Available online at http://www.journalijdr.com

ISSN: 2230-9926

International Journal of Development Research
Vol. 4, Issue, 10, pp. 2091-2093, October, 2014

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

INTEGRATION OF BIOTECHNOLOGY AND CONVENTIONAL BREEDING TO DEVELOP CITRUS SEEDLESS CULTIVARS IN VIETNAM

Dr. Ha Thi Thuy, Prof. Do Nang Vinh Msc. Tran Thi Hanh, Dr. Tran Ngoc Thanh, Dr. Le Quoc Hung, Msc. Vu Anh Tuan and *Dr. Tran Dang Khanh

Agricultural Genetics Institute, Pham Van Dong Road, Tuliem, Hanoi, Vietnam

ARTICLE INFO

Article History:

Received 18th July, 2014
Received in revised form
07th August, 2014
Accepted 30th September, 2014
Published online 25th October, 2014

Key words:

Citrus,
Embryo Rescue,
In Vitro, Seedless

ABSTRACT

Citrus is a major fruit product in Vietnam. However, most of citrus cultivars are high number of seeds that curb in developing citrus production and fruit quality. Seedless is an important trait in Citrus. Hence, the objective of current study was to integrate the conventional breeding and biotechnological approaches for developing Citrus seedless cultivars in the country. By integration between conventional and biotechnological breeding approaches, 5 new seedless cultivars have been released and approved. The method of tetraloid induction by colchicine *in vivo* treatment on mature buds and following interploid hybridization have showed highly effective for pummelos triploid breeding. Numerous triploids have been obtained in *Citrus nobilis* Cam Sanh by embryo rescue *in vitro* of aborted and small seeds and also developed by interploid crosses. The method of tetraploid induction on buds of mature diploid plants and grafting of young triploid buds on mature trees is two effective ways for shortening breeding circle in Citrus. The intensive field test is urgently needed for next 5 years to select and release the best triploids for production in the country.

INTRODUCTION

Vietnam is known to be the center of origin of many Citrus species. The climatic and soil conditions in entire country are suitable and favorable for Citrus production. The planted areas of citrus fruit are rapidly increased and citrus fruit production has significantly enhanced in some recent years. In 1990, the total areas of the citrus trees were over 19 thousand hectares, while in 2011, the areas of citrus production reached 135,000 ha with a production of nearly 1.35 million tons. Citrus is occupying the highest production areas in comparison with the other fruits (Bananas 113,900 ha, Longan 93,500 ha, Litchi 82.700 ha, Mango 87,900 ha, Pineapple 39,300 ha, Rambutan 23,100 ha). Among the grown varieties of citrus, Pummelos has been popularly developed and spread all along the country with many indigenous cultivars including spontaneously seedless cultivars (Nam Roi, Da Xanh) and many seedy ones such as Tan Chieu, Phuc Trach, Buoi Dien, Buoi Doan Hung etc. Cam Sanh is known as citrus nobilis King Mandarin, is one of most important citrus species native of Vietnam, with 28.7 thousands ha planted in Mekong River Delta and is most important fruit crop in the north of mountainous provinces of Vietnam. Many of these cultivars have high potential in local and export markets. The return of investment per area of this crop is higher than 3-6 times of growing rice (Minh, 1992; Chau and Hong, 1994). However, the disadvantages of most citrus cultivars in Vietnam are high density of seedy that are commercially unacceptable number of seed per fruit. Those citrus cultivars are strongly affected by the insects and diseases, especially Huanglongbing (HLB) and Tristeza disease. It is causing the fruit production significant reduction. Recently, interest of consumer in seedless of citrus fruits has paid much attention and increased. Hence, seeds with few seeds or seedless has currently become an imperative traits (Ali *et al*., 2013). Seedlessness, regular fruit bearing, tree size, disease resistance which are the goals of many citrus breeding programs in the world. Application of conventional cross breeding is undoubtedly continue to be the most important means to produce improved cultivars. Biotechnology is being applied in citrus breeding to enhance the conventional programs. However, in Vietnam, there are very few studies on citrus breeding to improve the fruit production as well as selecting the citrus seedless. Therefore, the objective of current research is integrating the conventional breeding and biotechnological approaches for developing Citrus seedless varieties in the country. Seedless is an important trait in

Corresponding author: Dr. Tran Dang Khanh
Agricultural Genetics Institute, Pham Van Dong Road, Tuliem, Hanoi, Vietnam

Citrus. The different breeding approaches have been applied in Institute of Agricultural Genetics as cultivar introduction; mutation breeding and somaclonal variations; development of seedless triploids by interploid backcrosses; development of triploids, spontaneously derived from small and aborted seeds. The combination of selected above-mentioned approaches is considering as key importance for Citrus seedless production in Vietnam.

