eTechnical Bulletin No. 6
(Ref.: No.F.PME/3-3/2021 dated 4.7.2021)

Next-Generation Breeding in Potato

Authors

Jagesh Kumar Tiwari

SK Luthra

Vinay Bhardwaj

Salej Sood

Dalamu

Tanuja Buckseth

Rajesh Kumar Singh

Manoj Kumar

ICAR - CENTRAL POTATO RESEARCH INSTITUTE, SHIMLA (HP)

1. Conventional potato breeding

The cultivated potato is autotetraploid (2*n*=4*x*=48) has four copies (homologues) of each of 12 unique chromosomes is highly heterozygous and suffers from rapid inbreeding depression. The heterozygosity in commercial cultivars is preserved by the clonal propagation of tubers. Breeding potato is a difficult task, as more than 52 desirable traits are to be combined in an ideal modern potato variety. These traits include morphological features, yielding ability, tuber characters, ability to withstand biotic and abiotic stresses, wider adaptability, quality parameters, consumer and industrial acceptability. It is perhaps an impossible task to combine all traits to obtain an ideal variety because of complex heterozygous nature of potato. The new variety thus, should be superior to existing in at least one important characteristic, without being significantly inferior to it in any other important traits. The genetic base of the released potato varieties grown on large areas is relatively narrow as compared to accessible genepool for conventional potato breeding. Only a fraction of useful genes from wild species has been successfully introgressed into modern potato varieties (nearly 4000 varieties). Modern varieties are the products of extensive breeding effort between different cultivar groups, cultivated and wild species of potatoes. History of conventional potato breeding reveals that many of important variety took nearly 30 or more years from hybridization and clonal selection to their release. The long breeding cycle in potato is due to quantitative nature of most of the desirable traits, rapid inbreeding depression and low intensity of selection in early generation. The low multiplication rate and amenability of potato clones to pathogens lead to degeneration of stock and reduces the quality of the tubers. Conventional potato breeding scheme involves, selection of trait specific parents, hybridization, phenotypic recurrent selection in seedling and clonal generations at targeted locations for a wide range of desirable characters, which nearly take 12-15 years. Thus the conventional potato breeding cycle take longer time, seek huge investment, involves more manpower and delay the accessibility of the targeted variety to stakeholder in changing climatic scenarios.

2. Next-generation potato breeding

Potato improvement through application of next-generation breeding techniques is essential to shorten usually longer time period (9-10 years) required for developing a new potato variety. Successful completion of potato genome sequencing enabled discovery of a large number of genes regulating multiple traits like biotic and abiotic stress tolerance, quality and yield attributes etc. Besides, bi-parental linkage mapping and population genetics by Genome Wide Association Studies (GWAS), Genome Editing and Genomic Selection (GS) coupled with Genotyping by Sequencing (GBS) and/or SNP chip markers platform with integrated High-throughput Genotyping (HTG) and High-Throughput Phenotyping (HTP) facility have emerged as powerful techniques for completion of breeding cycles in shorter time span. Though, potato is an autotetraploid with high heterozygosity and complex inheritance pattern, there is immense potential to apply these new breeding techniques for potato improvement (Fig. 36).

