eTechnical Bulletin No. 4
(Ref.: No.F.PME/3-3/2021 dated 4.7.2021)

Potato Transgenics

Authors

Jagesh Kumar Tiwari

Sundaresha S

Vinay Bhardwaj

Sanjeev Sharma

Rajesh Kumar Singh

SK Chakrabarti

Manoj Kumar

ICAR - CENTRAL POTATO RESEARCH INSTITUTE, SHIMLA (HP)

1. Genetic transformation

1.1 *Agrobacterium tumefaciens* mediated transformation

Availability of suitable regeneration protocol is a pre-requisite for undertaking genetic transformation and target specific genome editing work in any crop. A rapid and efficient *Agrobacterium tumefaciens* mediated transformation protocol based on direct organogenesis from inter-nodal stem explants of *in vitro* potato plants has been standardized. It ensures development of large number of transgenic plants in a short period of 4-6 weeks without formation of intermediary callus phase. The protocol gives about 70% regeneration efficiency and is being used routinely for transformation work at ICAR-CPRI, Shimla. Using the procedure, the institute has developed many transgenic potato lines possessing resistance to various biotic and abiotic stresses which are discussed in the later sections.

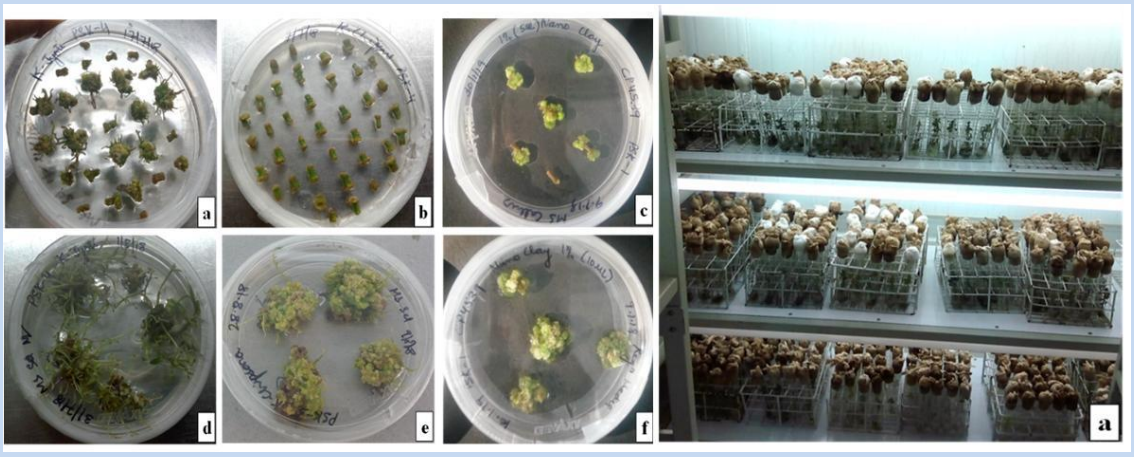

Fig. 1. Callus induction and shoot regeneration after transformation via *Agrobacterium* mediated transformation in potato

1.2 Gene gun mediated transformation

Biolistic (biological ballistics) plant transformation was initially developed in 1987 for transformation of monocots but later the technology was used in most crops. Gene gun is very much required for the protoplast transformation in any crop including potato. A gene gun-mediated transformation protocol has been successfully developed in potato at the institute. Though, potato is amicable to the *Agrobacterium* mediated or indirect method of transformation, use of gene gun not only must for plastid transformation but also enhances the transformation efficiency in general. Here tungsten or gold particles coated with the DNA are accelerated to a high speed to bombard the target tissue. The technique has been successfully used to transfer the plastid specific cassette for tuber-specific expression of *cry1Ab* and a fused *cry1Ab+cry1B* genes to develop transgenic potato resistant to potato tuber moth.

Fig. 2. Gene gun used for genetic transformation in potato

2. Gene cloning

The institute has adequate facility and expertise for cloning and characterization of genes, designing gene constructs for plant expression and also for gene silencing through RNAi technology. Most of the targeted gene sequences being used for genetic transformation have been cloned and characterized at the institute level. An osmotin-like gene has been cloned and sequenced from the late blight resistant wild potato species *Solanum chacoense*, which has been used for developing transgenics with late blight resistance and tolerance to water stress. Similarly, the invertase inhibitor genes (*inhh)* from tobacco has been cloned, sequenced and used to develop transgenics to reduce cold-induced sugar accumulation in tubers. Numerous other genes imparting heat tolerance (*HSP17*), abiotic stress tolerance (*StSOD*), tuberization (*StSP6*), anthocyanin synthesizing (*StAN*), vitamin C regulating (*StGalDH*), photoperiod regulating (*StCDF1*) etc. have been cloned, sequence characterized and being utilized for transgenics development.

Post-transcriptional gene silencing (PTGS) or RNA interference (RNAi) is a newly discovered mechanism of gene regulation in eukaryotes. In plants PTGS acts as surveillance system against the invading molecular parasites like viruses, transposons and transgenes. PTGS is induced by double-stranded RNA (dsRNA) intermediates. An endogenous multi-component enzyme complex called DICER degrades the dsRNA into 21-25 nucleotides small RNAs called small-interfering RNA (siRNA). siRNAs then guides another multi-component protein complex called RNA-induced silencing complex (RISC) for degradation of homologous mRNA. Because of consistent and profound inhibition of gene expression, PTGS is now being utilized for transgenics development against diseases causing pathogens by introduction of desirable trait (inhibiting unwanted gene expression) and functional genomics for elucidation of gene function. The institute has successfully utilized RNAi technique for reduction of cold-induced sweetening and development of fungi, virus and bacterial diseases resistant potato transgenics. A novel and efficient strategy was developed

and employed to inhibit potato vacuolar invertase by PTGS through expression of partial cDNA sequence of vacuolar invertase gene in inverted repeat orientation so that a double-stranded hairpin gene sequence of vacuolar invertase is expressed in transgenic potato lines for silencing of expression of potato vacuolar invertase gene at the post-transcriptional level and consequent reduction of cold-induced sweetening in potato. The steps involved in development of vector cassette for expression of hairpin gene construct are as follows:

RT-PCR amplification of partial cDNA sequence; ligation the partial gene sequence in inverted repeat orientation under the control of either constitutive or tuber-specific promoter, potato transformation with the gene construct and evaluation of the transgenics.

