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Research Article

SHOOT TIP GRAFTING (STG) IN COMMERCIAL PRODUCTION OF DISEASE FREE, QUALITY BUDGRAFTS OF CITRUS IN CENTRAL INDIA

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ABSTRACT

The Citrus growers are facing a serious problem of decline that is attributed to prevalence of Citrus virus and virus like diseases. The productive life span of Citrus orchards in tropical countries is less than 10 years because of these diseases. Hence, the need for healthy certified quality planting stock is recognized as basic to the establishment of a viable Citrus industry. Use of healthy planting material for establishing the new orchards will help in controlling the diseases and increasing the productivity. Shoot tip grafting (STG) has been proved effective in elimination of diseases and in establishment of healthy Citrus orchards worldwide (viz. Brazil, USA, Spain etc.). Keeping this in view, a comprehensive program based on STG to produce, multiply, maintain and distribute the disease free planting stock to Citrus growers was initiated nearly a decade ago. The program named as Citrus variety improvement program has following functions: 1. Restricted movement of bud wood. 2. Introduction of citrus exotic cultivars and cleaning through STG. 3. Maintenance of healthy mother trees in protected foundation block. 4. Continued testing and growth evaluation. 5. Discarding infected trees. Through successful transfer of STG technology from lab to land so far 4.5 lakhs of certified quality bud grafts of *Citrus reticulata* Blanco Nagpur mandarin were produced and distributed to Citrus growers which facilitated establishment of around 1500 hectares of healthy Citrus orchards in farmer's fields of Central India. Thus, paving the way for a sound and sustainable Citrus industry. In this paper the authoress presented status of STG based Citrus improvement program being implemented at CCRI, Nagpur, India.

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INTRODUCTION

Nagpur Mandarin (*Citrus reticulata* Blanco) is the most important fruit crop of India and is grown over an area of 10.55 Lakh Ha with a production of 127.46 lakh MT. The average productivity of 12.0 MT/ha (www.nhb.gov.in) is far below the potential productivity of 25-30 MT/ha. The Citrus decline is a major problem of Citrus cultivation leading to poor productivity, shorter productive life and serious economic losses. Various abiotic and biotic factors contribute to decline and viruses and virus like diseases are one of the major causes of this problem.

About 25 million bud grafts are produced in the region annually in various nurseries and supplied all over the country for raising new orchards or replacing the old orchards. Adequate attention is not paid towards quality and health of bud wood used for propagation. Though there are legal provisions to regulate the production and movement of bud wood, due to ineffective implementation, desired sanitation and quarantine levels could not be achieved. The bud wood

certification programmes started earlier did not yield desired results due to non availability of effective sanitation and diagnostic techniques for (a) facilitating large scale production of planting stock and (b) quick, effective detection of viruses and virus like pathogens. With the advent of biotechnological/diagnostic techniques like Shoot Tip Grafting, ELISA, PCR, it has become possible to implement Sanitation and Quarantine Programmes very effectively and efficiently. The status of STG based Commercial production programme of healthy, quality bud grafts of Nagpur Mandarin, being implemented since 2002 at National Research Centre for Citrus, Nagpur, India is summarized in this paper.

MATERIALS AND METHODS

The STG based Citrus Variety Improvement Programme being implemented at CCRI was initiated under the World Bank aided project during the year 1994 and later scaled up as a pilot project for transfer of technology using the funds made available by the National Horticulture Board and the

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Department of Biotechnology, Government of India. The programme comprises of the following steps.

Citrus Sanitation

Selection of mother trees: A large number of Mandarin orchards were surveyed in Nagpur District of Maharashtra and Chindwara District of Madhya Pradesh, discussions were held with the farmers and promising mother trees were selected on the basis of Yield (No. of fruits per tree), tree vigour, fruit quality parameters such as fruit weight, TSS and tree health. After observing and confirming the yield and fruit quality parameters, about 20 outstanding mother trees were finally selected for sanitation and further multiplication. Bud wood was collected from selected outstanding trees and propagated on vigorous rough lemon rootstock seedlings and maintained in screen house.

