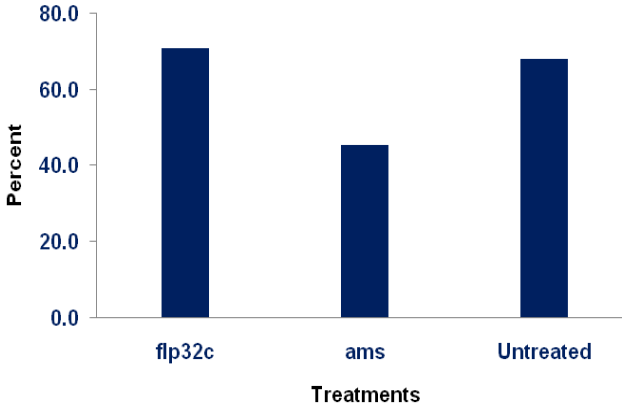
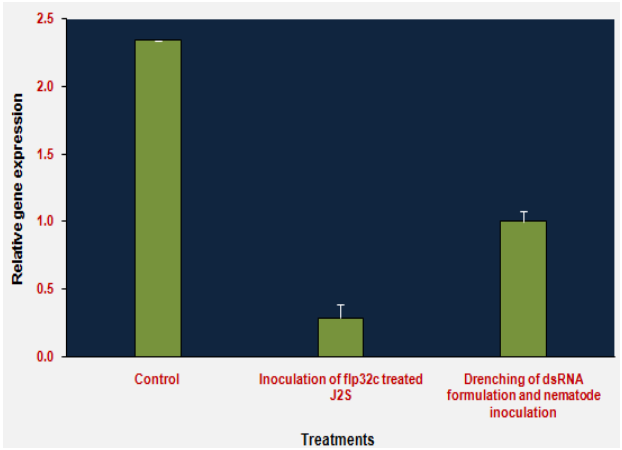
Number 80 *April-June, 2020*

Research Highlights

Novel dsRNA based nematicide formulation for the management of potato cyst nematodes (*Globodera* spp.)

The potato cyst nematodes (PCN) are major pests of potato worldwide with quarantine nature. Among the various management strategies evolved, host resistance is the most desirable and effective however, development of new virulence within the species makes resistant cultivars susceptible. Therefore, incorporation of blend of various management options like host resistance, chemical, biological and cultural methods is being advocated for profitable potato cultivation. However, farmers are mainly relying upon chemical control of PCN involving very harmful pesticides, though these are not feasible in long term due to the increasing concern about environment, hence requires knocking of eco-friendly management strategies. New novel gene targeted management strategy using dsRNA based nematicide formulation is an exciting and promising option. Based on the results of the RNAi approach, dsRNA based nematicide formulations were developed for selected pathogenecity genes responsible of locomotory [*flp*-32(c)] and sensory function (ams-1). The dsRNA based nematicide formulation was drenched in three week old plants in pots. The drenching of dsRNA in pot and inoculation of J_2s resulted increased infestation in case of flp32c, dsRNA drenched pot (71.0%) as compared to untreated control (68.0%). In addition, there was no further development of J_2s and resulted premature death. Whereas, ams-1 dsRNA treated pot resulted IN reduced infestation (45.5%) as compared to untreated control. The reduced infestation in case of ams1 dsRNA treated pot was due to silencing of sensory organs gene which is responsible for finding out the host. Further relative gene expression based on qRT-PCR analysis between untreated control, dsRNA treated nematodes and drenching of dsRNA formulation followed by nematode inoculation were determined using an unpaired two-tailed Student's