Similarly, PTGS has been targeted for *avr3a* gene against late blight disease caused by *Phytophthora infestans* and phosphatidic acid phosphatase 2 (*PAP2*) gene against *Ralstonia solanacearum* causing bacterial wilt of potato. Several viral gene sequences have also been cloned and characterized. The coat protein gene from an Indian isolate of potato leaf roll virus (PLRV) has been targeted through PTGS for imparting resistance. Similarly, the coat protein gene of PVY, PLRV and potato apical leaf curl virus (PALCV), replicase associated protein gene of PALCV, and movement protein gene of potato stem necrosis virus (Agarwal *et al.* 2007) have been cloned and sequenced and being used for development of virus resistant transgenics by RNAi technology.

To increase the harvest index in potato in hills during long days (summer season) along with high temperature where potato plant tends to grow more vegetatively than to divert the energy towards tuber, the GA_{20} Oxidase gene has been silenced using the RNAi construct. As a result dwarf potato transgenics with improved yields and harvest index have been developed. Genes encoding two putative protein subunits of potato ribonuclease P (RNase P) were cloned and characterized. Based on the ORF search complete cDNA sequences of putative potato RNase P protein subunits of *Pop5* and *Rpp25* were deduced and used for their over expression in *Escherichia coli* using N-terminal 6x his tag proteins using over-expression vector pQE 30 (Qiagen). Polyclonal antisera were developed against purified recombinant potato *Pop5* and *Rpp25* to be used for western blotting. The institute has developed easy and efficient protocols to carry out the southern, northern and western blotting with potato samples which are very much required for characterizing the transgenic events. Besides, a potato-specific plastid transformation vector with homologous target sequence from potato plastome has been designed and utilized for choloroplast transformation in potato. The efficacy of this vector for plastid transformation in potato is being evaluated by biolistic shooting. A gene cassette for tuber-specific expression of *cry1Ab* and a fused *cry1Ab+cry1B* genes has also been designed and used for genetic transformation.

Table 1. Genes/promoters cloned in the institute for use in genetic transformation

SN	Gene/Promoter	Source organism	Trait
1	*cry1Ab*/GBSS	*Bacillus thuringiensis*	Tolerance to potato tuber moth
2	*cry9Aa2*/CaMV35S	*Bacillus thuringiensis* subsp. *galleriae*	Tolerance to potato tuber moth
3	*osmotin*/CaMV35S	*Solanum chacoense*	Tolerance to late blight/salinity
4	*CP*/CaMV35S	Potato leaf roll virus	Resistance to PLRV
5	$glgC^{m}$/CaMV35S	*Escherichia coli*	Increase in starch quantity
6	*AmA1*/CaMV35S & *AmA1*/GBSS	*Amaranthus hypochondriacus*	Improvement of protein quality and quantity
7	RNAi construct of *GBSS*/CaMV35S	Potato	Improvement of starch quality
8	RNAi construct of rep gene	Potato apical leaf curl virus	Resistance to potato apical leaf curl virus
9	Plastid transformation vector (pSKC21)	*Bacillus thuringiensis* subsp. *galleriae*	Resistance to tuber moth
10	Gene cassettes with fused *cry1Ab+cry1B*	*Bacillus thuringiensis*	Resistance to tuber moth

3. Transgenics development

Genetic transformation also called genetic modification has many advantages for plant breeding, and these advantages are even more striking in crops with polyploid complex inheritance such as potato. While conventional breeding manipulates genomes in a largely uncontrolled fashion, requiring generations of selection to assemble and fix the maximum number of desirable traits, transformation offers a direct approach, allowing introgression of a single, distinct gene without linkage drag. Thus, genetic modification allows rapid and often powerful improvement of crop plants, and is not limited by compatibility barriers. In cases where genetic diversity among sexually compatible relatives of crop species is insufficient for a particular trait, genetic modification may represent the only possibility for improvement in that trait. Transformation offers a highly effective means of adding single gene to existing elite potato clones with no or very minimal disturbances. Potato, being highly amenable to genetic transformation, attracts attention of researchers to assess the impact of development of transgenic potatoes harbouring diverse traits. Genetic engineering in potatoes has a rather long history with the first transgenic potato developed about 30 years ago, and it is envisaged that many of the transgenic plant products to be commercialized in the present decade are likely to be potatoes with enhanced characteristics. CPRI has the policy of using genetic transformation technique for improving those traits that cannot be manipulated by conventional breeding. A few priority traits namely durable disease resistance (late blight, viruses, bacterial wilt and soil and tuber borne diseases), pests (aphids, white fly, potato tuber moth) reduction of cold-induced sweetening, yield improvement, nutritional enhancement,

abiotic stress tolerance (heat, drought and nutrient use efficiency) and tuberization under high temperature have been identified by the institute for improvement by genetic engineering.