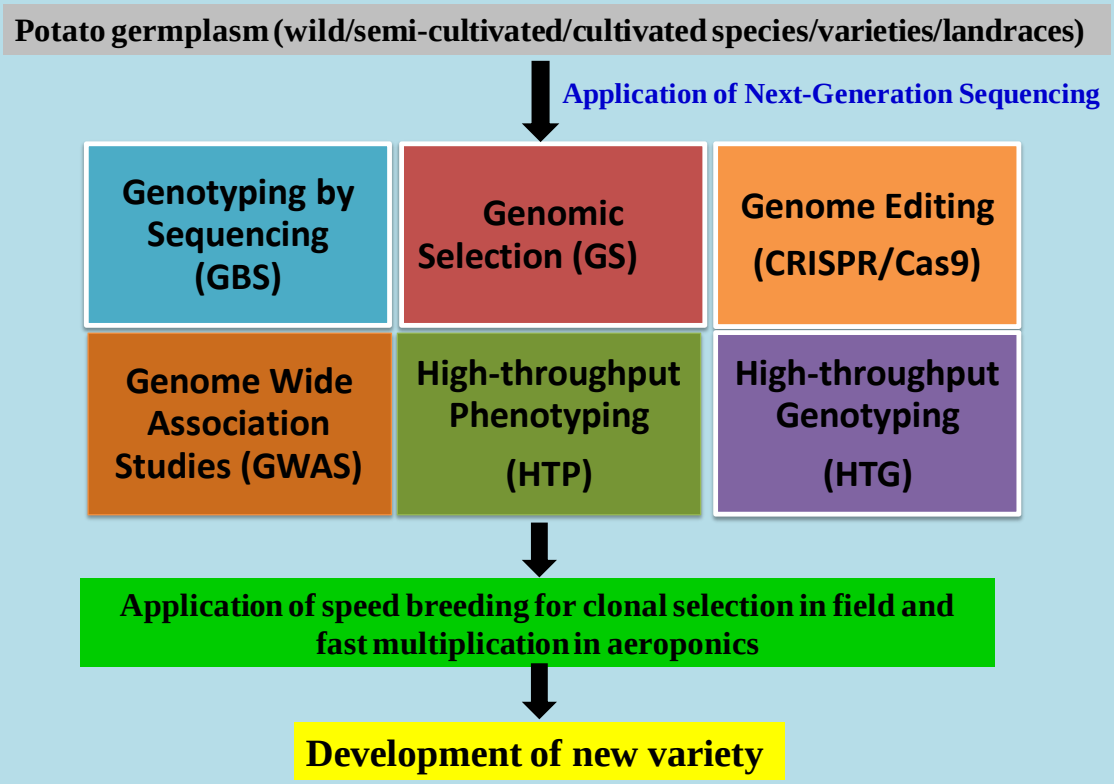

Fig. 36. Application of new breeding tools in potato improvement

3. Genomic Selection

In developing new potato variety, breeders have to deal with more than 50 characters (biotic stress, abiotic stress, quality traits, yield attributing and tuber traits), which takes over 10 years in this clonally propagated crop. Since long, marker-assisted selection (MAS) has been the powerful tool in plant breeding, and it has been applied in potato for traits like late blight, potato viruses and potato cyst nematode resistance etc. However, MAS has limitation for complex inheritance traits like yield and abiotic stress tolerance.

Genomic selection (GS) or genome wide selection or genomic-assisted breeding is a novel plant breeding method. It enables integration of phenotyping and high-throughput genotyping data of pedigree/segregating generations to enhance genomic selection in short period. With the discovery of potato genome sequences, there is immense opportunity to work at whole genome level. In that, GS is the strategy to predict breeding model at whole-genome level for rapid potato improvement. Without gene mapping, GS works on the principle of linkage disequilibrium (LD) with a minimum of one marker per loci in the breeding population. GS accelerate breeding cycles with increase in genetic gain per unit time and reduced cost as well. GS is well established in animal breeding and becoming important in plant breeding as well. In GS method first we estimate GEBV in Training Population (TP) based on HTG and HTP data; then genotype any unknown Breeding Population (BP) to select lines based on the genomic estimated breeding value (GEBV), determined in TP, without phenotyping of BP. TP a sample breeding population which is used to investigate future segregating generations/populations etc. to develop new varieties. GS depends upon genetic similarity

between TP and BP within the LD between marker and loci. Once, GEBV is predicted, it can be considered for selection of new breeding lines based HTG only (Fig. 37).