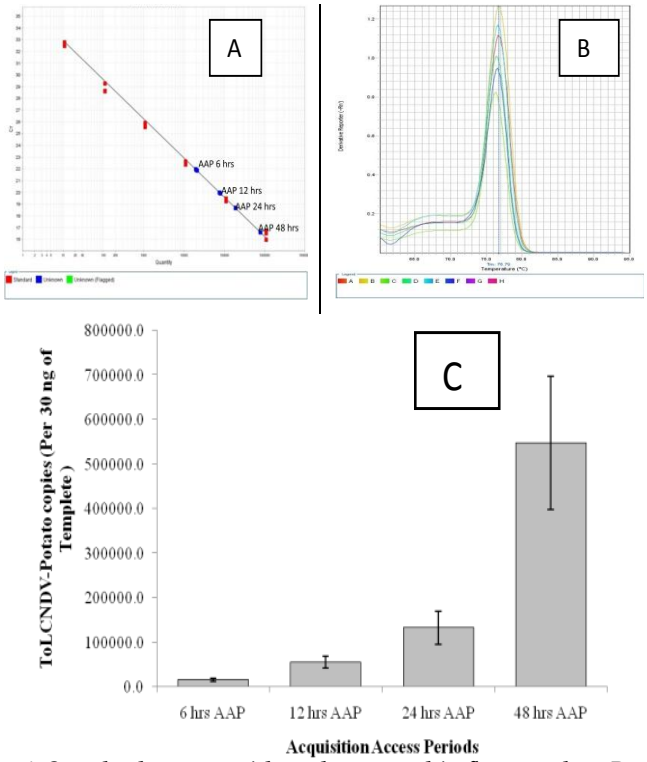
RT-PCR analysis of flp32c gene expression

t test (***, P\0.001). Compared to dsRNA treatment, there was a significant increase in level of the transcript in untreated control roots (> 7 fold) according to ABI real time SD RQ manager. Which shows that after silencing the relative expression was less in ds-RNA treated as compared to untreated control.

Aarti Bairwa, S Sundaresha, E P Venkatasalam, Bhawna Dipta & Sanjeev Sharma

Methodology for Absolute Quantification of *ToLCNDV- Potato* in Whitefly

The absolute quantification protocol using qPCR allows to determine the number of genomic copies (genomic units) of virus present in any experimental sample. Apical leaf curl disease in potato is caused by a strain of *Tomato leaf curl New Delhi Virus* (*ToLCNDV*) which is posing a serious threat to quality seed production of potato. The whitefly, *Bemisia tabaci* (Gennadius) is responsible for transmission of *ToLCNDV-potato* in circulative and persistence manner. To know the efficiency of any vector it is important to know the ability of a vector to acquire virus load.



A.Standard curve with unknown whitefly samples; B-Melt curve of standard dilutions; C- Viral copies in whitefly with different Acquisition Access Periods.

A method was standardized to quantify the absolute load of *ToLCNDV- potato* in the whitefly using standard curve generation. A linearized plasmid containing coat protein gene of *ToLCNDV –Potato* (771 bp) was used to generate the standard curve. The standard was prepared by making six serial dilutions (tenfold) starting with 1.1×10^{-6} copies of the plasmid. Number of copies per ng of plasmid carrying CP gene was calculated using a dsDNA copy number calculator (https://cels.uri.edu/gsc/cndna.html). Virus-specific primer ToLCNDV-CP-Q 5′ TAAGGTGCAGTCCTTTGAATC T3′ and 5′CTCCTCG GGTAACATCACTAAC3′ targeting coat protein gene was used for qPCR.