3.1 Late blight resistant

Late blight caused by the oomycetous fungus-like organism *Phytophthora infestans* is a major disease of potato cultivation. The disease has the history of causing catastrophic famine in Ireland where people depended heavily on this crop. In recent years, India and China emerged as the global leaders in potato production together contributing about 50% of world production. Popularity of potato in these two Asian giants is largely due to remarkable productivity of this crop per unit area and time. If one thing can mar this happy situation, that is an epidemic of late blight. Causes of concerns about this disease in India are already evident. In addition, occurrence of both A1 and A2 mating type of *P. infestans* resulting in sexual reproduction and survival through resilient oospores have been reported that may give rise to immense variability in the pathogen population, thereby endangering durability of a cultivar. Moreover, this population is gradually becoming tolerant to higher doses of the prophylactic fungicides. As a consequence of this hidden but serious population shift in *P. infestans*, Kufri Jyoti, the most popular Indian cultivar, has succumbed to this disease after a sustained performance for about 30 years. The other popular cultivar Kufri Bahar does not have any resistance to *P. infestans*. Together, these two cultivars along with Kufri Pukhraj occupy > 60% of potato area in India creating an imminent danger.

Race specific, major genes from the wild potato species *Solanum demissum* have been extensively used in resistance breeding programmes throughout the world including India. However, efficacy of such major genes had been too short-lived to justify their deployment. Therefore, thrust in late blight breeding has now shifted to deployment of multi-gene, horizontal resistance. Identification of candidate genes responsible for horizontal resistance and their pyramiding is a formidable task that can't be achieved in foreseeable future.

3.1.1 Late blight resistant potato RB transgenics: A major gene (RB) behaving like non-host resistance and effective against all known races of *P. infestans* has been mapped and cloned by two independent groups in the USA and the Netherlands. The RB gene has withstood the onslaught of *P. infestans* for more than five decades and transgenic clones of the potato cultivar Katahdin encoding this gene showed late blight resistance at Toluca valley, the center of origin of *P. inefstans*. The Agricultural Biotechnology Support Project-II operating from the Cornell University, USA initiated a programme to popularize the use of RB gene in South and South-East Asia. Two transgenic lines (SP904 and SP951) of the potato cultivar Katahdin were imported from the University of Wisconsin and evaluated under limited field trial at Shimla. Since, the event SP951 (male) showed better performance, it was used to cross with popular Kufri Jyoti (female) variety. The hybrids were tested and two of the hybrids namely KJ65 and KJ67 have showed promising results consistently since last 5-7 years.

The race non-specific resistance gene *RB* cloned from the sexually incompatible diploid species *Solanum bulbocastanum* was utilized to develop potato cultivars with durable late blight resistance. The Katahdin transgenic event SP951-derived five promising clones

(KJ-16, KJ-21, KJ-65, KJ-66, and KJ-77) carrying the *RB* gene and showing very high level of late blight resistance along with good agronomic characters and yield have been identified. We discovered that the genotypic background of the recipient plant is crucial for conferring RB gene mediated late blight resistance in potato. All five promising lines have potential to be released as cultivars and obtained permission for BRL trial.

