Standardization of DNA extraction procedure from single potato cyst nematode (*Globodera spp*) and whitefly (*Bemisia tabaci*)

Molecular techniques have become an important tool for the identification of species and races of insect pest and for that purpose various PCR-based techniques are applied. The basic requirement for such technique is isolation of quality genomic DNA and this becomes more challenging in case of tiny insects such as single whitefly and potato cyst nematode (PCN). In the PCN aspect, both the species (*Globodera rostochiensis* and *G. pallida*) comprises eight pathotypes (Ro1 to Ro5 of *G. rostochiensis* and Pa1 to Pa3 of *G. pallida*) therefore, identification of pathotypes using single cyst is important for the accurate results. There are various kits available for isolation of DNA from animals but did not provide satisfactory results in case of single PCN and whitefly and the cost of isolation of per sample remains always high. Apart from DNA extraction kits there are various conventional methods for extraction of DNA from single whitefly and PCN which is time consuming and does not yield satisfactory results during PCR reaction possibly be due to their small size. The efforts were made to standardized DNA isolation using Phenol/chloroform with a few modifications. Single cyst of PCN/ whitefly was crushed in the 200µl extraction buffer (TRIS HCl 10mM (pH-8), EDTA 5mM (pH-8), NaCl 50mM and 10mM β-mercaptethanol) using mortar & pestle. The homogenate was then transferred to the 1.5 mL eppendorf tubes and mixed with 100µl SDS (10%). The homogenate was incubated in dry bath at 65°C for 30 min. The lysate was then added with 50µl Potassium acetate and kept in ice for 10 minutes and centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was collected and placed in the separate collection tube and 140µl Isopropanol was added to the supernatant and kept for precipitation at -20°C for 25 min.

After that it was centrifuged at 10,000 rpm for 10 min. The supernatant was discarded and the pellet was re-suspended with 50µl TE buffer. Equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added and mixed thoroughly by inverting at least for 15-20 times and centrifuged at 10,000 rpm for 5 minutes. The upper aqueous layer was collected and precipitated with the 1.5 volume of ethanol and centrifuged at 10000 rpm for 5 minutes and the supernatant was discarded. The pellet was air dried, dissolved in 10 µl of nuclease free water and stored at -200C for further use. The DNA concentration and purity of samples was measured...
using nanodrop. The concentration of the DNA in the sample was 500 ng/µl for single PCN cyst and 300 ng/µl for whitefly. Purity of the DNA was determined by taking absorbance at A260/A280 ratio for protein and A260/A230 ratio for RNA impurities. The values recorded for protein and RNA impurities of PCN and whitefly are 1.78 & 1.78 and 2.05 & 2.07, respectively. PCR analysis was performed using 200ng DNA of single cyst of *G. rosotochiensis*, species specific forward (5’-GCAGTTGGCTAGCGATCTTC-3’) and reverse primers (5’-TGTTGTACGTGCCGTACCTT-3’) which generated 238 bp amplicon (Mulholland et al., 1996) for *G. rosotochiensis*. The DNA of whitefly (100ng) were used for PCR and approximately 850 bp of the mtCOI gene fragment was amplified using forward primer C1-J-2195 (5’-TTGATTTTTTGGTCATCCAGAAGT-3’) and reverse primer L2-N-3014 (5’-TCCAATGCACTAATCTGCCATATTA-3’).

Aarti Bairwa, Kailash Naga, Bhawna Dipta, Sanjeev Sharma, EP Venkatasalam & Priyank HM

