



IN VITRO SHOOT TIP GRAFTING IN MASS MULTIPLICATION AND SUPPLY OF DISEASE FREE PLANTING STOCK OF NAGPUR MANDARIN IN CENTRAL INDIA: A SUCCESS STORY

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ABSTRACT

The need for pathogen free propagative budwood for the production of healthy, certified trees is recognised as basic requirement for the establishment and the future of a viable citrus industry. In central India every year millions of citrus plants are multiplied and supplied all over India without any certification for quality and diseases. Lack of elite, planting material and inadvertent multiplication of diseases (Virus, virus like and bacteria) through nursery stock are considered as major reasons for low productivity and less longevity of orchards.

The average life span of citrus orchards in tropical countries is less than ten years, because of these diseases. The situation warrants for a modern technique or biotech break on which the foundation of the future citrus industry can be banked on. The ICAR-CCRI, Nagpur (erst while NRCC) has evolved the complete protocol for production and multiplication of disease free planting stock through STG, which is comparable to the citrus sanitation programme of advanced citrus growing countries viz. Spain, Brazil, and USA. In this paper present status of the Citrus disease free planting material production and release program based on STG in central India was described.

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INTRODUCTION

Mandarin Orange (*Citrus reticulata* Blanco) is one of the main commercial Citrus cultivars of India and accounts for 43 percent of all citrus fruits produced in the country. Nagpur mandarin is the prime fruit crop of central India (Vidarbha region of Maharashtra and some areas of M.P.) and is grown over an area of 10.55 lakh ha with a production of 12746000 MT. The average productivity of 12.0 MT/Ha (Horticulture Data base 2011) is far below the potential productivity of 30 MT/Ha. And the average productivity (25-30 tons/ha) of countries like USA, Brazil, Spain, Japan, Mexico etc. Various reasons viz., (i) Wide scale infections viz., virus and virus like diseases, fungus and bacteria (ii) Absence of reliable sources of certified, elite and disease free planting material (iii) Apathy towards available technologies and (iv) Lack of coordination between the stake holders etc are responsible for low productivity and less longevity of citrus orchards.

About 15 viruses and virus like diseases viz., CTV, Mosaic, Ring spot, Exocortis viroid and Greening bacterium etc., are reported from various citrus growing regions. Virus infected trees do not put on normal healthy growth, become stunted, show die-back of twigs and branches. Viruses are the most powerful influencing factors leading to citrus decline.

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These diseases cause huge economic losses by increasing the cost of orchard maintenance, reducing the production, productivity, and productive life of the orchards. The average life of Nagpur mandarin orchard may last for 20 years where as oldest orchards of 200 years are reported in Italy. Poor state of Citrus orchards is attributed to propagation of in-genuine and diseased bud wood on wrong root stocks.

In Vidharba alone (Sindurjanaghat and Tiwsa blocks of Amravati district), about 300 private nurseries raise 8 to 9 million plants every year. These plants are used by the citrus growers of M.P, Rajasthan, Punjab, Haryana, Tamil Nadu, Karnataka for raising/replacing citrus orchards. These nurseries are not paying attention towards the production potential, health of the bud wood material and also the sanitized methods of propagation leading to inadvertent multiplication and spread of infected material/ diseases from region to region through sale of infected plants. The state of Maharashtra had enacted the "Maharashtra Fruits Nurseries (Regulation) Act.1969" to provide for licensing and regulation of fruit nurseries in the private sector but it was not implemented properly for achieving the intended results. The status of implementation of Nursery Registration Acts in other states is similar and very little has been achieved in controlling the genuineness of the planting material and its freedom from diseases.