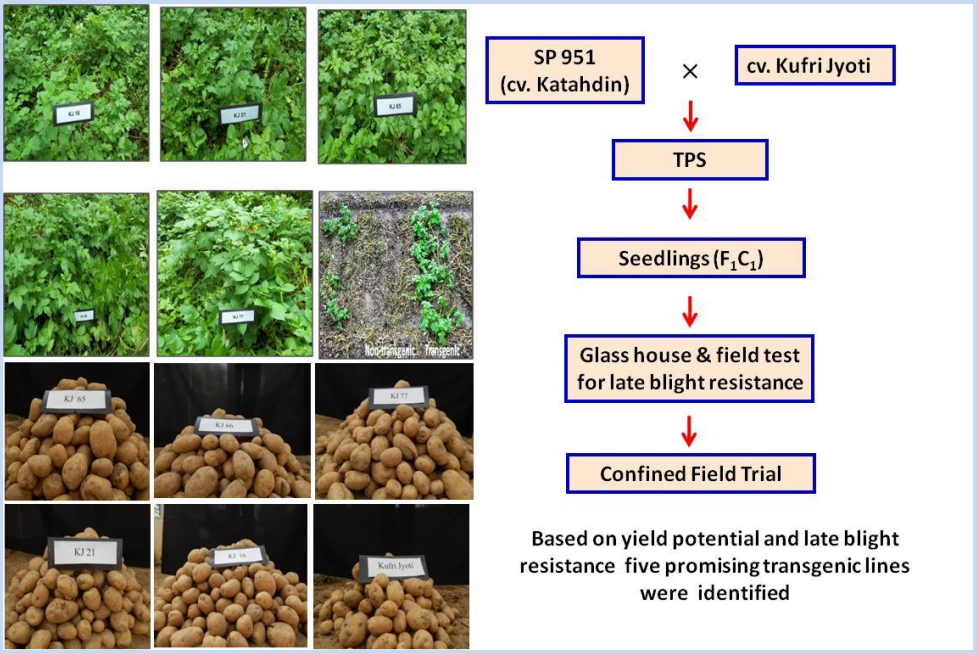

Fig. 3. Development of RB gene potato transgenics for late blight resistance

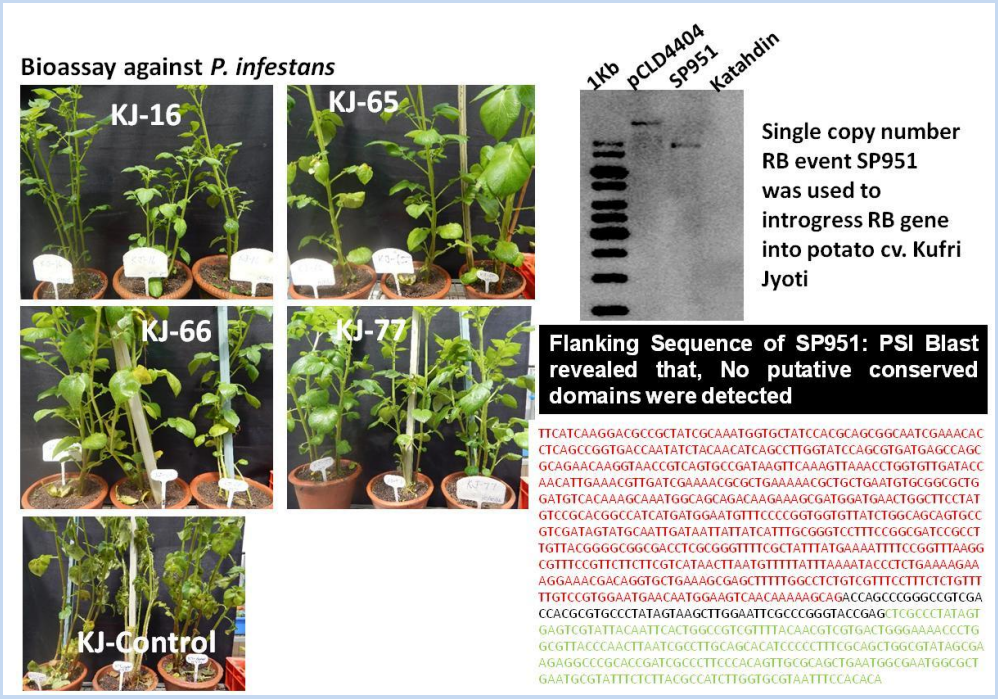

Fig. 4. Southern hybridisation of SP951 event showing integration of RB gene and single copy number

3.1.2 RXLR effecter gene (*Avr3a*) transgenics: Another technique which has been exploited in development of resistance against late blight is the RXLR effecter gene (*Avr3a*) of the pathogen that is responsible for pathogenicity. The silencing of pathogen effecter gene responsible for pathogenicity through host-mediated technique would prevent the pathogen establishment and spread. The *Avr3a* gene was targeted for silencing using siRNA (small interfering RNA) and amiRNA (artificial micro RNA) method using binary construct developed for transformation of Kufri Pukhraj variety. Five lines of siRNA and 3 lines of amiRNA showing resistance against late blight up to 90% have been identified and are being tested at the field level. Very recently, the institute has developed RNA solution consisting of dsRNA of the target pathogenicity gene for silencing that can applied to plant through spray to prevent the disease. This technology of dsRNA spray formulation to prevent the late blight disease has been applied for the patenting.

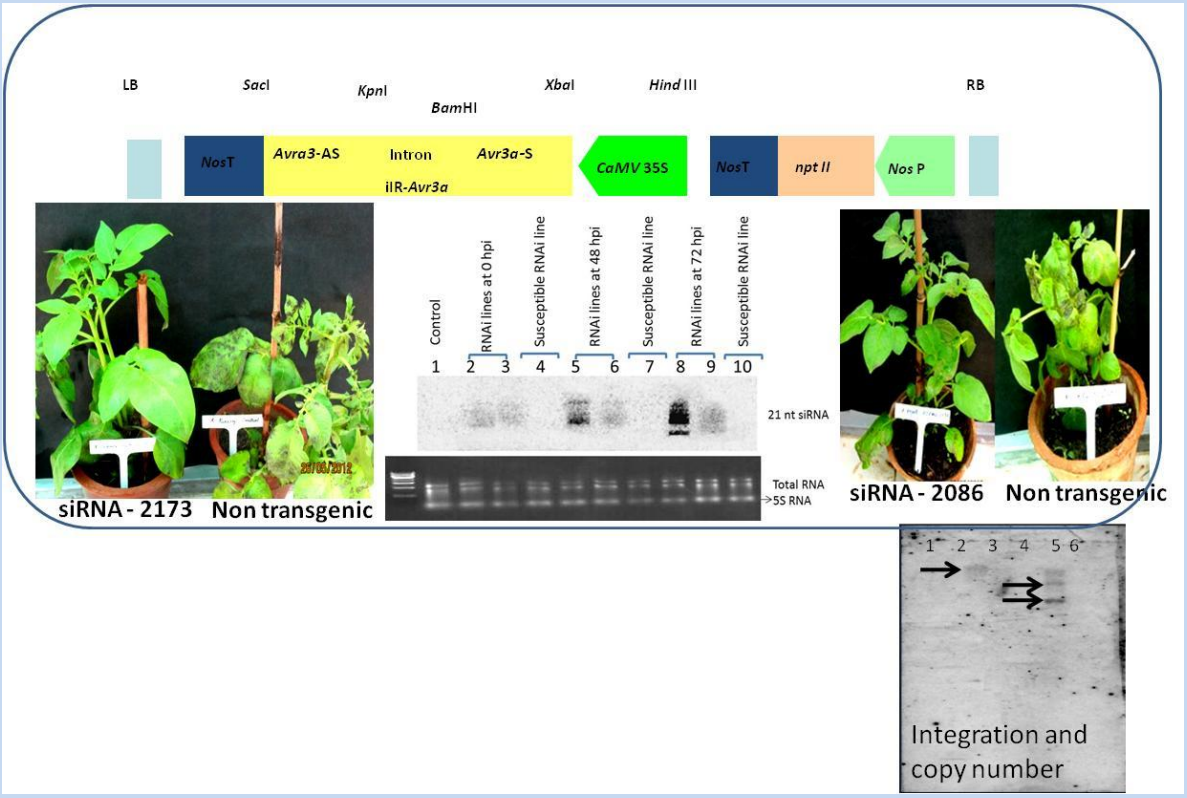

Fig. 5. Silencing of *Phytophthora infestans effector* Avr3a gene for late blight resistance