**Development of event PCR protocol for characterization of ToLCNDV transgenic potato events**

In an earlier studies, developed transgenic potato resistance to *Tomato leaf curl new Delhi virus* (ToLCNDV) causing apical leaf curl disease employed RNAi technology using replicase gene. Series of clonally propagated plants have been evaluated for their bio-efficacy against ToLCNDV in terms of inherent gene expression, integration as well as bioassay. To know the chromosome location of the integrated gene/T-DNA in the identified event, performed flanking sequence analysis using genome walking method in the selected promising ToLCNDV transgenics events KPLC2-53 (Kufri Pukhraj event) and GTLC2-127 (Kufri Badshah event). The Right border flanking sequence of the event KPLC2-53 showed 99% homology with the “*Solanum lycopersicum* chromosome 7” and that of event GTLC2-127 showed 98% similarity with “*Solanum tuberosum* group chromosome 11”. Based on the flanking sequence information designed primer to reconfirm the site of integration and specificity of flanking sequence. The designed primer is flanked to potato genome and T-DNA region of pBINAR. The authenticity of the identified sequences was confirmed in the selected events by event PCR using primers specific to the pBI121 T-DNA region and flaking sequence (potato genome sequence). The expected amplified product i.e 539 bp from KPLC2-53 and 756 bp from GTLC2 -127 in the selected events and the absence of the fragments in other events confirmed the integration of the T-DNA/transgenic DNA in the potato chromosome. Using flanking sequence, the standard genetic code will be used to determine whether these amino acid sequences were identifiable proteins from potato, each of them was used as a query sequence in the BLASTP sequence matching algorithm, using the default parameters and searching the non-redundant protein sequences database and species name *S. tuberosum* as a delimiter. Results indicated that no conserved domain have been detected for the obtained flanking sequences and inserted sites have not disturbed the potato genome.

Sundarasha S., Jeevalatha A, Ravinder Kumar, Priyanka Kaundal, Sanjeev Sharma & SK Chakrabarti

**Aeroponic minituber production through TPS**

TPS as a source of plant material for minituber production through aeroponics was tried for the first time. 150 lines were evaluated and 100 minitubers/plant productions were recorded. The TPS of a cross were planted in portrays containing soil coco-peat media (1:1). After one month, each germinated seedlings were transferred for hardening in a glass filled with soil: FYM (2:1) media for vigorous growth thereof. After 15-20 days the plants were ready for transplanting in the aeroponic boxes. The
roots were properly washed and treated with fungicides (Bavistin (1%) +Mancozeb (2%) mixture) to avoid any further media contamination. There was 100% survival of the plants. The first harvesting was done at 28-30 days of transplanting. This technique could serve the purpose of accelerated breeding to advance the generations in potato breeding.

Tanuja Buckseth, Vanishree G, Rajesh K Singh, Jagesh K Tiwari, Ashwani K Sharma, VU Patil & Vinay Bhardwaj

Development of a visual detection method for Potato virus M by reverse transcription loop-mediated isothermal amplification

The viral diseases of potato can be effectively managed by planting of healthy and virus free seed potato. This is possible only after deployment of effective detection method. Double antibody sandwich ELISA (DAS-ELISA), reverse transcription- polymerase chain reaction (RT-PCR) and multiplex RT-PCR are being used to detect PVM. Although, these techniques are robust, sensitive and efficient, but they are time consuming normally requiring two days. Now a days, reverse transcription-loop mediated isothermal amplification (RT-LAMP) is being used to detect viruses due to its simplicity, rapidity, high sensitivity and specificity. The main objective of this study was to develop RT-LAMP for detection of PVM in leaves and tubers and to prove its advantage over the RT-PCR. To optimize the RT-LAMP reaction, two sets of six novel primers (F3, B3, FIP, BIP, LF and LB) were designed based on highly conserved sequence of coat protein (CP) gene of PVM. RNA extraction was done followed by cDNA synthesis and RT-LAMP was carried out under isothermal conditions with Bst DNA polymerase. The assay was optimized using different concentrations of primers, MgSO4, betaine, dNTPs and Bst DNA polymerase. RT-LAMP assays could successfully detect positive infected plant leaves and tubers samples, considering the time, safety, sensitivity and simplicity. RT-LAMP detected PVM around in 60 min, compared to 150 min for RT-PCR and visual detection of RT-LAMP products was done using the SYBR Green I dye under UV light or ambient light, the additional step of gel electrophoresis was not required. A positive result in the reaction mixture was observed as orange colour with no amplification, whereas turns green with amplification using the dye. The assay successfully detected the virus in infected plants collected from potato fields whereas no cross-reactions were observed with healthy plants and other potato viruses. Overall, the developed assay was speedy, sensitive and convenient and could detect virus in infected potato tubers including asymptomatic plants.