MATERIALS AND METHODS

Production of tetraploid lines by colchicine treatments

Treatment on axillary buds in the branches of mature plants; *in vitro* treatment on somatic embryogenic callus derived from local orange and mandarin cultivars; colchicine treatment on mature seeds of different citrus cultivars. The main varieties used in experiments are as follows: Pummelo (*Citrus grandis*) elite cultivars: Phuc Trach, Buoi Dien, Nam Roi, Doan Hung, Red Pummelo; King Mandarin (*C. nobilis*): elite clone of Cam Sanh; Orange cultivars (*Citrus sinensis*): Van Du, Xa Doai. Different colchicine concentrations (0; 0.05; 0.1; 0.2; 0.3 and 0.4 percentages) with different treatment times (0h; 24h; 48h and 72h) have been used as the method developed by (Thuy, 2005).

Developing triploids by interploid hybridization

The experiments were conducted at Agricultural Genetics Institute farm, Van Giang, Hung Yen, Hanoi. The crossed combinations were as following: Buoi Dien (2x) X Phuc Trach (4x), Doan Hung (2x) X Phuc Trach (4x), Nam Roi (2x) X Phuc Trach (4x), Phuc Trach (2x) X Phuc Trach (4x). Crossing steps were followed the method of Thuy, (2010).

Embryo rescue

Small and aborted seeds from Cam Sanh, Cam Van Du and interploid hybrid combiners) Grafting new breeding lines on mature trees for shortening vegetative growth and early flowering of plants. Ploidy levels of plants are verified by flow cytometry analysis recommended by Partec Company (Thuy, 2005).

Statistical Analyses

All data were conducted at least thrice and analyzed by Excel ver.2010 and IRRISTAT software ver 2008.

RESULTS AND DISCUSSION

One of the shortest way for releasing seedless Citrus varieties for production is introduction of international Citrus varieties and following systematic field trials for selecting the best varieties adapted to different ecological regions of country. By this methods, it takes at least 12 years of research and active extension from experimental station of research institute to the farms of companies and from companies to large-scale production by the farmers. As the reported results, 5 seedless, highly productive and good fruit quality orange and mandarin varieties have been released and approved for production by Ministry of Agriculture and Rural Development (MARD). The disease-free materials of these varieties are available for large – scale production by applying shoot-tip grafting methods and protective greenhouses (Thuy, 2010). High Efficient protocol for producing tetraloids have been developed for Citrus: The most effective method for inducing tetraploids in Citrus is *in vivo* treatment of colchicine on the axillary buds of two-year old plants. Selected fully developed shoots, cutting off the top young shoot internodes and remove the leaves from stems for waking-up axillary buds.

Colchicine treatment have been started 3-4 days afterwards. The ploidy level of sprouting shoots has been identified by their morphology changes and followed by flow cytometry analysis. The most effective treatment has been observed at colchicine concentration 0.1% with treating time 24h. The buds of homogenised tetraploid stems are used for grafting to mature plants (3 year-old plants as root-stocks) for promoting faster flowering of grafted tetraploid buds. By this method, 8 tetraloid lines have been obtained from elite local cultivars (5 pummelos, 1 Cam Sanh and 2 local orange cultivars). While, only 2 triploid lines with seedless fruits were developed (Table 1). Numerous triploid lines have been obtained from interploid hybridization and embryo rescue of aborted and undeveloped seeds.

Table 1. Plants recovered from interploid hybrid fruits and their ploidy levels

Hybrid combinations	Number of ploidy analysed plants	Number of Diploid plants	Number of triploid plants	Number of tetraploid plants	Number of Triploid lines with seedless fruits
NR (2x) x PT (4x)	11	2	9	0	
DH (2x) x PT (4x)	142	9	131	2	
PT (2x) x PT (4x)	39	24	15	0	1
BD (2x) x PT (4x)	282	12	264	6	1
PT (2x) x PT (2x)	1317	1317	0	0	
Total	1791	1364	419	8	2

Note: NR: Nam Roi; PT: Phuc Trach; DH: Doan Hung; BD: Buoi Dien.

Table 2. Classification of seed types in Cam Sanh (*C. nobilis*) and Van Du (*C. sinensis*), according to their seed weights

Cultivars	Seed type	Seed weight (g)	Number of seed in group/ Total seed	Percentage of each type (%)
Cam Sanh	Aborted	< 0.06	24/ 473	5.07
(*C. nobilis*)	Small	$0.06 \leq m \leq 0.09$	58/ 473	12.26
	Normal	$0.1 \leq m \leq 0.17$	355/ 473	75.05
	Big	> 0.17	36/ 473	7.61
Van Du	Aborted	≤ 0.01	15/ 489	3.07
(*C. sinensis*)	Small	$0.01 \leq m \leq 0.04$	55/ 489	11.25
	Normal	$0.04 < m \leq 0.12$	377/ 489	77.09
	Big	> 0.12	42/ 489	8.59

Table 3. Ratios of monoembryonic and polyembryonic seeds in Citrus cultivars, cam Sanh (*C. nobilis*) and Van Du orange (*C. sinensis*)