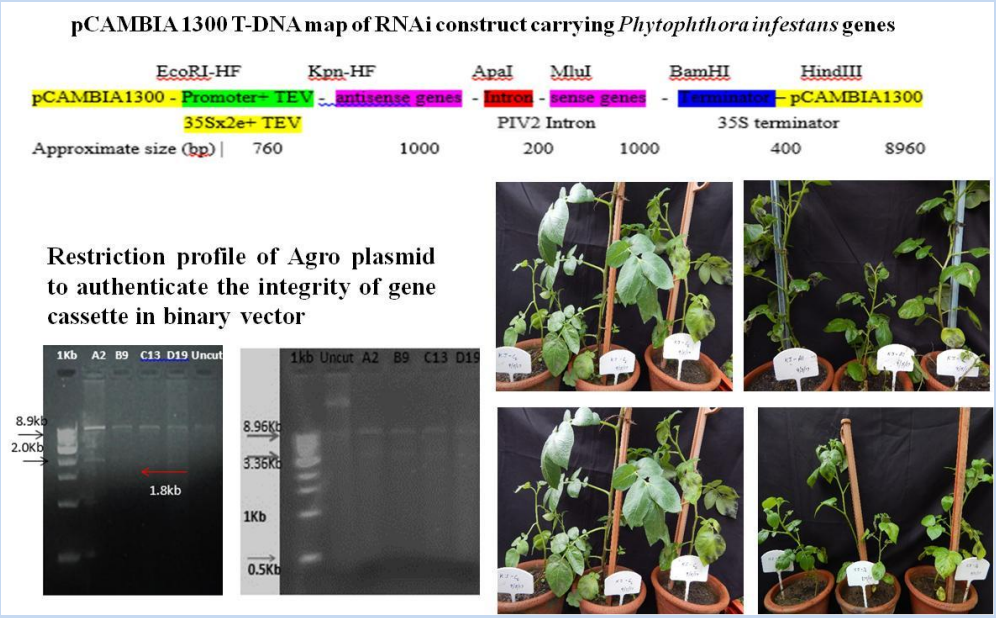

Fig. 6. Multiple siRNA expression cassette to confer late blight resistance

3.2 Bacterial wilt resistant

Bacterial wilt in India is caused by strains of phylotype I, II and IV of *Ralstonia solanacearum*. It is one of the most devastating diseases of potato, as observed in the Malwa region of Madhya Pradesh in India. Bacterial wilt is another chronic disease problem that does not have any reliable resistance source. Therefore, an antimicrobial peptide gene, bovine enteric β-defensin (*EBD*) has been used for conferring bacterial wilt resistance in potato. Transgenic lines of Kufri Badshah showed very high level of resistance to bacterial wilt in glass house screening. The gene has now been transferred to two commercial potato cultivars Kufri Giriraj and Kufri Jyoti. Kufri Giriraj was selected because of its popularity in Shimla and Nilgiri hills where bacterial wilt is prevalent. Kufri Jyoti is a popular variety in eastern plains where bacterial wilt is endemic. Twenty seven putative transgenic lines of Kufri Griraj and 12 lines of Kufri Jyoti have been developed.

Very recently, alternative approach for management of bacterial wilt caused by *Ralstonia solanacearum* in potato by silencing host susceptible gene was achieved. Since resistance host is not available, as an alternative management approach identified and isolated disease susceptibility gene (responsible for degradation of reactive oxygen species and hypersensitive response, as a negative regulator of defense) and developed RNAi transgenic lines. Through this a host susceptible gene phosphatidic acid phosphatase 2 (*PAP2*) has been silenced using RNAi technique and initial results have indicated imparting high level of resistance to the pathogen. The dsRNA based bacteriacide for management of same has also been developed and tested successfully. Many other methods of identification and developing the resistance against the bacterial wilt disease in potato are being followed at the institute.

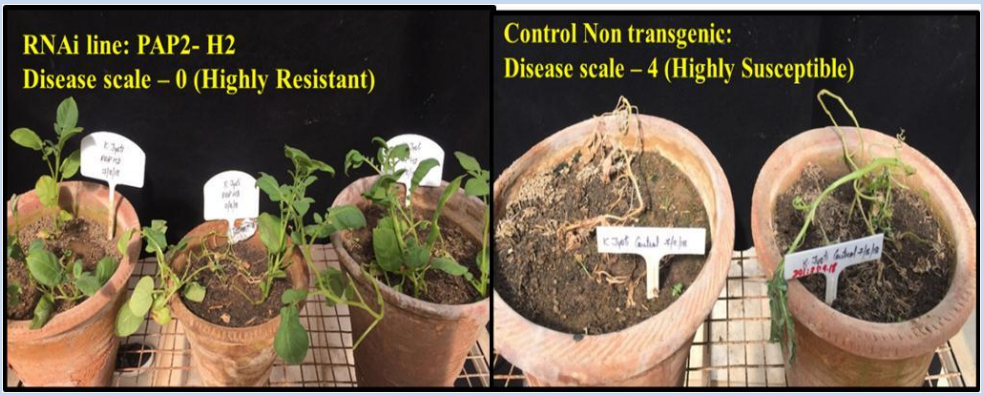

Fig. 7. Resistance response of RNAi Kufri Jyoti lines against *R. solanacearum*

3.3 Virus resistant

Disease caused by the viruses is one of the most important for potato production, especially for seed potato production as these viruses not only affect the quantity but also cause the degeneration of quality. Around 30 different viruses are known to infect potatoes, but only few contribute to degeneration of the crop. These viral diseases are the major limiting factors for the potato seed production and include, potato leaf roll virus (PLRV), potato virus X (PVX), potato virus Y (PVY), and potato apical leaf curl virus (PALCV). In addition to many known viruses infecting potato, newly emerging viral or virus-like diseases also threaten its seed production. Due to its vegetative mode of propagation, many of the viruses accumulate in seed tubers causing severe yield depression. PVY highly virulent mutant PVY^{NTN}, PLRV and the newly emerging ToLCNDV-Potato (also called PALCV) are the important viruses that cannot be managed by conventional breeding. Transgenic development by using pathogen-derived resistance is, therefore, being pursued for their management.