Ravinder Kumar, Priyanka Kaundal, Rahul Kumar Tiwari, Sanjeev Sharma & SK Chakrabarti

ICAR-CPRI Pensioner Portal

ICAR-CPRI Shimla has developed Pensioner’s Portal for the ICAR-CPRI pensioner’s. Pensioner’s portal is a one point solution to the pensioners, which acts as a pensioner’s directory, source of information and grievance redresser. The Pensioners Portal has Pensioner’s Directory where the pensioners can register themselves into the portal by giving details like name, designation at the time of retirement, retirement place, retirement date, PPO number, address, contact number and email-ID. The Pensioners/Users can view the details of pensioners in tabular format. All the circulars and other informations specifically related to the pensioners are uploaded into the portal in the circular section regularly. Feedbacks and any other query related to pensioners can
be easily sorted by using this portal. This portal also has the link to the “Government of India Pensioner’s Portal” where the grievance redressal system can also be used for redressing any kind of grievances. The portal has been designed using the ASP.NET with C# and the database for the same has been developed using SQL Server. This portal is hosted at in-house Application Server maintained at ICAR-CPRI and can be accessed through ICAR-CPRI website (https://cpri.icar.gov.in).

Shashi Rawat, Shefali Sood, VK Dua & SK Chakrabarti

Performance of high yielding table potato cv. “Kufri Mohan” under FLDs in Uttar Pradesh

The Frontline Demonstration (FLD) is one of the important extension approaches evolved by the Indian Council of Agricultural Research for transferring of latest technologies to the farmers. Kufri Mohan, a medium maturing, main season, high yielding table purpose potato variety was released in 2016 for cultivation in Indo-Gangetic plains (northern and eastern). In order to demonstrate the superiority of this variety over Kufri Bahar, the most popular variety in Uttar Pradesh, six FLDs (one each in Saharanpur, Bulandshahr, Agra, Baghpat, Meerut and Kanpur districts of UP) were laid out in 2017-18 crop season and ten FLDs (four in Meerut, three in Hapur, two in Aligarh and one in Muzaffarnagar district) were laid out in 2018-19 crop season. The FLDs were laid out in collaboration with the scientists of Krishi Vigayan Kendra (KVK) of the respective district. Regular advisory services were given to farmers and proper monitoring of the FLD fields were made by the scientists of ICAR-CPRI and KVKS. The performance of Kufri Mohan was better than Kufri Bahar in all the districts. The two years average yield (2017-18 and 2018-19) of Kufri Mohan was 421.1 q/ha which is about 30.4 percent higher than Kufri Bahar (322.8 q/ha). Thus, necessary arrangements should be made by the state and central governments for speedy dissemination and adoption of Kufri Mohan in these areas for improving potato productivity and farmers’ income.

NK Pandey, Pynbianglang Kharumnuid, Ashok K Chauhan, Manoj Kumar, Anuj Bhatnagar & SK Luthra

Transfer of Technology

ICAR-CPRI celebrated fifth International Yoga Day-2019

The 5th International Yoga Day was celebrated on 21st June 2019 at the CPRI, Shimla and its 6 regional stations. In Shimla, two very experienced Yoga teachers from Art of Living, Ms. Dhara Saraswati Ji and Shri Abhay Sharma Ji were invited to conduct the yoga session at Shimla. The Aasans, Pranayam and Dhyan of Yoga were demonstrated by the teachers and performed by about 230 staff members, research scholars of the institute and all participants of the ongoing training at the institute. The yoga teachers also explained the beneficial effects of all the demonstrated Aasans, Pranayam and Dhyan techniques. ICAR-CPRI regional stations at Modipuram, Gwalior, Jalandhar, Ooty, Patna, Kufri also celebrated International Yoga Day. On this occasion experienced Yoga teachers from Patanjali and other Yoga sansthans were invited to conduct the yoga sessions. All Station Heads and their staff members were present in large number on this eve.