GS determines genetic association and diversity with different landraces/ cultivars/ varieties/ breeding lines/ wild species with variation in topography and ecology. With the identification of genome rearrangements and SNP discovery at whole genome level, GS can be applied in near future. GS has been successfully applied in animals and reported to some extent in plants like maize, wheat, sugar beet etc. Specific SNPs or haplotypes can be used for genomic selection, as predicted frequency of SNP in potato is 1 in 24 base pair in the exons. Application of genomic selection in potato so far is very limited; might be due to unavailability of SNP markers distributed throughout genome, trait association, SNP calling rate and software uses in this tetraploid and heterozygous potato crop. Effect of SNP and haplotypes is searched throughout the genome to estimate genomic estimated breeding value (GEBV) of a training population which is then used to predict performance of other breeding lines avoiding phenotypic selection at every generation. Training population should be genotyped through as many SNP markers as possible. Availability of over 39,000 genes in potato genome helped in identifying large number of markers for GS. The rapid advancement in genotyping techniques (SNP and haplotypes), HTP and trait association would led to reality of GS of potato in near future.

Advantages of GS

- GS can resolve the complication of complex traits with MAS and it is applied to whole genome level and not to a particular trait.
- GS adds advantage where no reference genome sequence is available to genotype a large breeding population
- Single gene or multiple genes governing traits with complex nature can be handled.
- It reduces the length of breeding cycles to enhance genetic gain and selection efficiency with less resource input using molecular markers.
- It reduces cost of phenotypic screening and times.
- GS investigates whole genome level (on contrary to MAS is applicable for limited number of QTLs/gene)-based variance and avoids complications associated with GWAS and QTLs related markers.
- GS applies a number of markers distributed throughout genome and to develop genome wide marker maps (like earlier done using 10000 AFLP markers in potato).