3.3.1 PVY resistant transgenics

Coat protein (CP) gene of *Potato virus Y* (PVY) was used for engineering pathogen derived resistance against PVY disease in the potato cultivar Kufri Pukhraj. Identification of stability and integration of coat protein gene in Kufri Pukhraj transgenic lines expressing PVY-CP through Southern Hybridization was carried out. Copy number analysis of CP gene was performed according to standard protocol. The presence of *npt*II gene was detected by random prime labelling radioactive method as mentioned by Amersham Random prime labelling kit. Data collected was *npt*II gene copy number in the plant genome of each transgenic line. The southern blot analysis result shows the copy number of *npt*II gene in the selected promised transgenic (KPYS1, KPYS13, KPYS18, KPYS20, KPYS21, KPYS26 and KPYNT3). Therefore, by referring to *npt*II fragment, CP gene copy number was same among the transgenic lines. It confirmed as a co-integration of the gene in the selected transgenic lines indicating the integration and stability of transgenes over the clonal propagation. The copy number analysis confirmed the integration of gene in the genome. These lines were subjected to bioassay by sap inoculation method. The lines were examined for their resistance

against PVY by observing the symptom expression over control (Fig. 4). Among the lines, KPYS-1, KPYS-13 and KPYS-20 showed resistance as the symptoms were not expressed in these selected lines. PVY transgenic lines KPYS-1, KPYS-13 and KPYS- 20 along with a non-transgenic healthy control were sown and raised under glass house conditions. These lines were regularly observed for their general growth. Out of three line KPYS-1 did not germinate i.e., zero per cent germination was observed. Whereas, the other two lines (KPYS-13 and KPYS- 20) along with non-transgenic healthy control showed 90 per cent germination. The above ground growth of KPYS-13 and KPYS- 20 was better than non-transgenic healthy control.

Fig. 8. Potato virus Y resistant transgenics

3.3.2 Potato apical leaf curl virus resistant

Potato apical leaf curl disease is an emerging gemini viral disease in tropics and subtropics. We investigated the potential use of RNAi for obtaining resistance against this DNA virus in potato using hairpin construct of replication-associated protein gene (*AC1*) of the virus. Transgenic lines subjected to agroincoculation showed higher level of resistance compared to non-transgenic control. AC1 (replicase associated protein) gene of PALCV has been targeted to silence through RNAi technique. Potato apical leaf curl virus (PALCV) resistant transgenics namely GTLC2-90 and GTLC2-127 of Kufri Badshah; KPLC2-13, KPLC2-37, KPLC2-53, KPLC2-54 of Kufri Pukhraj are multiplied under tissue culture. Out of these, two promising transgenic lines GTLC2-127 and KPLC2-53 were screened through agro inoculation technique and the result showed that transgenic lines did not show ToLCNDV-potato symptoms as against non-transgenic plants.

We have developed transgenic potato resistance to Tomato Leaf Curl New Delhi virus (ToLCNDV) or potato apical leaf curl (PALCV) virus causing apical leaf curl disease employing RNAi technology using r*eplicase* gene. Series of clonally propagated plants have been evaluated for their bioefficay against PALCV, gene expression and integration. To

know the chromosome location of the integrated gene/T-DNA in the identified event, performed flanking sequence analysis using genome walking method for selected promising ToLCNDV transgenics, i.e. KPLC2-53 and GTLC2-127 events. 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’’.

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 (Fig. 1) and 756 bp from GTLC2 -127 (Fig. 2) 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 no conserved domain have been detected for the obtained flanking sequences. Indicates that inserted site have not disturbed the potato genome. Southern blot hybridization analysis of the *NptII* gene copy number in the transgenic lines and genomic transgenic lines was digested with *Hin*d III is shown here.