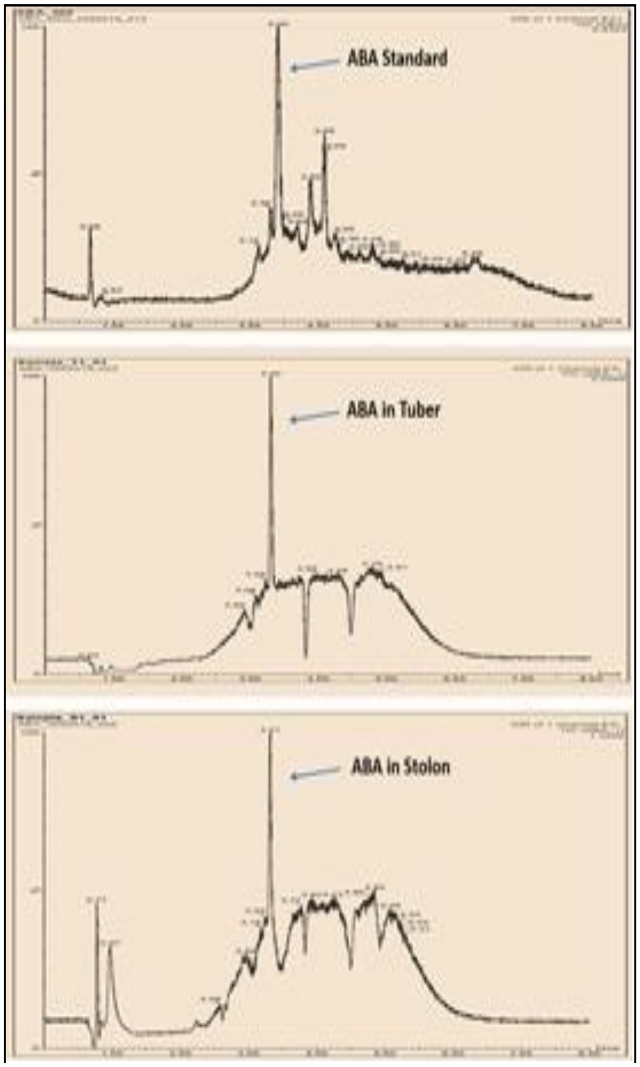
To check the virus titre in whitefly adults were allowed to acquire the virus from *ToLCNDV* positive potato plant at different acquisition access periods (6, 12, 24 and 48 hrs). Whitefly DNA was isolated and used for virus load estimation using Step One Plus real time PCR (Applied Biosystems) along with standard in the same reaction plate. The qPCR reaction was carried out and the virus load was successfully carried out in whitefly; it acquired a 15658.4± 2963.0, 55730.9± 14012.9, 131870.1± 37144.1, 547158.7± 149184.5, numbers of virus copies in per 30 ng of DNA sample at 6, 12, 24, and 48 hrs of acquisition periods respectively. The outlined protocol is potentially suitable for various applications, such as plant breeding for resistance, testing response of various chemicals on virus replication and virus-vector interaction studies.

Kailash Chandra Naga, Rahul Kumar Tiwari, Subhash S, Ravinder Kumar, Aarti Bairwa, Gaurav verma & Sanjeev Sharma

Modified LC-MS method for Abscisic acid (ABA) quantification in potato

Plant hormones play a crucial role in controlling plant growth and development. ABA is required for both the initiation and maintenance of tuber dormancy. Accurate quantification of ABA is required for tuber sprouting studies in potato. An LC-MS based protocol for plant hormones estimation was standardized. Two grams of frozen potato sample was ground to a powder using

liquid N_2 and homogenized in methanol (100%). The samples were extracted and purified at several steps and the dry residue containing hormones was dissolved in 1 ml of mobile phase and injected into LC-MS.



LC-MS chromatogram of ABA in potato

The standards of ABA (Sigma-Aldrich) were run using different concentrations to make standard curve. This method may be used for quantification of ABA in potatoes.

Sushil S Changan, Som Dutt, Pinky Raigond, Dharmendra Kumar, Milan K Lal & Brajesh Singh

Photoautotrophic micro-propagation of sprouts for easy sampling in tuber indexing protocol

Potato tuber indexing is the backbone of disease free quality seed production under conventional system. It involves testing of a single scooped eye out of a 4 clone set belonging to Stage I tubers for freedom from viruses using PCR and ELISA techniques. These scooped eyes are grown in pots during May to June after chemically breaking the dormancy. However, in the North-western plains, this period is marked by drastic rise in temperatures, which causes appearance of soft rot in scooped eyes grown in pots, despite rigorous temperature regulation under hardening chamber. Due to the small size of scooped eye plug the disease becomes difficult to manage and valuable testing material is lost during this stage, posing a bottleneck in further selection of respective clones for planting in stage I, which in turn have to be rejected in the absence of reliable testing. As such, not only the reliability of results gets reduced but only 60—70% of the clones are effectively tested for the disease.

