CONTENTS

भारत सरकार
विज्ञान और प्रौद्योगिकी मन्त्रालय
बायोटेक्नोलॉजी विभाग
ब्लाक–2,7 वां तल, सी० जी० ओ० कम्प्लेक्स
लोदी रोड, नई दिल्ली–110003

GOVERNMENT OF INDIA
MINISTRY OF SCIENCE & TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY
Block-2, 7th Floor C.G.O. Complex
Lodi Road, New Delhi-110003

FOREWORD

India is the second largest producer of black pepper 'the king of spices'; however its productivity is the lowest among the major producing countries. The major limiting factor is dearth of quality planting materials and severe diseases infestation. Plant tissue culture is a key technology for enhancing the capability for production of large quantities of quality planting material. Department of Biotechnology took the initiative to support R&D programmes for developing micro propagation protocols in black pepper. Viable protocols for large scale *in vitro* multiplication of black pepper were developed by Kerala Agricultural University, Thrissur and Indian Institute of Spice Research, Calicut. Subsequently a programme was launched to demonstrate field performance of tissue culture derived black pepper plants over an area of 100 ha in the traditional and non traditional areas of black pepper cultivation in the country. The tissue culture derived plantlets were distributed to over 1,000 farmers in the states of Kerala, Karnataka, Tamil Nadu, Andamans, Sikkim, Assam, Meghalaya and Tripura. The programme was implemented by KAU, IISR and Spices Board.

The success of this programme is due to the effective leadership of the coordinator Prof. K.V. Peter, Former VC, KAU and stringent monitoring under the Chairmanship of Dr. C.K. George. The better performance of *in vitro* derived black pepper plants in the traditional and non traditional areas open up great opportunities for tissue culture technology and improvement of black pepper productivity in the country as a whole. This booklet highlights salient features of the programme.

I am confident that the know how generated and demonstrated would be of great use to the end users, primarily our farmers.

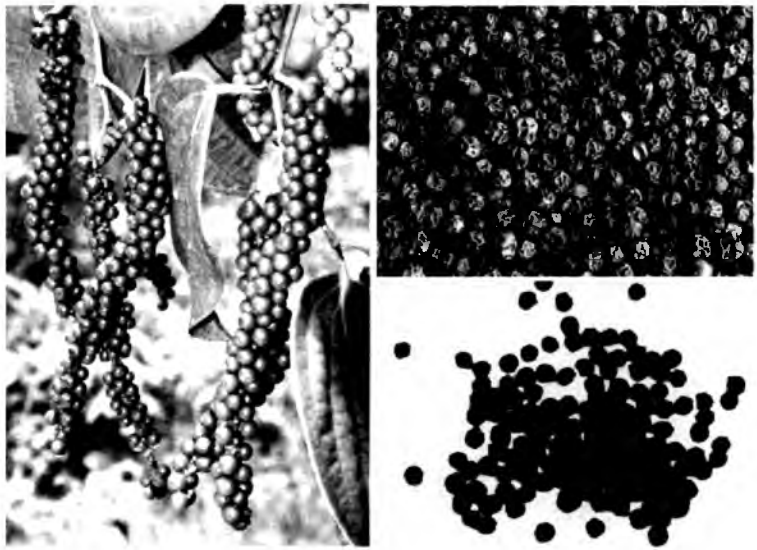
(Dr. Renu Swarup)
Advisor

Website: http://www.dbtindia.nic.in http://www.btisnet.gov.in
दूरभाष / Telephone : 24363012, 24362329 फैक्स / Fax : 011-24362884

MICROPROPAGATION IN BLACK PEPPER A SUCCESS STORY

PRELUDE

Black pepper, the dried berries from the perennial climber *Piper nigrum L.* is the most important spice of the world and is often referred as 'King of Spices'. India is the major producer and exporter of black pepper grown mainly in the states of Kerala, Karnataka and Tamil Nadu. Though Indian pepper has the monopoly in production and trade, its productivity in India is the lowest. It is cultivated in India in an area of 257020 hact. and during 2005-06, India exported 16700 tonnes worth Rs. 14050 lakhs. The production has decreased tremendously during the 2005-06 years due to diseases like phytophthora foot rot and slow decline caused by nematode attack. The poor genetic stock, predominance of old and senile vines in the traditional pepper growing areas and the disease incidence were identified as the major factors contributing to the low productivity of Indian pepper.