Indexing of mother trees: The bud grafts raised from scion of selected mother trees were maintained in screen house and were indexed for major pathogens associated with citrus decline in India such as Citrus tristeza virus (CTV), ring spot, mosaic virus, citrus exocortis viroids (CEVd), citrus yellow corky vein viroid, and greening bacterium, both biologically and serologically (CTV). Biological indexing was done according to standard procedures (Roistacher 1991) and indexing of CTV was done by indirect DAS-ELISA using CTV specific antibodies (Cambra *et. al.* 1990).

Elimination of pathogen by STG: Superior mother trees found carrying one or more of the five major pathogens were cleaned using procedure of STG developed by Murashige *et al.*, 1972 and Navarro *et al* 1975 and adopted to Nagpur Mandarin (*Citrus reticulata* Blanco) by Vijayakumari *et. al.* 1994 and Vijayakumari and Shyam Singh 2000 was used for elimination of pathogens and production of Shoot tip grafts of Nagpur Mandarin. These Shoot tip grafts were re-indexed for confirming disease freeness as per procedures mentioned earlier.

Rough lemon (*C. jambheri* Lush) was used as *in vitro* rootstock. Scion shoot tips of 1 cm length were excised from elite mother trees of Nagpur mandarin, surface sterilized with 0.25% sodium hypochlorite solution followed by thrice rinsing with sterile distilled water. Pre- treatments of shoot tips and rootstocks with growth regulators. (BA and 2,4 D) were accomplished by dipping the shoot apex and decapitated rootstock prior to grafting for 10 min. (Vijayakumari and Shyam Singh 2000). The shoot tip comprising of apical meristem and two or three leaf primordia, is excised and with the aid of microscope, was transferred to the cortex in apical triangle cut (Vijayakumari *et. al.* 1994) at the top of decapitated rootstock. The micro grafts were placed in liquid basal medium supplemented with modified Whites vitamins and maintained in a culture room at 27°C and exposed daily to 1000 Lux illumination for 16 hours. After sprouting, when the graft takes two or three leaves, was transferred by direct transfer in sterilized soil rite or by double grafting on vigorous green house grown Rangpur lime and rough lemon rootstocks as described by De lange (1978).

Re-indexing and Establishment of field and protected foundation blocks: Healthy, elite shoot tip grafted plants after re-indexing were maintained in protected and field foundation

blocks to serve as repositories of disease free mother trees for further multiplication (Vijayakumari *et al.* 2000). These plants were re-indexed periodically as required to ascertain health. Each tree in the foundation block was examined several times each year for overall health, stability, fruit quality and symptoms of disease and other abnormalities Further each tree of the protected foundation block is tested for CTV and Greening once in a year, and for viroids once in 3 year by Sero, bio-diagnostic and molecular techniques.

Multiplication: Multiplication blocks were made under low cost green house. Solarised disinfected potting mixture was only used for filling the poly bags. The rootstocks were grown from disinfected seed, which breaks the cycle of virus transmission. The scion wood collected from virus free foundation blocks was used for raising bud grafts for supply to farmers.

Evaluation of field transferred STG plants: The STG derived, quality healthy bud grafts planted in farmers orchards in different districts of central India were evaluated (on 6 years old trees) in terms of vegetative growth and yield parameters through properly laid out experiments in Randomized block Design and the data analyzed statistically

RESULTS AND DISCUSSION

Best candidate trees were selected based on superior horticultural characteristics and being maintained in the green house as a source of scion wood. Through *in vitro* STG (Fig. 1) produced more than 300 micro grafts, indexed and healthy micro grafts were being maintained in protected foundation block (insect proof screen house) and in field foundation block as a repository of mother trees (Scion sources) for future large scale multiplication (Fig. 2).