Sprouts cultured on defined PAM medium for initiating growth on day 1 after culture

Keeping this in view, a methodology of photoautotrophic micro-propagation (PAM) of sprouts was developed. The sprouts were simply excised out of sprouted tubers using a sterilized scalpel and cultured in sugar free MS medium on sterilized coco peat medium under artificial growth conditions with aprox 50-60 µmol/m2/s PAR from white fluorescent lights, 22 ± 2° C temperature and 16 hr photoperiod in magenta

boxes with closed lids. Response of the six varieties Kufri Jyoti, Kufri Badshah, Kufri Khyati, Kufri Chandramukhi, Kufri Surya and Kufri Himalini were evaluated under this system. This was compared to the growth parameters of scooped eyes planted in pots kept under net house. It was observed that the sprouts showed vigorous growth under photoautotrophic control conditions, requiring minimal manual management until the shoots reached the height of the container (magenta box), by 6-7 days when the lids were opened to allow further growth upto 20 days when

Cultured sprouts after 20 days of planting using the PAM technique. Sprouts cultured on defined PAM medium for initiating growth on day 1 after culture

ample sample could be withdrawn for testing. In comparison, to the varieties planted in pots, the PAM cultured sprouts showed 4.5 and 2.7 times higher shoot lengths at 10 and 15 days averaging 6.31cm and 7.81cm, respectively. Besides, these plants had an average of 6 leaves at 15 days. The samples were ready to be tested by 20 days after planting. The PAM conditions promote the sprouts to grow photoautotropically/ naturally as compared to use of conventional method where scooped tuber portion is used as the source of nutrition for initial growth of sprouts.

The most important benefit of the technique is that the testing conditions are controlled eliminating the inconsistency arising due to appearance of diseases under hot weather conditions. It is a simple and clean procedure ensuring phytosanitary conditions with 100% survival of the sprouts under testing, which can be easily adopted. Using the technique testing time can be easily be delayed until natural sprouting is achieved, eliminating the role of chemicals for dormancy breaking. Based on these observations, this methodology is proposed for indexing of tubers for disease free seed production.

RP Kaur, MA Shah, AK Singh, Raj Kumar & RK Singh

Value added product: Gluten-free potato muffins

Muffins are a small cake type baked product usually made from a mixture of wheat flour, buttermilk, curd, milk, egg, sugar, salt, shortening, and baking powder. More than a thousand variants of the muffins are being commercially prepared worldwide. To target the present post-harvest losses in potatoes, ICAR-CPRI also has developed and standardized the process for chemically leavened potato-based gluten-free muffins having more than 60% of potato flour. The process technology involves batter preparation, moulding and then baking. At ambient temperature conditions, the shelf life of the muffin is 3 days.

By using permitted natural or synthetic preservatives the shelf life can be extended up to 15-20 days. Interestingly potatoes of any shape,

Gluten free potato muffins

size, colour, sugar and dry matter can be used for the preparation of muffins. Moreover, partially damaged or cold-stored potatoes can also be utilized. Such type of products is consumed by the

population of all age and income groups. Therefore, there will be huge business opportunities. Technology is ready for commercialization and can be easily adopted by the processors involved in the production of bakery items.

Arvind Jaiswal, Pinky Raigond, Milan Kumar Lal & Brajesh Singh

Live Phone-in Programme at Doordarshan

Scientists from ICAR-CPRI, Shimla participated in the live phone programme during April-June, 2020. The detail of the topic alongwith experts are given below:

Month	Topics	Name of the Expert
June, 2020	Different potato varieties in Himachal Pradesh	Dr. NK Pandey

Important Meetings, Events & Visitors

26th Regional Committee Meeting for region-1 held through Video Conferencing

ICAR-CPRI, Shimla organised 26th Regional Committee-I meeting virtually of the Indian Council of Agricultural Research on 30th June, 2020 through video conferencing. The committee covered two states i.e. Himachal Pradesh and Uttrakhand and two union territories i.e. Jammu & Kashmir and Laddakh. The regional Committees meet biennially to discuss and review the current

status of agricultural research, education and extension to critically examine various problems faced by the region either in the execution of the approved programmes or in tackling the emergent problems and identify gaps for research by Agricultural Universities or in transfer of known technologies by extension agencies. The committee provides a good forum for a meaningful dialogue amongst research and development agencies and also to bridge the gap between research and extension in the fields of agriculture, horticulture, animal husbandry, fisheries and agro-forestry. It helps in forging an effective liaison and coordination amongst ICAR Institutes, State Agriculture Universities and Departments of Agriculture, Horticulture, Animal Husbandry and Fisheries of the respective state governments/ UTs. This year almost 170 participants took part in this meeting. The meeting was chaired by Dr. T. Mohapatra, DG, ICAR and Secretary, DARE, Govt.