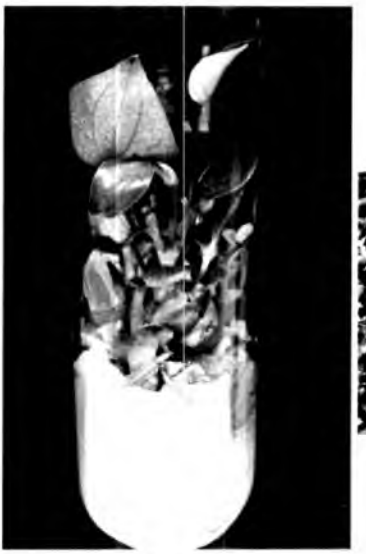
Production of large number of disease free planting materials was thus felt as the need of the hour to rejuvenate the black pepper production and productivity in the country. Though vegetative methods of propagation are available for cloning of black pepper, the

systemic microbial load and already infected nurseries failed to cater the need. Micropropagation protocols developed in different laboratories were considered as an alternative along with exploitation of plant growth promoting rhizobacteria (PGPR) that could control root pathogens and augment soil quality.

This story tells the success of micropropagation in black pepper and the performance of tissue culture derived plants in major producing centres all over the country as a DBT initiative.

BACKGROUND

Considering the importance of black pepper at national/international level and understanding the constraints in productivity; the Department of Biotechnology, Govt. of India supported R&D programmes for developing viable protocols for mass multiplication of elite types in black pepper. In the year 1996, the Centre for Plant Biotechnology, College of Horticulture, Kerala Agricultural University and Indian Institute of Spices Research, Calicut came out



with successful protocols for *in vitro* regeneration in Black Pepper with different explants. Heavy load of systemic bacteria, polyphenol interference and low multiplication rate were identified as the major hurdles to be tackled in micropropagation of black pepper. Pilot studies were conducted by KAU with DBT support to evaluate the performance of TC regenerants in demonstration plots at different locations. The encouraging results in the initial evaluation urged for a large scale on farm trial for evaluating the performance of TC derived black pepper plants.

THE ONFARM TRIALS

The Department of Biotechnology, Govt. of India offered financial support for Onfarm evaluation of TC derived black pepper plants in the traditional and non traditional areas. Three Centres in Kerala- Centre for Plant Biotechnology and Molecular Biology, Kerala Agricultural University; Indian Institute of Spices Research, Calicut and Spices Board, Cochin participated in the programme with specific targets for each centre. The project period was from April 2002 to March 2007. The total area covered under the programme was 100 hactares all over India in the states of Kerala, Karnataka, Tamil Nadu, Andaman, Sikkim, Assam, Meghalaya and Tripura. The objectives identified are:

1. *In vitro* mass multiplication of selected types of black pepper utilizing the protocol already developed.

2. Distribution of tissue culture regenerants to various research centres and selected farmers (1000) in an area of 100 hect.

3. Evaluation of field performance using morphological, biochemical and molecular markers.

Distribution of TC black pepper in India

PRODUCTION OF PLANTING MATERIALS

The protocols developed at Centre for Plant Biotechnology and Molecular Biology, Kerala Agricultural University & IISR, Calicut were utilized for mass multiplication. Nodal segments were used as explant and were multiplied en route enhanced release of axillery buds avoiding an intermediate callus phase. The participating centres IISR & KAU produced the plantlets required to meet their target while Spices Board outsourced it from KAU, IISR, Grow more biotech, Hosur and BMFC, Kazhakoottam.

Details of *in vitro* multiplication adopted is as follows.

Explant	- Shoot tip, nodal segments
Surface sterilization	- 0.1% $HgCl_2$ (5-10 mts.)
Culture establishment	- 1. ½ MS, 1mg l^{-1} BAP 2. MS, 0.5 mg l^{-1} Kinetin (Cephotaxim at 250 mgl^{-1} or $CuSO_4$ 100 mg l^{-1} could avoid systemic bacterial Contamination)
Propagule multiplication	- 1. ½ MS, 1mg l^{-1} BAP, 1 mg l^{-1} IAA. 2. ½ WPM, 3 mg l^{-1} BAP, 1mg l^{-1} kinetin
Rooting	- ½ WPM without growth regulator ½ MS, IBA 1mgl^{-1}
Multiplication rate	- 1 : 4
The varieties selected for *in vitro* multiplication	Panniyur 4 & Panniyur 5 (High yielding varieties released by KAU) - Panchami & Subhakara (High yielding varieties released by IISR)

The *in vitro* derived plantlets were planted out in potting mixture and hardened in mist chamber with >95% success rate.