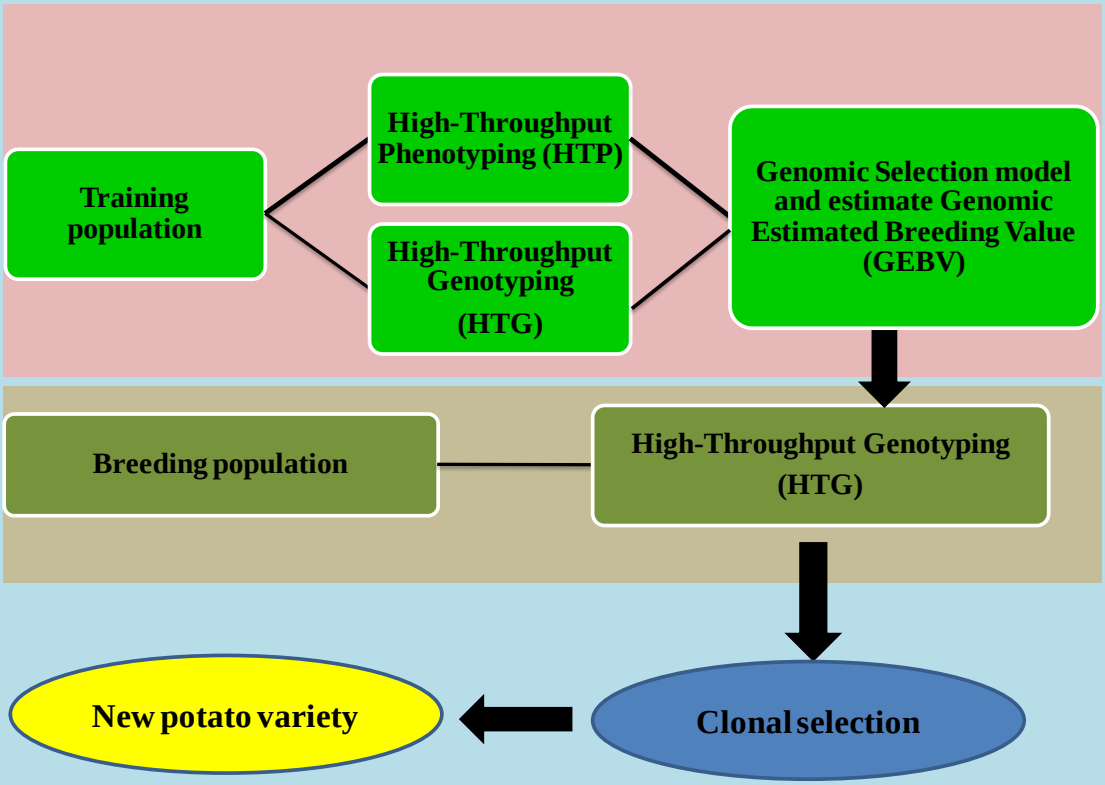

Fig. 37. A schematic outline of Genomic Selection in potato

4. Genotyping by Sequencing (GBS)

GBS is one of the HTG techniques currently being used to generate high-throughput genotyping data for several crop species for SNPs identification and genotyping. With the reducing next-generation sequencing cost, a huge amount of high-throughput data are being generated. It enables discovery of a large number of SNPs for high-throughput genotyping. GBS has been designed for several studies like genetic analysis, population studies, molecular characterization of germplasm, SNP discovery etc. GBS is being studied at single to whole genome level. This is a quick and cost-effective method for high-throughput genotyping of breeding/segregating population, linkage analysis, diversity studies, molecular markers, genomic selection etc. To breed varieties, knowledge about genes and environment and their interaction is important for using GBS method to select advance breeding lines with desirable traits. Besides GBS, SNP chip-based markers are also another HTG platform available in potato for genotyping using various platforms such as 20K SNPs Affymetrix Axiom (SolSTW array), Infinium 12K V2 Potato Array (Illumina platform), and 8K SNPs (Illumina Infinium BeadChip).

Advantages of GBS:

- Identification of genome wide thousands of markers and generate adequate data to cover the whole genome using NGS technology
- It has improvement in terms of markers resolution efficiency and development of reference assembly by GBS to develop markers for multiple traits.

- It helps in development of linkage map of segregating generations.
- It is cost-effective, less price, and genome level sequencing.
- Development of whole genome level SNP markers for a large number of application with chromosome wise genomic region, linked with desirable traits information.
- It has wider application in terms of population genetics studies.

5. Genome Wide Association Studies (GWAS)

GWAS has been applied in many crops including potato to examine simple and complex traits to analyse genetic diversity in populations. GWAS is a family based linkage mapping approach to identify markers and associated traits. It consists of a large number of individual like landraces, wild and cultivated species, varieties, core collection etc. It takes advantage of meiosis over the evolutionary process in hundreds of genotypes having genetic diversity based on linkage disequilibrium (LD) value. Association mapping (AM) uses association of markers and QTLs within LD and linked in the ideal case. In this method, population structure is determined based on the genetic markers using software like Structure to determine marker trait association (MTA). It has been conducted in several crops for complex traits and in potato for traits like late blight resistance, maturity, *Verticilium* resistance, yield, tuber quality, chips, starch, tuber bruising, tuber shape etc. Over the time GWAS has improved a lot due to various population structure, parents/kinship, evolutionary relationship etc.

Advantages of GWAS

- Better mapping due to large number of meiosis events in the diverse population unlike linkage mapping where recombination in a single mapping population is searched
- Large number of alleles per locus can be found.
- Markers are directly applicable for breeding application.

6. Genome Editing: a paradigm shift in potato breeding

Genome editing is the targeted alteration in a genome, which creates new allelic variation. Sequence specific nucleases (SSNs) have been applied for genome editing and genetic manipulation in the genome. The SSNs technology is rapidly becoming important in plants, which uses three major nuclease systems like Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR- associated proteins (CRISPR/Cas9). Among these, CRISPR/Cas9 is the most widely used today for genome editing. The CRISPR/Cas9 is a RNA-guided method to target DNA sequence. Site directed mutagenesis (SDM) and gene silencing are two major concepts in crop improvement, which work on principles of double-stand breaks (DSBs) creates mutations and repaired endogenously by non-homologous end joining (NHEJ) or homologous recombination (HR). This is used widely due to its simplicity, multiplexing capability, cost-

effectiveness, and high efficiency. The issue with the technology is the off-target mutations due to mismatch base pairing between gRNA and DNA.

Genome editing is an important tool to create new variants with desirable gene combination. Unlike GM technology which creates stable integration in line by cisgene or transgene, this new breeding tool of genome editing offers crop improvement where no foreign gene is introduced and the selected line is not likely to be treated as GMOs. In potato TALENs and CRISPR/Cas9 has been used to some extent for traits like cold induced sweetening, glycoalkaloids (solanine and chaconine), acetochalactate synthase, granule bound starch synthase etc. CRISPR/Cas9 is known for site directed mutagenesis and gene silencing in potato (Table 38).