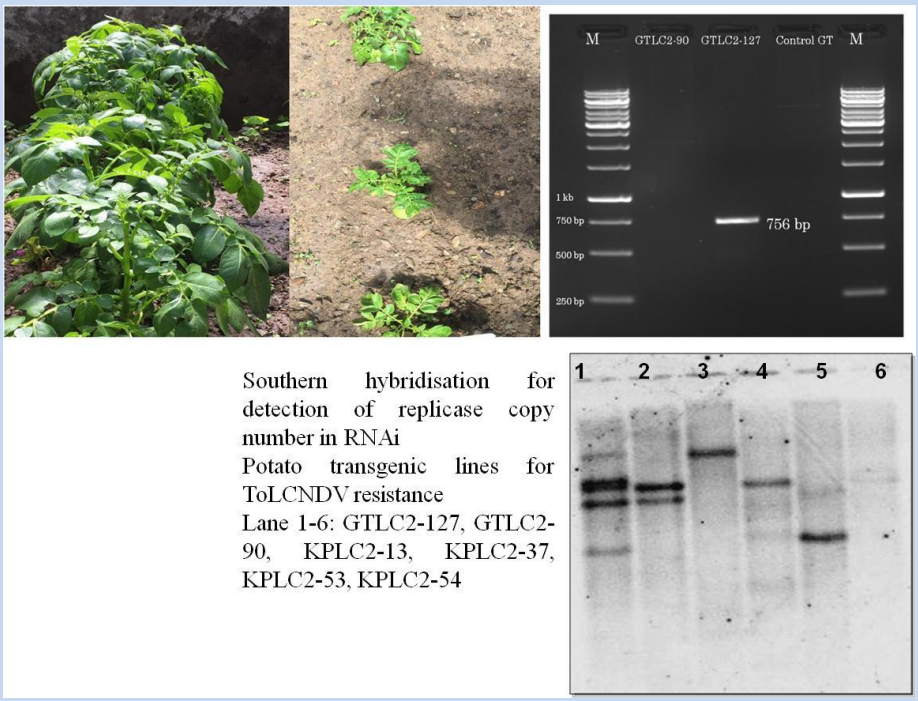

Fig. 9. Potato apical leaf curl virus (PALCV) resistant transgenics (GTLC2-127) and event PCR analysis in GTLC2-127

3.4 Reduction of cold-induced sweetening for chipping

Cold storage of potato is an integral requirement of post-harvest handling of this semi-perishable crop. Unfortunately, potato accumulates high amount of reducing sugars during cold-storage, a physiological phenomenon known as cold-induced sweetening. Processed

products like chips, French fries, etc. when prepared from sweetened potatoes develop brown coloration and are not preferred by the processing industry and consumers. Potatoes suitable for processing are not available round the year. This poses a serious impediment to fast emerging potato processing industry in India. It is, therefore, important either to develop varieties that do not accumulate reducing sugars under cold storage or to improve the processing attributes of existing potato cultivars to meet the growing demand of processing units. The biggest hurdle towards development of cold resistant potato cultivars through conventional breeding is lack of suitable germplasm to be used as parents.

Genetic engineering of Indian popular potato cultivars for prevention of cold-induced sweetening offers an easier and cheaper alternative as potato is highly amenable to biotechnological tools. Cold-induced sweetening in potato is a complex physiological process that involves interplay of different cellular controls. Different strategies need to be adopted to get viable and stable reduction of cold-induced sweetening, and also to prevent breaking down of any single strategy when the transgenic lines will be exposed to vagaries of nature in field. Two different biotechnological approaches through metabolic engineering have been adopted to block the step leading to conversion of sucrose to reducing sugars either by inhibiting the vacuolar invertase activity, the enzyme responsible for synthesis of reducing sugars, or silencing of one or more genes encoding for enzymes responsible for sugar accumulation in potato like vacuolar invertase (*INV*) and UDP Glucose Phosphatase (UGPase) at post transcriptional level. A gene encoding tobacco invertase inhibitor (*Nt-Inhh*) has been used for *StINV* inhibition in potato by over expressing the *Nt-Inhh* under the control of two different promoters, constitutive promoter, CaMV 35S, and tuber specific promoter, GBSS. While for the second strategy *StINV* and *UGPase* genes have silenced using the RNAi technique (both SiRNA and amiRNA). By inhibiting the expression of this gene using RNAi technology, it has proven that the transgenic plants prevented the cold induced sweetening of potato tubers upon cold storage even up to 135 days. A total of 7 transgenic lines of Kufri Chipsona-I with inhibited expression of INV were selected from more than 300 transgenic events and confined field trials (CFT) at CPRS, Jalandhar (BT/BS/17/22/97- PID). Single line KChipInvRNAi-2214 was selected because it has significantly lower soluble sugars, superior chipping characters, and on par yield compared with the control. This transgenic event exhibited >80% reduced hexose accumulation during cold storage. Moreover, during the frying process, the transgenic Event KChipInvRNAi-2214 showed no browning up to 135 days of cold storage compared to the wild type with significant reduction in acrylamide formation. These results suggest that inhibiting the expression of vacuolar invertase in potato tubers have greatly improved the processing quality. This product is in the last stage of obtaining the Indian patent (Application: 2762/DEL/2009).

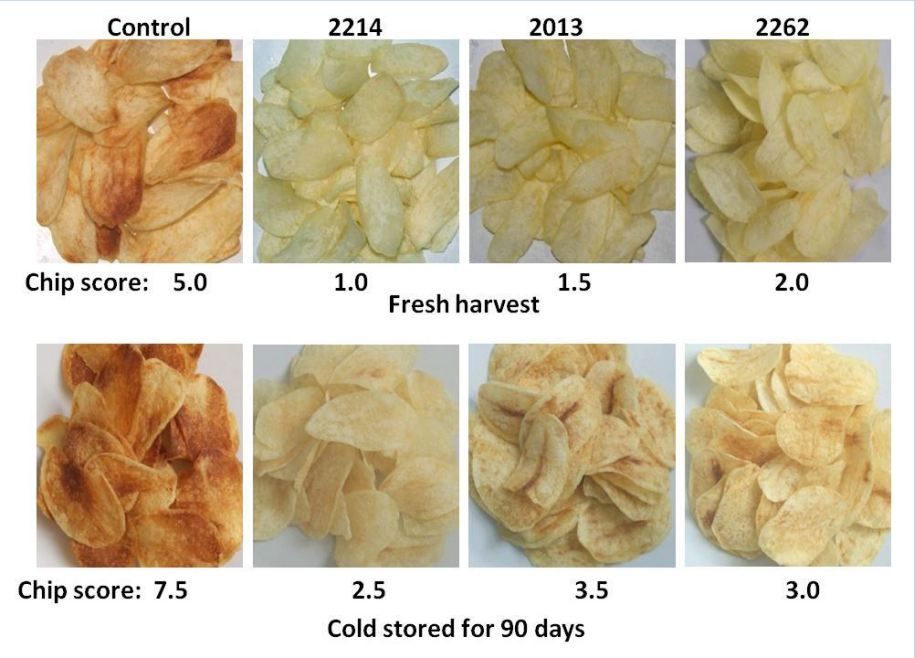

Fig. 10. Potato transgenics KChipInvRNAi-2214 with reduced cold-induced sweetening for better chipping quality