of India. On this occasion Hon'ble Minister of State for Agriculture and Farmers Welfare, Govt. of India Sh. Parshottam Rupala and Sh. Kailash Choudhary were present. The chief guest of the meeting Sh. Narendra Singh Tomar Hon'ble Minister of Agriculture and Farmers Welfare, Govt. of India in his presidential address stressed upon organic farming and value addition for enhancing farmers' income. Sh Subodh Uniyal and Sh. Ram Lal Markanda the Ministers of Agriculture of Uttrakhand and Himachal Pradesh respectively also elaborated about the farmer's situation, their problems and ways for enhancing farmer's income. The Vice Chancellors, Directors Research, Scientists from Himachal Pradesh, Uttrahkand, Jammu & Kashmir and Laddakh, Member ICAR General Body, Farmers from all four states/UTs were also present in the meeting. Several recommendations emerged out after

thorough discussion. Publications related to package of practices for respective states were also released during this meeting. Virtual plantation at ICAR-CPRI, Shimla was also organised. The meeting ended with a vote of thanks to the chair person.

International Day of Yoga (IDY) Celebration at ICAR-CPRI, Shimla

International Day of Yoga (IDY) was celebrated by all staff members of Institute along with their family members on 21st of June 2020 from their respective homes due to COVID-19 pandemic in the county. Two experienced Yoga teachers (Art of Living), Ms. Dhara Saraswati Ji and Shri Abhay Sharma Ji were invited to conduct the yoga session. The Aasans, Pranayam and Dhyan of Yoga were demonstrated online by the teachers and performed by all staff members, research scholars 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. Various online resources pertaining to IDY available on the Ministry of AYUSH website were utilized by several staff members for performing the Yoga. In total, around 400 persons of the Institute participated in the IDY-2020 celebrations.

Human Resource

Scientific

Transfers

1. Ms. Preeti Singh, Scientist, ICAR-CPRI, Shimla relieved on 19.6.2020 to join at ICAR-IARI, Goria Karma, Hazaribagh, Jharkhand.
2. Dr.(Mrs.) Pooja Praful Mankar, Scientist, ICAR-CPRI, Shimla relieved on 20.6.2020 to join at ICAR-CPRI RS, Modipuram.
3. Dr. Subhash S, Scientist, ICAR-CPRI, Shimla relieved on 20.6.2020 to joint at ICAR-CPRI, RS, Modipuram.

Technical

Promotions

1. Sh. Yogesh, ACTO, ICAR-CPRI, Shimla promoted to CTO w.e.f. 01.02.2019.
2. Sh. Arjun Kumar Sharma, Sr. Tech. Officer, ICAR-CPRI, RS, Patna promoted to ACTO w.e.f. 08.06.2019.
3. Sh. Akhilesh Kumar Singh, Sr. Tech. Officer, ICAR-CPRI, RS, Jalandhar promoted to ACTO w.e.f. 24.07.2019.
4. Sh. Avinash Chaudhary, Sr. Tech. Officer, ICAR-CPRI, RS, Modipuram promoted to ACTO w.e.f. 30.06.2019.
5. Sh. Subhash Chand, Sr. Tech. Officer, ICAR-CPRI, RS, Modipuram promoted to ACTO w.e.f. 16.09.2018.
6. Sh. Krishan Pal Singh, ACTO, ICAR-CPRI, RS, Modipuram promoted to CTO w.e.f. 12.03.2019.

Transfers

1. Smt. Nisha Verma, Hindi Translator, ICAR-CPRI, Shimla transferred to ICAR-CPRI, RS, Gwalior on 12.06.2020.

Resignations

1. Sh. Rajat, Technical Trainees, ICAR-CPRI, Kufri resigned from Councils service on 23.6.2020 (AN)

Administrative

Promotions

1. Sh. Ashish Kalyan, LDC, ICAR- CPRI, Shimla promoted to the post of UDC w.e.f. 06.06.2020.
2. Sh. Girish Thakur, Stenographer Grade-III, ICAR-CPRI, Shimla promoted to the post of Personal Assistant w.e.f. 26.06.2020.