Table 1: Application of breeding tool like CRISPR/Cas9 and TALENs in potato

Objective	Target trait	Editing tool	Reference
Altered starch quality	Granule-bound starch synthase (GBBS)	CRISPR/Cas9	Andersson et al., 2017
Targeted mutagenesis	Aux/IAA gene family (StIAA2)	CRISPR/Cas9	Wang et al., 2015
Targeted mutagenesis	Acetolactate synthase1 gene (StALS1)	CRISPR/Cas9	Butler et al., 2015
Targeted mutations	4-alpha-glucan branching enzyme (SBE1), Vacuolar invertase 2 gene (StvacINV2)	TALENs	Ma J. et al., 2017
Altered starch composition	Granule-bound starch synthase (GBBS)	TALENs	Kusano et al., 2016
Reduced anti-nutrient element	Vacuolar invertase gene (VInv)	TALENs	Clasen et al., 2016
Targeted mutagenesis	Acetolactate synthase gene (ALS)	TALENs	Nicolia et al., 2015
Reduced anti-nutrient element	Sterol side chain reductase 2 (SSR2)	TALENs	Sawai et al., 2014
Targeted mutation	Acetolactate synthase1 gene (ALS1)	Geminivirus replicon (GVR)-mediated TALENs and CRISPR/Cas9 delivery	Butler et al., 2016

Adapted (modified) from Hameed A, Zaidi SS, Shakir S and Mansoor S (2018) Applications of New Breeding Technologies for Potato Improvement. Front. Plant Sci. 9:925. (see for detailed references)

7. High-Throughput Phenotyping platform

After the huge work on MAS, linkage mapping, GWAS, GS and genomics, genome editing has taken up led in the area of plant breeding to enhance crop production/ productivity. This has enhanced our knowledge in plant breeding. However, high-throughput precision phenotyping is essential to utilize ultimate potential of genotype. Present methods of phenotyping is slow, time taking, laborious and less precise, often destructive or has limited phenotyping ability. High-throughput phenotyping platform is essential for precision phenotyping and breeding application.

High-throughput phenotyping (HTP) platforms are usually based on automation, sensors, high resolution imaging capability, robotics etc. In potato, a few technologies have been applied to roots and shoot traits (Fig. 38). For example, Phenofab and Keytrack System (KeyGene, The Netherlands) have been developed using multiple imaging system and thermal sensors with automated handling under controlled environments for measuring plant growth and other traits. However, correlation between pots and field grown plants are essential.

Fig. 1. High-throughput Phenotyping facility at the Plant Accelerator, University of Adelaide, South Australia, Australia

8. Speed Breeding in Potato

As conventional potato breeding cycle take nearly 12-15 years for the development of variety, the new proposed **s**peed breeding scheme in potato to aims to reduce breeding cycles with fast multiplication of tubers and multi-location evaluation of clones to enhance selection process within less time to develop new varieties. The traits specific selection of parents

through genomic selection, targeted hybridization, screening of population up F_1C_3 stage and rapid multiplication and efficient faster multi-location evaluation added with modern techniques may results in delivery of end product in shorter period. A schematic outline is depicted in Fig. 39. Some points are:

- The first requirement for speed breeding is multiplication of F_1C_4 advanced hybrids (around 50 clones) in aeroponics during offseason, and multiplication of selected F_1C_4 advanced hybrids in seed production unit (SPU) during main crop season.
- Multi-location evaluation of F_1C_5 advanced stage hybrids in three environments for trait specific selection
- Identified promising F_1C_5 advanced stage hybrids for foreground selection for specific traits using marker assisted selection.
- Introduction of advanced stage hybrids (F_1C_6) in AICRP for multi-location testing across the country.
- Release of potato variety following the crop improvement protocols under AICRP (potato).
- Aeroponic facility for 500 plants might be exclusively required for speed breeding purpose.
- The process is expected to shorten the breeding cycle in potato at least by 1-2 years.

