spring, Summer, Fall and Winter sprouts) and one flowering phase. This is significant for gardeners to intervene timely technical measures such as nutritional supplements; fertilizer spray before the blooming flowers, full blooming flowers and after flowering, rates of fructification and productivity of experimental lines/cultivars have increased.

2. In terms of capacity of creation of seedless fruits of experimental lines/cultivars, it is namely

2.1. The phenomenon of multiple embryos

Pomelo fruit has 100% single-embryo seeds, and there is a certain ratio of single-embryo seeds and multi-embryos seeds of the lines/cultivars of oranges and mandarins and this ratio depends on each the year. When multi-embryos seeds are sowed for multiplication, the ability to produce seedless fruits is very high.

2.2. Biological characteristics related to male sterility capability

In the citrus line/cultivars experimented, germination rates of pollen grains of lines of TN1, TN6 and TN13 are very low. Rates of the malformed anthers and flowers bearing malformed anthers of these lines are relatively high; these lines are identified as incomplete male-sterility lines showing incomplete. The other lines/cultivars have not male sterility ability such as XB-130 line having a low germinating rate of pollen grains. The reasons for this is the triploid line, so, the division process of chromosomes to form gametes (pollen grains) is disordered that make pollen grains lose germination capacity. Research results have show that three male sterility lines mentioned above can be used as hybrid materials or continue selectively to produce new cultivars

2.3 The influence of different pollen grain sources to the ability to create and produce fruit seeds:

In term of the line/variety of Pomelo,

Self-pollination produces fructification with a high rate and seedless fruits for 6 lines/ cultivars of pomelos (Da Xanh, Nam Roi, 2X- B, TN4, TN5, XB130). These lines/cultivars, when produced, are not necessary to intercrop.

Self-pollination provides fructification with a low rate and seedless fruits for four lines/cultivars of pomelos (Phuc Trach, Buoi Do, TN3, TN15). These lines/cultivars, when self-pollinated, are to produce seedless fruits. Therefore, in production for these lines/cultivars, it is necessary to use intercropped measure to improve productivity and fruit quality.

When naturally pollinated and pollinated with pollen grains of different sources, fructification rates of 10 pomelo lines/cultivars are equivalent to those in formula of naturally pollinated and seed fruits.

In term of the line/variety of oranges and mandarins,

Self-pollination: There are two lines (TN1, TN13) producing fructification with a high rate and seedless fruits.

Remaining lines/cultivars of oranges and mandarins (TN6, TN12, XB-6, Xa Doai orange, Tuyen Quang orange, Duong Canh mandarin, Bac Kan mandarin), when self-pollinated, produce fructification with a high rate and seed fruits.

When naturally pollinated and pollinated with pollen grain of different sources, orange lines /cultivars produce fructification with a high rate and seed fruits

2.4 Assessment of the ability to bring seedless fruits of experimental lines/cultivars related to the self-incompatible feature

There are two lines of TN1 and TN13 oranges available self-disharmony feature. Therefore, perianth, or male elimination and perianth have pollination process. However, because of self-disharmony feature, it has not the process of fertilization. They produce seedless fruits, through data showing that capable of carrying seedless fruits of these two lines rated is a "high to very high rate" when self-pollinated and eliminating male of perianth.

There are 7 lines/cultivars of oranges and mandarins (including TN6, TN12, XB-6, Xa Doai orange, Tuyen Quang orange, Duong Canh mandarin, Bac Kan mandarin) are these lines/ cultivars with harmony feature, as perianth without emasculation of perianth, pollination and fertilization occur normally. Rates of fructification and the fruit weight are equivalent to those of pollination and perianth with seeded fruits.

Seven lines/cultivars of oranges and mandarins (including TN6, TN12, XB-6, Xa Doai orange, Tuyen Quang orange, Duong Canh mandarin, Bac Kan mandarin) are cultivars with self-compatible feature. Therefore, when emasculation of perianth and flower envelope, they are not capable of carrying of seedless fruit. When emasculation of perianth and flower envelope, 100% flowers have fallen off and without the ability to bring seedless fruits.

3. A number of technical measures to intervene

- The lines/cultivars (including Da Xanh, Năm Roi, 2X-B, TN4, TN5, XB-130, TN1, TN13) have perianth producing fructification with a high rate and seedless fruits. In production, these lines/cultivars planted purely or perianths of flowers produce seedless fruits.

- The lines/cultivars (including Phuc Trach, Buoi Do, TN3, TN15) produce fructification with a low rate and seedless fruits. To get high yield, these cultivars should intercrop with cross- pollinated crops.

- The lines/cultivars of oranges and mandarins (including TN6, TN12, XB-6, Xa Doai orange, Tuyen Quang orange, Duong Canh mandarin, Bac Kan mandarin) are lines/cultivars with harmony feature. Therefore, when pure plantation or intercropped plantation, these lines/cultivars still produce high yield.

Through research by spraying with GA3 growth regulator, kinds of foliar fertilizers for the experimental line/cultivars provide an exogenous auxin amount to help increase the proportion of fructification, fruit weight and reduction of number of seeds per fruit.

RECOMMENDATIONS

- It continues to study mechanisms of the process of creation of seedless fruits and capacity of increase of productivity, quality and commercial value of citrus fruits to apply measures of appropriate cultivation techniques.

- It continues to study intervened measures such as nutritional supplements through leaves in improving productivity and quality for Citrus fruits.

LIST OF PUBLICATION RELATED TO PhD.DISSERTATION

1. Hoang Thi Thuy, Ngo Xuan Binh (2015), “Research on biological characteristics related to emasculation of perisnth in Citrus trees (*Citrus*), *Journal of Agriculture and Rural Development*, (16), pp. 55 - 61.
2. Hoang Thi Thuy, Nguyen Huu Tho, Le Tien Hung, Ngo Xuan Binh, Akira Wakana (2014), “Self-incompatibility in Pumelo (*Citrus grandis* L. Osbeck) with focus on Vietnamese cultivars with and without Parthenocarpy”, *Journal Fac. Agriculture Kyushu University*, 59, pp. 65-70.
3. Nguyen Huu Tho, Hoang Thi Thuy, Ngo Xuan Binh (2014), “Research on the actual production of Dien pumelo in Thai Nguyen Province”, *Journal of Science and Technology, Thai Nguyen University*, (16), pp. 95 - 100.
4. Nguyen Huu Tho, Hoang Thi Thuy, Le Tien Hung, Ngo Xuan Binh (2014), “Research results on relationship between age of maternal branches and the growth of fruit branches to productivity of fruit of Dien pummelo (*C. grandis*)”, *Journal of Agriculture and Rural Development*, (1), pp. 43 - 48.
5. Hoang Thi Thuy, Nguyen Huu Tho, Le Tien Hung, Ngo Xuan Binh (2011), “Research on the process of sexual reproduction related to the ability of fructification in Phuc Trach pummelo (*Citrus grandis*).”, *Journal of Agriculture and Rural Development*, (19), pp. 12 - 18.