Retirements

1. Sh. Jai Ram Thakur, AAO, ICAR-CPRI, Shimla retired on 30.04.2020.
2. Smt. Bimla Salhotra, UDC, ICAR-CPRI, RS, Jalandhar retired on 30.05.2020.

Skilled Supporting Staff

Retirements

1. Sh. Kailash, SSS, ICAR-CPRI, RS, Gwalior, retired on 30.04.2020.
2. Sh. Shiv Singh, SSS, ICAR-CPRI, RS, Gwalior, retired on 30.04.2020.
3. Sh. Madan Lal, SSS, ICAR-CPRI, RS, Kufri, retired, on 30.06.2020.

From the Director's Desk

Potato (*Solanum tuberosum* L; 2n = 4x = 48) belongs to the family Solanaceae that includes more than 2000 species, in which about 200-250 species are of potato. The genus Solanum is a reservoir of genetic resources of wild and cultivated species ranging from diploid to hexaploid. Potato is a tetraploid crop with complex tetrasomic inheritance and suffers from acute inbreeding depression on selfing. On the contrary, it is highly amenable to tissue culture and other biotechnological tools that can be readily used for improvement of this crop. A large number of biotechnological tools like tissue culture and micropropagation, QTL mapping, association mapping, gene tagging, marker assisted selection, transgenics, RNAi and its application, genome sequencing, high-throughput genotyping, genotying by sequencing, transcriptomics, proteomics, metabolomics etc. have been used in India for improvement of potato; and more recently attempts are also being made on genomic selection and genome editing technologies at ICAR-CPRI, Shimla. These techniques have led to better understandings of problems associated with biotic and abiotic stresses, quality characters, tuber traits and yield. Molecular markers have been used for various applications like genetic diversity analysis, mapping, marker-assisted selection (MAS), genetic stability, gene tagging, development of markers for various traits, genetic fidelity study, germplasm characterization etc. A new potato cv. Kufri Karan has been developed using MAS and released recently. SSR markers have been developed for fingerprinting of potato varieties and wild species. Advanced hybrids (LBY-15 & LBY-17) having combined resistance to late blight and PVY have been developed through marker-assisted breeding. Also identified potato genotypes having multiple resistance genes of late blight (R1&R3), PVY (Ryadg) & cyst nematodes (HC, H1 & Gro1) using molecular markers.

The genome sequence of potato was deciphered using a homozygous doubled monoploid (DM1-3 518 R44 or 'DM') as well as a heterozygous diploid line (RH89-039-16 or 'RH') by The Potato Genome Sequencing Consortium where ICAR-CPRI was also a partner. This resulted in annotation of 31,039 protein coding genes, which has opened up new opportunities to rapidly identify candidate genes in regions associated with traits of interest. Further, it has facilitated application in functional genomics to discover novel genes and markers for potato improvement. Besides, resequencing of wild potato species and sequencing of various pathogens have been undertaken at the institute. It resulted in elucidation of genome sequences of the dihaploid potato 'C-13', *Phytophthora infestans* (late blight), *Ralstonia solanacearum* (bacterial wilt), *Rhizoctonia solani* (stem canker), *Fusarium sambucinum* (dry rot) etc. The sequence data provided a catalogue of candidate genes for multiple traits. Moreover, targeted re-sequencing of many wild species of potato enabled identification of several other genes in potato. Genome wide expression profiles of potato using RNA-sequencing and microarray technologies have been used at the institute to discover genes for various traits like late blight resistance, apical leaf curl virus resistance, bacterial wilt resistance, heat and drought stress tolerance, low and high N conditions etc. Microarray techniques have been applied in potato varieties and somatic hybrids for understanding late blight resistance and tuberization. Development of SNP markers/chip is in progress for high-throughput genotyping application like 8.3 K or 20K SNP array, Illumina developed at global level. These innovative biotechnological applications would certainly make it easier for genetic enhancement and variety development of potato in the present century thus increasing production and productivity of this crop to meet the sustainable development goals and ensure food and nutritional security of the world.

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