Thus, there is immense opportunity to apply these modern breeding tools like genomic selection and genome editing for potato improvement for complex traits.

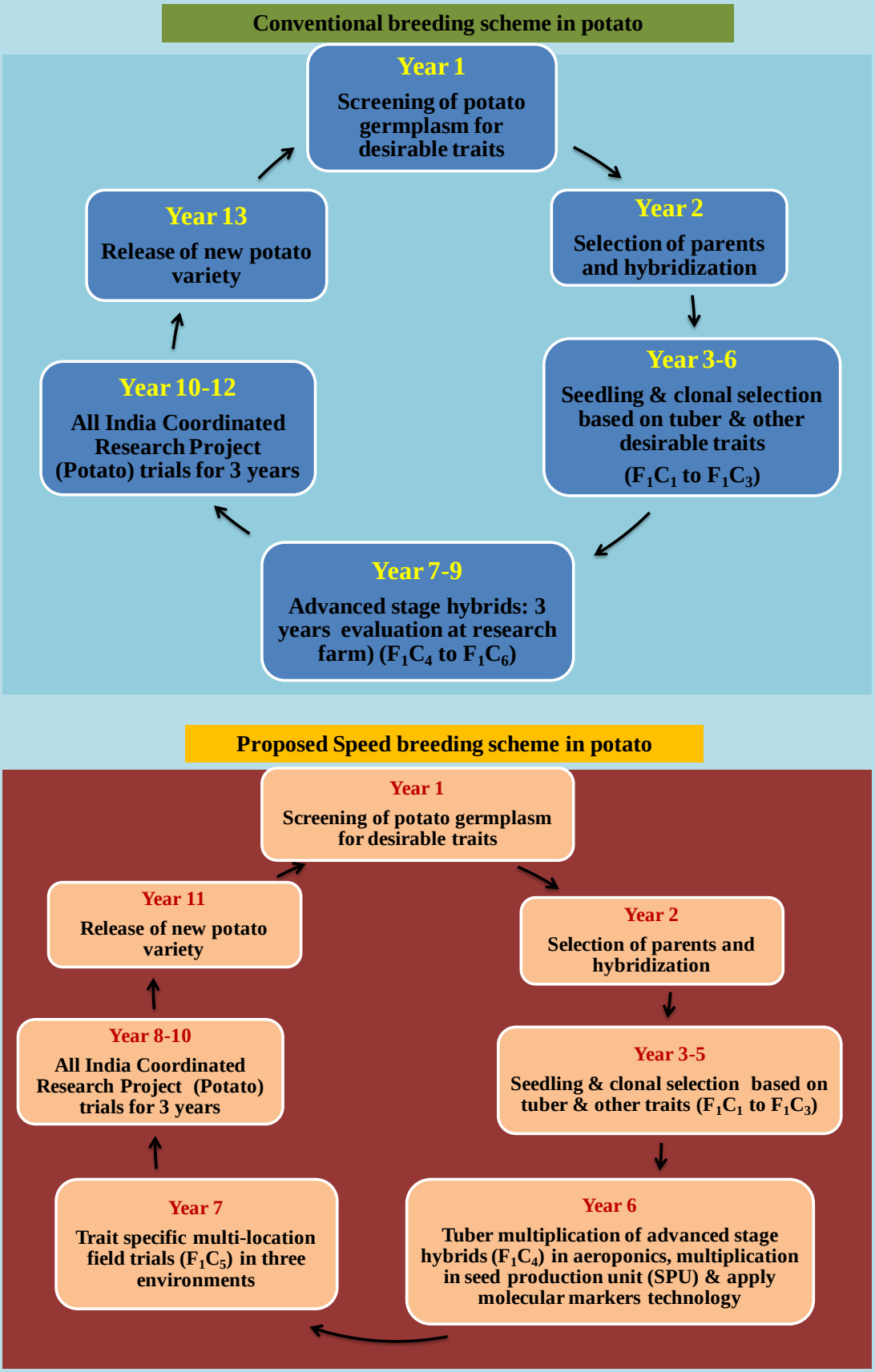

Fig. 2. Conventional and speed breeding schemes in potato