3.5 Protein enhancement

Potato tuber contains about 1-2% protein on the fresh weight basis, which is insufficient for a staple food. In terms of protein quality, potato protein has been given an intermediate score by WHO. This is because potato protein is deficient in certain essential amino acids like methionine/cysteine, leucine, isoleucine, and threonine. A collaborative project between ICAR-CPRI, Shimla and NCPGR, New Delhi to improve nutritional quality of potato protein by expressing the high quality seed storage protein (*AmA1*) of *Amaranthus hypochondriacus* cloned and patented by both the institutes. On the basis of gene expression analysis in putative transgenics, 40 lines were selected for preliminary evaluation of tuber yield at ICAR-CPRI RS, Modipuram. Based on tuber yield and expression of *AmA1* gene, 14 lines of 7 potato cultivars were selected for further limited field trial at Modipuram and Jalandhar. Crude protein content of 14 transgenic lines along with their respective controls was estimated in dry powder made from peeled potato tubers by Kjeldahl's method at Modipuram. Out of the above nine promising transgenic lines selected on the basis of yield performance, increase in crude protein content was observed in Kufri Chipsona 1/18 (11.36%), Kufri Chipsona 1/21 (4.57%), Kufri Chipsona 2/15 (38.81%), and Kufri Badshah/5 (16.02%). Increase in protein content was also confirmed by NCPGR, New Delhi in case of Kufri Chipsona 1/18 (20.72%), Kufri Chipsona 2/15 (34.20%), and Kufri Badshah/5 (46.65%). Therefore, on the basis of improvement in protein content, 3 transgenic lines, viz. Kufri Chipsona 1/18, Kufri Chipsona 2/15 and Kufri Badshah/5 may be selected for further analysis. Amino acid composition of 14 transgenic lines along with their respective controls was determined in freeze dried potato powder using HPLC at NBPGR, New Delhi.

Table 2. Composition of essential amino acid (g/16g N) of the above three selected lines along with their respective controls

Essential amino acid	Kufri Chip1-Control	Kufri Chip1/18	Kufri Chip2-Control	Kufri Chip2/15	Kufri Badshah-Control	Kufri Badshah/5
Histidine	2.71	2.51	2.40	1.90	2.32	2.57
Arginine + Threonine	14.60	13.27	12.46	11.12	11.58	13.03
Valine	4.74	4.42	4.42	4.87	4.03	4.50
Methionine	0.79	0.67	0.63	0.66	0.48	0.61
Lysine	3.94	4.35	3.90	3.72	4.42	3.90
Isoleucine	2.79	2.60	2.58	3.14	2.08	2.37
Leucine	5.30	4.73	4.53	5.56	4.09	4.58
Phenylalanine	4.34	3.56	4.03	4.70	3.31	4.27

In case of Kufri Chipsona 1/18, improvement only in lysine content (10%) was observed; other essential amino acid contents either decreased or remained unchanged. Improvement in valine (10%), methionone (5%), isoleuucine (22%), leucine (23%), and phenylalanine (17%) was observed in case of Kufri Chipsona 2/15 compared to non-transgenic control. Similarly, improvement in histidine (11%), arginine+threonine (12%), valine (12%), methionine (27%), isoleucine (14%), leucine (12%), and phenylalanine (29%) was observed in Kufri Badshah/5 compared to its non-transgenic control. Therefore, two transgenic lines, viz. Kufri Chipsona 2/115 and Kufri Badshah/5 may be selected for food safety analysis (Chakraborty et al. 2010). Very recently, with an intention of biofortification to improve nutrition quality of potato *StGalDH* for vitamin C, *StAN* for anthocyanin, are being over expressed in potato to improve respective nutrients. Efforts are on to biofortify potato with improved iron and zinc content.

3.6 Dwarf architecture potato

Potato plants when grown under long days or high temperatures conditions accumulate gibberellic acid and grow tall leading to reduced partitioning of dry matter to the tubers. Potato varieties grown under short days during winter in plains have high harvest index of about 80% whereas the same varieties when grown under long days during summer in hills grow very tall and have lower yields with an harvest index of about 50%. To reduce internal gibberellic acid content and consequently excessive vegetative growth, we have silenced *GA20-oxidase1* gene (involved in the synthesis of gibberellic acid) through post transcriptional gene silencing (PTGS) to obtain plants with reduced GA content. Glass house trials were conducted with 75 IRGA, 35 sense and 34 antisense plants. Based on the yield performance for two years as well as plant height, 10 IRGA lines, 5 sense lines and 5 antisense lines were selected for further trials.

Fig. 11. Dwarf potato transgenics

3.7 Transgenic potatoes for other traits

Many other characters have been targeted for improving the yield in potato through transgenics development. For example, potato tuber moth is an insect pest of potato prevalent in certain pockets where potato is stored under country storages without refrigeration. No conventional source of resistance has so far been reported against the pest. Genetic engineering is, therefore, is an alternative for developing resistance cultivars. The synthetic *Cry1A*b gene of *Bacillus thuringiensis* has been used to confer resistance to potato tuber moth. Transgenic tubers of five lines (KB-1, KB-22, KS-6, KJW-3 and KL-2) showed good level of resistance on laboratory evaluation. However, PTM resistance in transgenic tubers was lost with increase in storage time. Since *Cry9Aa2* was the most effective Bt toxin for PTM, attempt was made to check the efficacy of the native gene by chloroplast transformation of potato and characterized the transchloroplastic lines with *cry9Aa2* gene.