STG of Nagpur mandarin



Protected foundation block having STG derived healthy mother stock



STG derived planting material ready for release

From this repository of registered mother trees, produced and released 4,50,000 elite, certified, disease free plants (Fig. 3) to citrus growers/nursery men of Maharashtra, M.P, Gujarat, Rajasthan, Haryana, Punjab, Sikkim, Orissa, A.P, Tamil Nadu, Karnataka and Kerala states. More than 1500 Hectres of healthy orchards were established in the farmer's field predominately in Central India.



Conventional budded trees (6 years old), Nagpur, Maharashtra

STG derived planting stocks (6 years old), Nagpur, Maharashtra

Recorded data on vegetative and fruit yield of STG derived and conventionally budded plants at the orchards of the farmers located in Chindawada districts of Madhya Pradesh [table 1]. Nagpur and Bhandara districts of Maharashtra [Position of table 2 &3] and presented the results.

Table 1 Comparative field performance of 6 year old STG derived plants Vs. Conventional budded plants at Sausar (Chhindwara District)

Sr.No.	Type of Budding	Plant Height (m)	Stem Height (cm)	Stock Girth (cm)	Scion Girth (cm)	Canopy Volume (m ³)	No. of Fruits
1.	STG derived	4.59	38	62.16	58.66	36.49	376.07
2.	Conventional Budded Plants	4.14	32	57.02	54.83	21.78	155.00
3.	C.D. values at 5%	0.72	NS	NS	NS	3.95	25.63

The data presented in tables indicated that planting stock derived from STG established well in the field and showed significantly higher plant height, stem height, stock girth and scion girth compared to conventionally raised plants procured from (a) private nursery sources of Chindwada district of Madhya Pradesh state, Nagpur and Bhandara districts of Maharashtra State.(Fig. 4) and (b) Government agencies. Further the results were statistically significant for canopy volume and also fruit yield. Evidences are available to suggest the definite yield advantages, prolonged bearing life and increased longevity of plants raised from disease free bud wood. (Navarro *et al.* 1981, Santos Filho *et al.*, 1984 and Gumpf *et al.* 1996) through the citrus variety improvement programs at Spain, Brazil and USA. At CCRI, Nagpur the technique was successfully modified, improved with growth regulators and applied in the citrus improvement program (Vijayakumari *et al* 1994, Vijayakumari and Shyam Singh, 2000) Shoot tip grafting has the advantage of obtaining thornless plantlets, avoiding the nucellar juvenility phase and performed true to type in the field. Other studies (Navarro and Juarez 1977 and Starrantino *et al* 1984, Vijayakumari, 2009, 2010, 2012, Vijayakumari and Karihaloo, 2012) also reported similarly on this subject. Indisputably starting a new orchard using disease free planting material obtained though STG has greater advantages. Foundation stock of disease free budwood

source and cleaning of virus from time to time through in vitro STG plays a key role in disease management in Citrus.

Based on success story of STG in CCRI, Nagpur, the technology and associated infrastructure is being replicated in major citrus growing regions of India viz. Vidharbha, Marathwada regions of Maharashtra, Chindwada district of Madhya Pradesh, Chittorghad, Ganganagar, Jhalawad and Bikaner regions of Rajasthan state and Coorg mandarin growing region of Karnataka state.

There is a need to (a) widen the genetic base by adding additional Clones to the variety improvement programme (b) develop diagnostic kits for easy detection of viruses and (c) take up capacity building trainings for technical staff, field functionaries and nursery men.

CONCLUSION

A comprehensive Citrus Variety improvement program was implemented, Sanitized elite selections through *in vitro* STG, established protected foundation block, released 4,50,000 healthy and quality bud grafts to citrus growers/nurserymen till date and generated a revenue of Rs. 170 lakhs to CCRI by Sale of planting material.

About 1500 hectares of healthy orchards were raised by the farmers and the demand for STG derived bud grafts is increasing every year. The institutional linkages between the Central and State Research Organizations, State Horticulture Departments and Nursery men need to be strengthened to implement a coordinated Citrus Variety Improvement Programme. Further the Nursery Acts of various states have to be implemented effectively to regulate the production and movement of bud wood grafts to achieve the desired sanitation and quarantine objectives and boost the Citrus Industry.

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