The potting media were fortified with *Trichoderma harzianum* (50 g/vine) and *Glomus fasciculatum* (500 600 propagules/plant).

In vitro production, hardening and molecular profiling

VIRUS INDEXING

The virus diseases found associated with black pepper are mosaic disease and stunt disease. The explant source and samples (0.1%) from TC derived plantlets were screened at different Centres (at parent Institutes KAU& IISR and at the Central facility for Virus indexing IIHR) to ensure freedom from Virus infection.

MOLECULAR PROFILING

The genetic fidelity and clonal stability of TC plants were ensured through RAPD and iSSR assay at KAU & IISR. The TC regenerants distributed were genetically uniform as indicated in the RAPD & ISSR profiles.

SELECTION OF FARMERS AND DISTRIBUTION OF PLANTS

Wide publicity was given for the progrmme through mass media and through Department of Agriculture in the respective states. Applications were obtained from interested farmers, their plots visited by the project staff & locations identified. A block of 100 plants was identified as one unit and this would contain 80 TC derived plants and 20 conventional propagules; both distributed by the participating centres. Plants were distributed during the monsoon season and details of distribution are as follows.

Participating Centre	State	Area (hact.)
KAU	Kerala	40
IISR	Kerala	10
	Karnataka	15
Spices Board	Tamil Nadu	15
	Karnataka	5
	Andamans	5
	Sikkim	4
	Assam	2
	Meghalaya	2
	Tripura	2

The selected farmers were supplied with the biocontrol agents for field application and the pamphlets - pertaining to the cultural operation in local language.

Fortification with bioagents

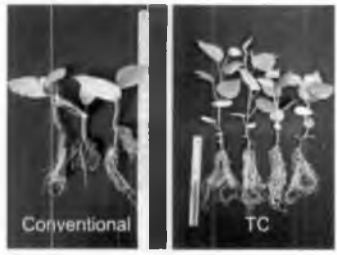
TRAINING TO FARMERS

The selected farmers were educated through group discussions and trainings regarding the management practices for TC black pepper.

PERFORMANCE EVALUATION

The performance of TC derived plants and conventional propagules was evaluated observing the growth parameters and recording them in a preset proforma approved for the purpose. Plant height, no. of leaves. leaf size, inter nodal length, no. of laterals, no. of spikes/unit area, length of spike, berry wt. and volume, litre weight, driage, oil and oleoresin content were the parameters evaluated.

The initial establishment and survival rate of TC plants were far better than the conventional propagules irrespective of variety or location studied. The observation recorded by all the three participating centres in different parts of the country indicated 10 to 20% better establishment for TC derived plants.



The better establishment can be attributed to the better root system and low microbial load on TC derived plants. It was also interesting to observe the faster growth of TC derived plants. This was indicated by better plant height, no. of leaves and early production of laterals-the bearing shoots. Though planting was taken up in different years in different parts of the country, the TC plants out yielded the conventional plants as per the data recorded independently by KAU, IISR & Spices Board.

3 year old TC plants in Kerala

The yield data recorded by KAU also indicate superiority of TC plants over the conventional propagules. TC plants recorded early production of laterals, better no. of spikes/lateral, better spike length and also higher yield. The oil and oleroesin content were on par with the values reported for the variety. More than 50% TC plants started bearing in the second year in Kerala while it was only 35% for conventional propagules. There was an advantage of 76% in no. of laterals, 71% in no. of spikes/unit area and 87% in total yield in the third year. The early bearing in TC plants was also observed in Tamil Nadu, Karnadaka and Assam as per the evaluation report of Spices Board.