Cultivars	Seed groups	Monoembryonic (%)	Polyembryonic seed (%)
Cam Sanh (*C. nobilis*)	Normal	30.33 ± 2.33	69.67 ± 2.33
	Small	46.67 ± 2.77	53.33 ± 1.63
	Aborted	40.67 ± 2.33	59.33 ± 2.43
Van Du (*C. sinensis*)	Normal	2.67 ± 0.33	97.33 ± 0.33
	Small	4.44 ± 0.93	95.56 ± 3.33
	Aborted	4.67 ± 1.33	95.33 ± 2.61

Table 4. Identification of ploidy level of plants regenerated from small and aborted seeds of Cam Sanh (*C. nobilis*) and Cam Van Du (*C. sinensis*)

Cultivars	Undeveloped seeds	Number of plants identified	Number of polyploid plants			
			3n	Percentage (%)	4n	Percentage (%)
Cam Sanh	Small seeds	679	38	5.59	0	0
(*C. nobilis*)	Aborted seeds	258	5	1.94	0	0
Van Du	Small seeds	128	0	0	1	0,78
(*C. sinensis*)	Aborted seeds	73	0	0	0	0

Two triploids pummelos have given seedless fruits and are on the way of further extension and trials. Currently, there are some methods to breed for seed lessness such as crossing diploid (2n) with tetraploids (4n), or contrarily bred (4n) with (2n) (Raza *et al*., 2003), embryo rescue, radiation, mutagenesis and transformation etc (Ali *et al*., 2013). Cam Sanh (*C. nobilis)* and Van Du (*C. sinensis*) are widely grown in Vietnam. As shown in Table 2, aborted seed type of Cam Sanh was reached 5.07%, while, Van Du was 3.07%. The small seed types of both cultivars were 12.26 and 11.25% respectively. The highest normal seed types were 75.05% in Cam Sanh and 77.09% in Van Du. To compare the seed weight of the big seed type between two cultivars was at 7.61% and 8.59% (Table 2.) As indicated in the Table 3, the percentage of monoembryonic of the aborted seed group was 40.67%, and small seed was 46.67% in Cam Sanh, while Van Du was shown lower at 4.67% and 4.44%. However, polyembryonic seeds were exhibited opposing (Table 3.). The small and aborted seeds with embryo were sterilized and conducted embryo rescue for regenerating plants and identifying the ploidy and polyploidy levels.

As shown in Table 4, the number of plants identified in small seeds of Cam Sanh was 769 plants and aborted seeds were 258 plants, respectively. In addition, number of polyploidy plants reached 5.59% and 1.94%. While, in Van Du cultivar was 128 in small seeds and 73 plants in aborted seeds which showed lower to compare with Cam Sanh. In the fact that the conventional methods have often dealt with numerous problems because of specific reproductive physiology of citrus and was also time exhaustive, the recent advanced techniques such as embryo rescue, protoplast fusion, irradiation, inter specific or genetic cross, flow cytometry and citrus transformation have reduced time and labor force and more productive as well as obtaining rapid results which can be attained in short time period and produce less seeded or completely seedless citrus cultivars (Ali *et al*., 2013). Research on producing citrus with seedless has been conducted sporadically. Our results may provide good information for further specific studies to enhance citrus production in Vietnam.

Conclusions

By integration between conventional and biotechnological breeding approaches, 5 new seedless varieties of orange and mandarin varieities have been released and approved by MARD for large –scale production. The method of tetraloid induction by colchicine in vivo treatment on mature buds and following interploid hybridization have showed high effective for pummelos triploid breeding. Numerous triploids have been obtained in Citrus nobilis Cam Sanh by in vitro embryorescue of aborted and small seeds and also by interploid crosses. The method of tetraploid induction on buds of mature diploid plants and grafting of young triploid buds on mature trees is two effective ways for shortening breeding circle in Citrus. The intensive field test is urgent needed for next 5 years to select and release the best triploids for production in the country.

REFERENCES

Ali, S., Khan, A.S., Raza, S. A, Rehman, R.N. 2013. Innovative breeding methods to develop seedless citrus cultivars. *Inter J Biosci*. 3,191-201.

Chau, N.M., Hong, L.T.T. 1994. Citrus Huanglongbing in Vietnam: Problems and some research achievements, *JIRCAS Working Report*, No 55, 41-46

Minh, D. 1992. Result of investigation on gardening economy in Can Tho, Vinh Long and Dong Thap (In Vietnamese), Approved project submitted to the Government of Vietnam, pp.1-17.

Thuy, T.H. 2010. Study on seedless of some citrus cultivars. *Final report of MARD*, 323 pages

Thuy, T. H. 2005. Stud on seedless trait and method to develop polyploidy in citrus. Ph.D dissertation, Hanoi National University, 145 pages.

Raza, H., Khan, M.M., Khan, A.A. 2003. Seedlessness in citrus. *Inter J Agric Biol*. 5,388-391.

****