Training for stenographers /personal assistants/private secretaries and personal secretaries of ICAR HQ/Institutes

A six days training programme on “Enhancing Efficiency and Behavioural Skills for stenographers/personal assistants/private secretaries and personal secretaries of ICAR HQ/Institutes” was organized during June 20-25, 2019 by ICAR-CPRI, Shimla in collaboration with ICAR-NAARM, Hyderabad. A total of 30 trainees from
different ICAR institutes attended the programme. The core contents of the training programme were noting and drafting, stenographic skills, office procedures, conduct rules, leave rules, official language policy, communication skills, interpersonal skills, computer skills, stress management, motivation and positive thinking and personality development. A number of training methodologies like lecture cum discussion, practical sessions, skill demonstration role play and video film shows, etc were implemented during the training.

Live Phone-in Programme at Doordarshan

Scientists from CPRI, Shimla participated in the live phone-in programmes during April to June, 2019. The details of the topics along with experts are given below.

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<tr>
<th>Month</th>
<th>Topics</th>
<th>Name of the Expert</th>
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| April | Potato varieties and planting in the higher hills of Himachal Pradesh | Dr. Rajesh Kumar Singh  
Dr. Ashwani K. Sharma |
| May   | Cultural operations for potato cultivation in the higher hills of Himachal Pradesh | Dr. VK Dua  
Dr. Jagdev Sharma |
| June  | Potato storage and marketing in the mid-hills hills of Himachal Pradesh | Dr. NK Pandey  
Dr. Brajesh Singh |

Important Meetings, Events & Visitors

Official Language Workshop at ICAR-CPRI, Shimla

A day long Official Language Workshop on “Rajbhasha Niti Evam Karyanvyan tatha Vitt Karya mein Rajbhasha ka Prayog: Kathnayian Evam Samadhan” was organized on 18.6.2019 at ICAR-Central Potato Research Institute, Shimla for the administrative category. Mr. Anil Tripathi, Assistant Director (Official Language) and Secretary Town Official Language Implementation Committee, Shimla and Mr. Zakir Hussain, Sr. Finance & Accounts Officer, ICAR-CPRI, Shimla were the Chief Speakers for this workshop. Workshop was inaugurated with address of Chief Guest, Dr. Brajesh Singh, Officiating Director, ICAR-CPRI, Shimla. Chief Guest welcomed the speakers and appealed the officers/staffs to participate in the workshop with great enthusiasm. On this occasion he lauded the efforts made by the Hindi Section in promoting the Hindi usage in the institute and congratulated for bagging the 3rd Rajbhasha Puruskar 2017-18 of the Department of Official Language, Ministry of Home Affairs, Govt. of India, for best implementation of Official Language policy at the institute. Assistant Director (OL) and Secretary, Town Official Language Implementation Committee, Shimla in his address focused elaborately on the provisions made in Indian Constitution Part 5, 6 and 17 and total 11 Articles mentioned therein with regard to Official Language policy and its implementation. Mr. Zakir Hussain Khilzi, Sr. Finance & Accounts Officer in his address discussed in details on use of Official Language in financial works/transaction and also the problems arised there from and solutions thereof. The workshop concluded with the address and vote of thanks from Dr. Rakesh Mani Sharma, In-charge, Official Language, ICAR-CPRI Shimla.
## Human Resource

### Technical

#### Promotions


#### Retirements/Resignation


### Administrative

#### Promotions

2. Granted 3rd MACP to Sh. Amar Chand, Assistant, ICAR-CPRI, Shimla w.e.f. 19.08.2019.
7. Granted 2nd MACP to Smt. Sneh Lata, UDC, ICAR-CPRI, RS, Modipuram w.e.f. 05.05.2019.