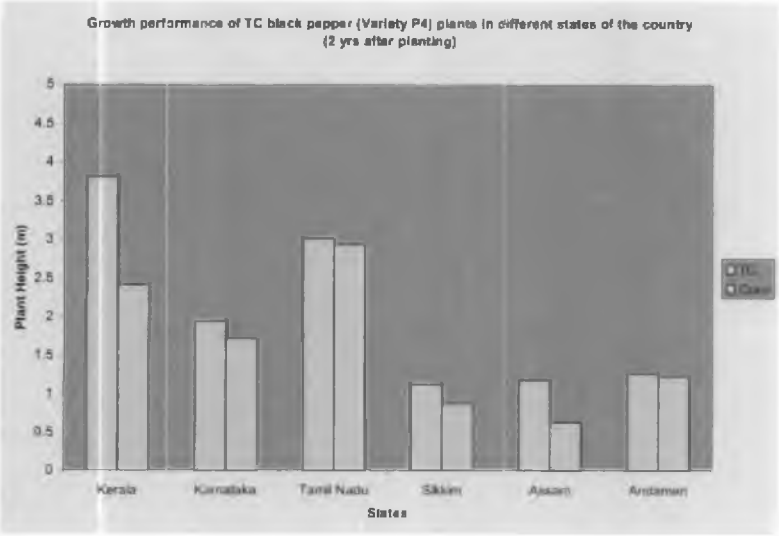
TC Plants in Assam

TC Plants in Karnataka

TC Plants in Sikkim

TC Plants in Meghalaya

TC Plants in Tripura



2 year old TC Plants in Idukki District of Kerala

2 year old TC Plants in Palakkad District of Kerala

The nature of growth in other parts of the country also indicates better advantage for TC plants and yield data are yet to be recorded, since the units in these area completed only two year of vegetative growth.

The better performance of TC derived plants was observed irrespective of the varieties, locations, standard used and soil types. They have many advantages over rooted cuttings in the field establishment and subsequent growth performance that contribute to yield.

The onfarm trials highlight the viability of tissue culture technology for mass multiplication of black pepper and to make the 'black gold glitter' rather than perish due to senility and microbial infection.

THE FACTORS UNDERLYING SUCCESS

The success story could be viewed in two different angles, the success in project implementation and the success of TC black pepper.

The project monitoring system

Department of Biotechnology constituted 2 committees for the project evaluation- the Programme Monitoring Committee (PMC) and the Scientific Advisory Committee Consortium on Micropropagation Research and Technology Development (SAC CMRTD). The project Monitoring Committee under the chairmanship of Dr. C.K. George with members from DBT and representatives from different states had regular review meetings at different participating centres. The teams visited different plots under evaluation for critical reviews and mid term corrections.

PMC review

SAC members visiting farmers field

The SAC-CMRTD under the chairmanship of Dr. P.S. Rao evaluated the project once or twice in an year. The principal Investigators regularly reported to this committee and the progress report presented at DBT.

Despite all the support given by DBT, the creative suggestions and mid term corrections given by Dr. Renu Swarup, Advisor, DBT was the key factor that led this project to a success story.

THE IMPLEMENTING CENTRES

The co-ordinated effort of the three implementing centres KAU, IISR & Spices Board provided a strong basement for the project implementation. The research facilities at KAU & IISR and Development activities of Spices Board helped a lot for its smooth functioning. The Institutional support from all the three centres also contributed much for the distribution of plants and evaluation of its performance all over the country. The co-ordinator Dr. K.V. Peter had a pivotal role in its successful implementation.

THE SUCCESS OF TC PEPPER

Till 1995, the totipotency was little exploited in black pepper due to the high phenol content and systemic bacterial contamination. The advantage of tissue culture was also not much envisaged in black pepper which is a vegetatively propagated crop in which elite types could be multiplied through rooted cuttings.

Critical evaluation of the current status of black pepper production in the country warranted something new that could save the crop through replacement of old and senile vines with disease free healthy plants. Though the protocols developed was not so perfect as it was for banana or orchids, the performance of TC derived black pepper plants indicated an initial advantage in the on farm trials.

Healthy plants with little microbial load and with a very good root system made them do much better than the conventional propagules. A single TC plant per standard can perform much better than 3-4 conventional plants per standard. This holds good in all soil types in all types of standards and in all parts of the country.

Thus the TC black pepper is really successful indeed!!

Urgent efforts are to be put for its large scale multiplication and distribution.

ACKNOWLEDGEMENTS

The Investigator group at the three participating centres gratefully acknowledge the financial support and encouragement extended by Department of Biotechnology, Govt. of India. The implementation of such an extensive programme in the traditional and non traditional areas of pepper cultivation with participation of farmers, scientists & private agencies was possible only with the technical and administrative support from the Directorates of all the three implementing centres.