Virus free planting material, control of vectors, sanitation and cross protection are some of the approaches advocated to

minimize the losses caused due to these diseases. Since no control measures are available, the role of virus free planting material for starting of healthy orchards has been emphasized from time to time. Virus free planting material is the primary requirement for the effective management of the virus diseases. It is proven that the citrus orchards established with the healthy planting material had higher productivity and longer longevity than the orchards established with the infected planting material. The virus free plants may get infected in the field through the vectors but the stage at which the first infection takes place has a definite bearing on the degree of losses. Once a plant is established, it can sustain the losses under a good system of management on the contrary, an infected plant may not attain even the stage of production. Use of healthy planting material for raising new orchards will help in controlling the diseases and enhancing the productivity and longevity of orchards.

Several schemes were started in the past to produce and supply certified bud-wood but worthwhile results have not come forward owing to many reasons. The schemes did not succeed due to non-availability of (i) efficient and cost effective virus elimination technologies and (ii) quick diagnostic techniques to frequently monitor the mother tree health had restricted the large scale multiplication of healthy planting stock. With the advent of biotechnological/diagnostic techniques like Shoot Tip Grafting, ELISA, PCR new vistas had opened to overcome the limitations.

The ICAR-CCRI, Nagpur (erst while National Research Centre for Citrus (NRCC) was started at Nagpur by the ICAR during the year 1985 to undertake focused research on Citrus crops. Various research projects on Varietal improvement, Germplasm collection/introduction etc were initiated and ICAR-CCRI, Nagpur (erst while National Research Centre for Citrus (NRCC) was recognized as “National Citrus Repository. It was decided to harness the new biotechnological techniques for promoting Citrus Sanitation and Citrus Quarantine programmes on the lines of USA, Brazil, and Spain etc. Under the World bank aided project (1992-96) a new Tissue Culture Laboratory was established and the research work on Shoot Tip Grafting (STG) of Nagpur Mandarin was started. The protocols for cleaning the elite trees and the sanitized production of disease free bud grafts were further refined under the institute project (1998-2002). In view of the promising results, National Horticulture Board and the Department of Biotechnology came forward to fund the development of additional infrastructural facilities, such as insect proof screen houses, ênet houses, glass houses, required to scale up the planting stock production programme as a pilot project (2003 -2009) and to transfer of technology from lab to land. Based on the success of pilot project, “Technology Mission on Citrus” was formulated for producing and supplying quality planting material of citrus on commercial scale to the farmers.

The programme comprises of the following steps.

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 (number of fruits per tree),fruit quality (fruit weight, TSS),Tree vigour and Tree health. After observing

and confirming the yield and the fruit quality parameters, about 20 outstanding mother trees were finally selected for sanitation and further multiplication. Bud wood was collected from these trees and propagated on vigorous rough lemon rootstock seedlings and maintained in screen house.

Indexing of mother plants : The bud grafts raised from scion of selected mother trees are maintained in screen house and are 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 as per the standard procedures (Roistacher 1991) and testing of CTV was done by indirect DAS-ELISA using CTV specific antibodies (Cambra et al 1990, Vijayakumari et al 2006)

Elimination of pathogens by STG: Superior mother plants 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 reticulate Blanco) by Vijayakumari et al 1994 and Vijayakumari and Shyam Singh 2000, and pathogens were eliminated. 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 at the top of decapitated rootstock. Successful grafts were transferred by double grafting on vigorous green house grown Rangpur lime and Rough lemon rootstocks as described by De lange (1978), Vijayakumari et. al.1994. Re-indexing and Establishment of field and protected foundation blocks: Produced more than 300 healthy, elite shoot tip grafted plants through STG and after re-indexing were maintained in protected (in insect proof screen house) and field foundation blocks to serve as repositories of disease free mother trees for further multiplication.(Vijayakumari et al 2000) These plants are re-indexed periodically as required to ascertain health. Each tree in the foundation block is 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. About 4,80 lakhs disease free bud grafts were supplied during 2018 to 2019 to citrus growers/nursery men of Maharashtra, M.P, Gujarat, Rajasthan, Haryana, Punjab, Sikkim, Assam, Orissa, A.P, Tamil Nadu, Karnataka and Kerala states .An amount of Rs.190 lakhs was realized as revenue from the sale of STG derived bud grafts.