#### Transfers

3. Sh. Ratnesh Kumar, CAO relieved w.e.f. 20.05.2019 to join as Registrar at ICAR-IARI, New Delhi.

### Skilled Supporting Staff

#### Promotions

5. Sh. Ashok Kumar, SSS, ICAR-CPRI, Shimla, granted 2nd MACP w.e.f. 22.4.2019.
7. Sh. Padam Chand, SSS, ICAR-CPRI, Shimla, granted 2nd MACP w.e.f. 22.4.2019.

#### Retirements

India is the second largest producer of potato in the world contributing about 12.52% of production from 11.29% area under potato with an average yield of 22.31 t/ha. Potato is grown in almost all the states in India under diverse agro-climatic conditions in total area of about 2.18 mha. Availability of assured quality seed is the most important production constraint in all the potato growing regions of the country. A well-organized scientific system of Breeder seed production was envisaged in 1962-63 jointly by ICAR and DAC& FW through clonal selection, tuber indexing and field multiplication of healthy indexed tubers in subsequent four generations in the farm of ICAR-CPRI under direct supervision of technically qualified staff. Usually, potato is grown vegetatively using tubers as planting material and the average seed multiplication rate (SMR) is only 1:6. For the last three decades ICAR-CPRI is producing about 3,000 metric tonnes of Breeders seed and supplying about 2,400 metric tonnes each year to the states and other seed producing agencies. If this stock is multiplied in three stages, i.e. Foundation 1, Foundation 2, and Certified grades, about 5 lakh metric tonnes of certified grade seed potatoes can be obtained, which constitutes about 10% of total seed requirement if certified seed is used every year by the farmers. It is virtually impossible to increase the quantity of certified seed being produced through the conventional system due to limitation in terms of available land and manpower.

Keeping that in view, ICAR-CPRI, Shimla has standardized a number of hi-tech seed production systems based on tissue culture and micropropagation techniques. Adoption of these systems of seed production will improve the quality of breeder seed, enhance seed multiplication rate and reduce field exposure of seed crop by at least 2 years. The systems were thoroughly tested at seed production farm of ICAR-CPRI before passing them on to farmers and other stakeholders. The latest hi-tech seed production system standardized by the institute is based on the concept of soil-less, aeroponic technology. The aeroponic system of seed production has the potential to once again revolutionize potato seed sector after about 50 years of introduction of “seed plot technique” by the institute. Just to shorten the span of almost 2 years in the potato breeder seed production and production of clean material are the major advantages of Aeroponic system which could revolutionize the potato seed industry in the country. Certification of seed potato tubers, minitubers and true potato seed is essential so as to ensure seed quality and prevent seed degeneration as well as spread of pests and diseases. Seed certification is managed by Central Seed Certification Board though ‘Seed Act’ which provides a set of rules for regulation of quality and purity of the seed material. The rules framed by the Central Seed Certification Board are followed by the State Seed Certification Agencies for carrying out the certification. The responsibility for the quality standards of seed that enters the trade market lies with the state seed certification agencies. The proposed schematic representation of the seed potato production through micropropagation (involving aeroponic tubers/ minitubers/ microtubers) followed at ICAR-CPRI is: Micro-plants/ micro-tubers (Pre-nucleus Seed) –to- Aeroponically produced minitubers/ minitubers under net house (Nucleus seed/ (Gen. -0) -to- Field Generation-1 (Gen.-1) Pre-breeder seed – Stage-III –to- Field Generation-2 (Gen.-2) (Breeder/Basic seed) Stage-IV -to- Field Generation-3 (Gen.-3) (Foundation seed-1) -to- Field Generation-4 (Gen.-4) (Foundation seed-2) –to- Field Generation-5 (Gen.-5) (Certified seed). Under the proposed scheme a total of 5 field multiplications are involved for the production of certified seed potatoes involving hi-tech system in the country. Its adoption by all agencies shall help in enhanced availability of certified seed for the country.
Global Potato Conclave 2020
Roadmap for a Better World

Mahatma Mandir, Gandhinagar, Gujarat, India
28-31 January, 2020

Organizers