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 (1 to 7 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. The planting stock derived from STG established well in the field and showed higher plant height, stem height, stock girth, scion girth and

canopy volume compared to conventionally raised plants procured from private nursery sources. The results are statistically significant for canopy volume and also fruit yield. (Vijayakumari N., 2009, Vijayakumari and Karihaloo, 2012).

Citrus Quarantine: Movement of vegetative material of Citrus is indispensable for various purposes like propagation; germplasm exchange etc. but there is an inbuilt risk of spreading the diseases. Traditional quarantine methods are very slow and require green house facilities remote from citrus production areas. Standardization of STG (shoot tip grafting) based citrus quarantine at ICAR-CCRI, Nagpur (erst while National Research Centre for Citrus (N.R.C.C.) has paved the way for safe introduction and widening the gene pool. Through Quarantine programme a large number of exotic accessions were introduced, cleaned and added to the citrus gene pool .

Using STG as post entry quarantine check, 23 exotic citrus cultivars introduced from USA, France, Australia were cleaned and are maintained in the Citrus repository. Mandarin introductions (Dhakula local, Corsica No.1 and No.2 Marissol, Clementine Monreal, Frost owari) Sweet orange introductions (Riogrande navel, lane late navel orange, Campbell Valencia, Cutter Valencia, shaumouti orange, Ruby blood orange) grape fruit introductions (Ray Ruby, Flame) and Pummelo (US - 145).were cleaned . Due to limited availability of exotic scion bud wood, flush produced from the potted plants of exotic germplasm that were housed in protected green house were used. (Vijayakumari et al 2006).

Out of 23 exotic cultivars 20 micro-grafted cultivars were recommended for field evaluation. On successful adaptation, can be used for both scientific and commercial purposes. Out of the above three exotic scion cultivars viz, USA-145 pummelo, Flame grapefruit and Cutter Valencia were released as varieties. The principal benefit to the Indian citrus industry is the availability of budwood of superior high yielding varieties or clones which meet the fruit quality requirements of export market. The healthy citrus repositories created through in vitro shoot tip grafting are the insurance policies for the future health and economic viability of the Indian Citrus industry.

CONCLUSION

A comprehensive Citrus Variety improvement program was implemented, Sanitized elite selections through in vitro STG, established protected foundation block, released 4,80,000 healthy and quality bud grafts to citrus growers/nurserymen till date and generated a revenue of Rs. 190 lakhs to CCRI by Sale of planting material. Sanitized propagation procedures ensure elimination of other soil borne pathogens too. This technology / protocol can be adopted for other Citrus species with assured results About 1500 hectares of healthy orchards were raised by the farmers and the demand for STG derived bud grafts is increasing every year. CCRI has signed five consultancy MOUs with private agencies for the transfer of this technology and many fold increase in availability of quality planting stock to the farmers.

Initial field performance of STG derived plants is better compared to conventionally budded plants. Exotic varieties are made available for field evaluation and crop improvement. Through Quarantine programme a large number of exotic accessions were introduced, cleaned and 23 citrus exotic

cultivars were added to the citrus gene pool . Out of the above three exotic scion cultivars viz, USA-145 pummelo, Flame grapefruit and Cutter Valencia were released as varieties.

There is an urgent need to establish an inter institutional arrangement between all the stakeholders to define the roles and coordinate the efforts for boosting the Citrus Industry. ICAR institutes, SAUs need to undertake research works related to varietal improvement, sanitation, establishment and maintenance of protected foundation blocks, registered nursery men need to establish model nurseries on scientific lines and the line departments need to implement the Nursery Registration Act. The scope of existing seed testing laboratories may be widened and strengthened or referral labs may be established by the ICAR institutes, SAUs to undertake health certification.

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